Comparative Study of Freely Suspended and Immobilized Microalgae for Palm Oil Mill Effluent (POME) Treatment

by

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15248

Dissertation submitted in partial fulfilment of
the requirements for the
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Universiti Teknologi PETRONAS,
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Perak Darul Ridzuan

CERTIFICATION OF APPROVAL

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Approved by,

_____

(Dr. Azizul Buang)

UNIVERSITI TEKNOLOGI PETRONAS
BANDAR SERI ISKANDAR, PERAK

September 2015

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 acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

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NAZIRA SYAZWANA BINTI MOHYEN

ABSTRACT

Palm Oil Mill Effluent (POME) generated as by-product during clarification and purification process to produce Crude Palm Oil (CPO), contains harmful heavy metals, high Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD) and mineral contents such as Nitrogen, Phosphorous which can cause severe pollution to the environment. To encounter this problem, the utilization of microalgae can solve the problem of POME remediation. However, the major challenge to utilizing algae is the challenges faced in harvesting and drying the algae after the remediation process. In this research, freely suspended and immobilized microalgae species *Nannochloropsis Oculata* and *Chlorella sp*. has been used. The main objective of this study was to evaluate the potential of immobilized microalgae for POME treatment. The changes of parameters in COD, BOD, TN and heavy metals have been tested after 8 and 16 days of treatment. The influence of different concentrations of filtered and centrifuged POME in sea water (1, 5, 10, 15 and 20%) on microalgae cell growth, lipid contents and POME remediation has been investigated. Immobilized *Chlorella sp.* and *Nannochloropsis Oculata* had enhanced cell growth and lipid accumulation at 10% POME with maximum specific growth rate (0.21 d^{-1}, 0.108 d^{-1}), doubling time (3.96 d^{-1}, 6.41 d^{-1}) and lipid content (31.67%, 31.45%), respectively, after 16 days of shake flask cultivation. Immobilized microalgae cultivation with POME media also enhanced the removal of heavy metals such as Fe(III) and Mn(II), COD (91-99%), BOD (82-99), and TN (78-98%).

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LIST OF ABBREVIATION

μ_{max}	=	Specific growth rate
x_2	=	Cell density at day 18
x_1	=	Cell density at day 4
t_d	=	Doubling time
A_i	=	Initial concentration
A_f	=	Final concentration

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CHAPTER 1
INTRODUCTION

1.1 Background of Study

For the last decade, Malaysia has controlled almost 45% of the palm oil production in the world. The main by-product in crude palm oil production processes (sterilization, clarification and nut cracking) is liquid sludge waste or known as Palm Oil Mill Effluent (POME). POME is a thick brownish colloidal suspension that has unpleasant odour and it is considered harmful to the environment if discharged untreated. According to Department of Environmental (1999), for every Fresh Fruit Bunch (FFB) Processes, 0.75 tonne of POME is generated.

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. At present, 85% of the POME treatment is using anaerobic and facultative pond system, followed by open tank digester attached with extended aeration in a pond (Vijayaraghavan et al, 2007). The conventional treatment system consists of aerobic and anaerobic ponds treatment which is not cost effective. A tertiary treatment is required using an environmentally friendly and economical method and this can be achieved with microalgae. Therefore, biological treatment by microalgae for POME has been reviewed and has gained importance in recent years. POME contains abundant of organic waste, heavy metals, nitrogen, phosphorus, etc. that later can be assimilated by microalgae for its growth.

The main advantages of utilizing microalgae for wastewater treatment are they only require short generation times, high growth rates, can produce biodiesel as renewable energy through its biomass, minimal land requirement needed and using wastewater bodies as nutrient feed with no herbicides and pesticides. The challenge is to make it more economical through effective and efficient growth and culture conditions and harvesting of the microalgae. Expensive media are being currently used for culturing microalgae which makes the overall process a costly affair. In addition, traditional method utilised free-suspended microalgae in the body of wastewater for treatment activities. Hence, large quantities of chemicals and high energy driven processes are used for harvesting of the microalgae biomass which made the process not economically attractive. Hence, to solve the problem, the objective of this study is also to develop microalgae cell immobilization system i.e. through the method of entrapping microalgae biomass in polymers formed with sodium alginate. Immobilization is among techniques that could lead to continued use of algae over prolonged period.

Basically, algal wastewater treatment systems can be viewed as two-component symbiosis systems of interaction between algae and wastewater. Consequently, the incorporation of immobilization technology into algal wastewater treatment systems introduces a third component that is alginate beads to the systems to improve the conventional method that is free suspension microalgae for wastewater treatment. Immobilization of microalgae in the alginate beads has significantly enhanced their populations when those beads were suspended in wastewater and also it is suitable for repeated process of wastewater treatment. The interactions between microalgae and alginate beads, i.e. how the immobilization affects the algal cells, includes morphological changes, growth characteristics and the metabolic adsorption activities of algae. The success in using immobilized microalgae for wastewater treatment depends upon many factors such as carbon and nutrients (N and P) available, algal species, immobilization matrix, cell and bead concentration, aeration, retention time, temperature, light intensity and pH for optimum microalgae growth.

1.2 Problem Statement

POME contains high Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD) and mineral contents such as Nitrogen, Phosphorous and heavy metals which can cause severe pollution to the environment. The conventional treatment system consists of aerobic and anaerobic ponds treatment which is not cost effective. An environmental friendly and economical tertiary treatment is required and this can be achieved with microalgae. Therefore, biological treatment by microalgae for POME has been reviewed and has gained importance in recent years. POME contains abundant of organic waste, heavy metals, nitrogen, phosphorus, etc. that later can be assimilated by microalgae for its growth. From the previous research, one of the major problems in algal treatment systems is harvesting or separation of algal biomass from the treated water discharge. The conventional methods used for harvesting are simple microfiltration, intensive centrifugation and chemical flocculation which have some advantages and weaknesses that need to be improved. Large scale use of chemicals and energy driven processes had caused harvesting of microalgae an uneconomical process

1.3 Objectives

The main objectives of this study are:

1) To develop optimized media formulation based on POME and seawater for optimal algal cell growth and lipid content during cultivation period.
2) To develop suitable immobilization technique based on sodium alginate for microalgal cells harvesting and POME remediation.
3) To carry out comparative study of free and immobilized microalgae cells for integrated POME remediation.
4) To solve harvesting problem from conventional harvesting method.

1.4 Scope of Study

The scopes of studies are as following:

1) Cultivation of two species of microalgae namely *Nannochloropsis Oculata* and *Chlorella sp.* under optimum parameters such as rate of stirring, light intensity, hygiene and temperature.
2) Monitoring and comparing the microalgae cell growth and density before and after treatment by using microscope.
3) Preparing adequate POME samples by doing filtration by using separatory funnel (to remove large contaminants), sterilization by using auto-clave (to kill the existing bacteria) and dilution with distilled water (to prepare suitable concentration).
4) Preparation of sodium alginate beads contained microalgae to develop the immobilization technique.
5) Monitoring the changes of significant characteristics of POME (COD, BOD and TN readings and heavy metals intensity) after treatment by using lab equipment.

CHAPTER 2
LITERATURE REVIEW

2.1 Palm Oil Mill Effluent (POME)

Palm oil industry is one of the major profit earner and largest producer in Malaysia. As demand of palm oil keep increasing from year to year, it is not surprising that very large production of effluent become main source of water pollution in Malaysia. POME is generated from 3 main processes of crude oil production; sterilization, clarification and nut cracking process. In Malaysia, it is estimated that at least 60 million tonne of POME was generated in the year 2009 alone (Ng, 2011). Fresh POME is a hot, acidic (pH between 4 and 5), brownish colloidal suspension containing high concentration of organic matter, high amounts of total solids (40,500 mg L^{-1}), oil and grease (4,000 mg L^{-1}), COD (50,000 mg L^{-1}) and BOD (25,000 mg L^{-1}) (Ma, 2000).

2.2 Microalgae in Wastewater Treatment

Wastewater treatment by microalgae has been used to remove organic matter and inorganic nutrients in many small communities due to its low cost and efficiency (Oswald, 1988; de la Noue et al., 1992). Microalgae have growth efficiency 10-50 times more compared to terrestrial plant and they only require inexpensive substrate which can be found vastly in wastewater, carbon dioxide and solar light. Among all unicellular algal species, *Chlorella* is a common and effective species for the immobilization and nutrient removal purposes (Robinson et al., 1988; Megharaj et al., 1992; Mallick and Rai, 1993; Tam et al., 1994; Lau et al., 1997,2008). Therefore, the types of microalgae that will used are green algae species from *Chlorophyceae* class

named *Chlorella sp.* and *Nannochloropsis Oculata* to compare the effectiveness of treatment by using different species of microalgae.

2.3 Conventional Wastewater Treatment by using Microalgae

The conventional methods used for harvesting microalgae are filtration, sedimentation and centrifugation. One of the major problems in using microalgae for wastewater treatment is their recovery from the treated effluent (de la Noue et al., 1992; Liberte et al., 1994). For example, filtration method is the most simple and cost effective harvesting method. The principle of filtration called microstraining that used a rotating fine-mesh screen and backwash to harvest microalgae. However, filtration is only suitable for harvesting fairly large microalgae (e.g. spirulina platensis) and unable to separate bacteria size microalgae like scenedesmus, dunaliella or chlorella species (Mohn, 1980). Centrifugation and microfiltration require huge amount of energy and may not be applicable in large-scale microalgae culturing farm. Chemical flocculation may be more economical but requires high concentrations for harvesting (Chen, 2011). Flocculants from multivalent salts (e.g. polyaluminium chloride) can contaminate the biomass with adverse effects on product quality (Harun et al, 2010, Azma et al, 2011).

2.4 Immobilized Sodium Alginate Beads

The use of immobilization technology is interesting technique to entrap microalgae in a matrix for prolonged use. The most common way is through gel entrapment method, in which natural polysaccharides such as agars, carrageenans and alginates are used due to their low toxicity and high transparency (Moreno-Garrido et al, 2008; Ignacio et al, 2008; Cao et al, 2010). Alginate has become the most commonly-used polymer for microbial entrapment, because of its high diffusivity, low production hazards, low costs, and a simple and fast immobilization process (De-Bashan and Bashan, 2010). Immobilization of cells can solve the problem of harvesting and increase cell retention time within bioreactors for higher metabolic activity (Azma, 2011).

Immobilization strategies enhance nutrient and heavy metal removal and one of the applications of bacteria and algae for treating hazardous contaminants (Moreno-Garrido, 2008). Some studies also suggested that immobilized cultivation system is more convenience in harvesting process of the microalgae. Immobilization technology, which entraps the microalgal cells into a matrix, solves the harvest problem (Chevalier and de la Noue, 1985). Higher nutrient removal efficiency has been recorded in the immobilized algal biomass than the freely suspended cells of the same algal species (Chevalier and de la Noue, 1985; de la Noue and Proulx, 1988).

According to (Tampinon,1987), an immobilized cell is defined as a cell that by natural or artificial means is prevented from moving independently of its neighbours to all parts of the aqueous phase of the system under study. Entrapment is one of the immobilized techniques used, by far the most frequently used immobilized technique in laboratory experiments and there are some examples of industrial process based on entrapped alginate cells. Alginate is the most commonly used polymer for immobilizing microorganisms within small cavities in its matrix. This method is based on the confinement of the cells in a three-dimensional gel lattice where the cells are free and the pores in the material allow substrate and products to diffuse to and from the cells. Sodium alginate, has been used to entrap microbial cells as alginate beads, is a very simple and cost-effective immobilizing matrix. Immobilization provides microorganisms with several major advantages over free-living suspensions. According to (Bashan,2010), the advantages include the uninterrupted supply of nutrients without competing with other microorganisms and the protection against environmental stress, bacteriophages, toxins, UV irradiation, soil contaminated by hydrocarbons and possible grazing by zooplankton. It has been reported that the immobilized biomass shows comparable heavy metal uptake to the free, non-immobilized biomass (Alejandro et al, 2010).

CHAPTER 3
METHODOLOGY

3.1 Process Flow of the Study

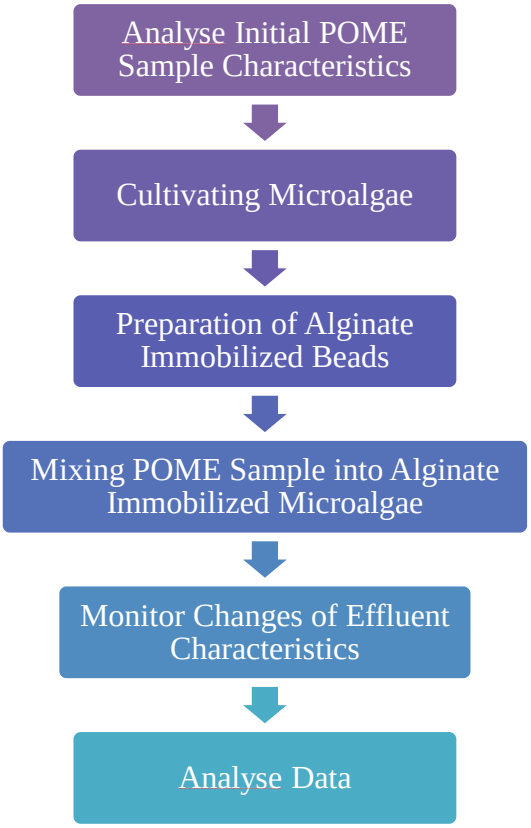


FIGURE 3.1 Diagram flow throughout this project

3.2 Materials Preparation

3.2.1 POME Sampling

A few samples of POME were taken from FELCRA Nasaruddin, Bota, Perak. POME was stored in the chilled room at 4°C to avoid microbial biodegradation activity and composition change before being filtered with 55mm filter paper to remove large 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 filtered POME will be autoclaved at 121°C for 30 minutes during sterilization process to kill the bacteria inside the sample. The initial characteristics will be identified before conducting the project to analyse the difference and reduction of COD, BOD and heavy metals in POME samples after the treatment.

FIGURE 3.2 POME sample from FELCRA Nasaruddin, Bota, Perak

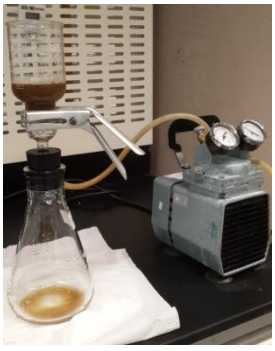


FIGURE 3.3 Filtration of POME by using vacuum pump and 55mm filter paper

FIGURE 3.4 Sterilization process in autoclave.

3.2.2 Culturing of Microalgae

The strains will be sampled from Fisheries Research Institute, Pulau Sayak, Sungai Petani Kedah, Malaysia. Two types of microalgae, *Chlorella* sp. and *Nannochloropsis Oculata* are cultivated in a conical flask containing filtered seawater. The pH of the medium is monitored within range of pH 6.5-8. The mass culture is aerated by filtered air at an appropriate speed and maintained at temperature of 23±2ºC, with suitable light intensity.

The stock culture (*Chlorella sp.* with density of 38.4 x 10^6 cells mL $^{-1}$ and *Nannochloropsis Oculata* with density of 68.56 x 10^6 cells mL $^{-1}$) were inoculated into 250 mL Erlenmeyer culture flask to get 10% (v/v) inoculum density. Conway media was used for Control culture and maintenance. *Chlorella* sp. were cultured in sterilized freshwater, enriched with Conway medium prepared as follows (g) [MacLachlan 1979]: Mineral solution in 1 L – $NaNO_3$ 100, Disodium EDTA 45, H_3BO_3 33.6, $NaH_2PO_4 4H_2O$ 20, $FeCl_3$ $6H_2O$ 1.30, $MnCl_2 4H_2O$ 0.36; Trace metal solutionin 100mL – $ZnCl_2$ 2.10, $CoCl_2.6H_2O$ 2, $(NH_4)_6MO_7O_2.4H_2O$ 0.90, $CuSO_4.5H_2O$ 2, and vitamin solution in 1000mL – Thiamine chlorohydrate, B1 0.2 Cyanocobalamin, B12 0.01 in 100 mL and KNO_3 116g. Media in culture flasks were autoclaved at 121ºC, for 15 min and all transfer of media and culture took place in aseptic environment in a laminar flow cabinet.

The standard conditions for control culture were 7 ppt NaCl for *Chlorella* and 30 ppt NaCl for *Nannochloropsis oculata* and initial pH 8, under illumination from fluorescence white light (Phillips) of 90 µmol photons $m^{-2}s^{-1}$ intensity. For

experiments, all the flasks were kept under the cycle of 12 h photoperiod and 12 h dark for 18 days. The culture flasks were grown on an orbital shaker at 80 rpm, at 26 ± 2 °C. All the glass -wares used in the experiment were sterilized by autoclaving at 121^{o}C for 20 mins, and all media constituents were added aseptically a laminar flow cabinet. Three replications were used both for the culture and control media.

Algal growth and lipid content were recorded every alternate day. After harvesting, the algal samples were centrifuged (Avanti J-251 Centrifuge) at 3000 rpm for 10 mins. The pellets were analyzed for cell growth, and the supernatant for chemical properties.

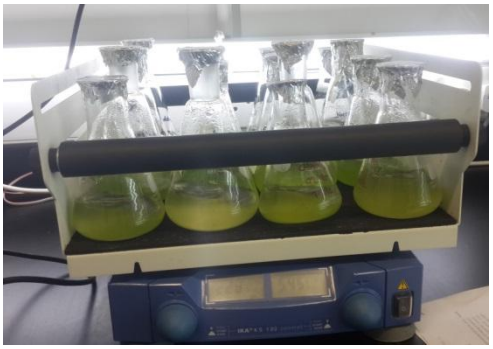


FIGURE 3.5 Microalgae (*Chlorella sp. and Nannochloropsis Oculata*) culturing

3.2.3 Preparation of POME Medium

POME was filtered to remove sand and dust particles and then centrifuged (Avanti J-251 Centrifuge). The supernatant of the effluent was used as algal culture medium and the pellet kept for future analyses. Immobilized algae and free-living algae were cultured in 250 ml flasks filled with 1, 5, 10, 15 and 20% POME composition in Fresh water under illuminated by fluorescent tubes, giving a mean light intensity of 80 μmol m^{-2} s^{-1} under a 12 h:12 h light:dark (L:D) cycle. pH level of POME medium was adjusted to pH 7– 8.

3.2.4 Preparation of Immobilized Microalgae Beads

Sodium alginate beads and polyvinyl alcohol foam will be used for microalgal cell immobilization. The sodium alginate beads will be prepared by using modified methods. The microalgae cells will be harvested in exponential phase and centrifuged. The suspension will be mixed with isopycnic 4 w/v% sodium alginate solution previously autoclaved for 20 min at 121 ^{o}C and cooled at room temperature. The alginate–alga mixture was transferred to 50 mL will be dropped slowly into a stirred 2% $CaCl_2$ (w/v) solution using a separatory funnel. Spherical beads of about 3–4 mm diameter will be produced and allowed to harden for 1 h before washing three times with distilled water to remove any remaining $CaCl_2$.

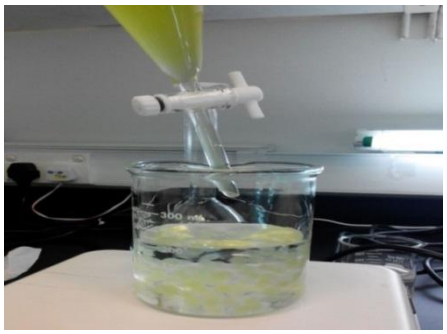


FIGURE 3.6 Beads contained microalgae formation

FIGURE 3.7 Spherical beads of 3–4 mm diameter produced and harden for 1 h

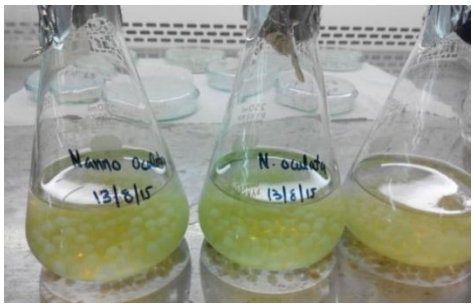


FIGURE 3.8 Beads soaked in microalgae solution to ensure its growth

3.3 Method Analysis

3.3.1 Determination of Cells Density for

a) Freely Suspended Cells Growth

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). Cells were then be harvested after 16 days by removing 100 mL samples and then later centrifuged at 3000rpm (Avanti J-251 Centrifuge).

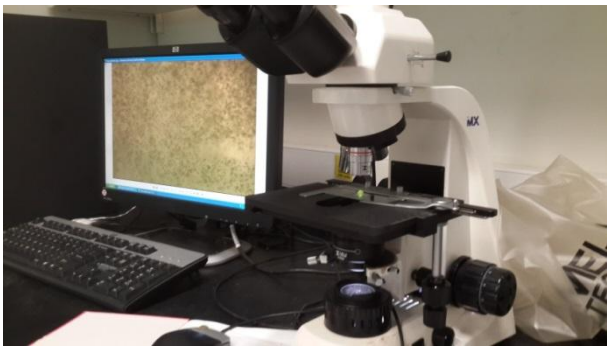


FIGURE 3.9 Cell counting under microscope

b) Immobilized Microalgae Cells

The biomass were washed with deionized water, dried at 80^{o}C in an oven for 8h, cooled in desiccator and weighed. Five beads were taken from each flask and solubilized by immersing in 1mL of a 4% $NaHCO_3$ solution for 30 min (Perez-Garcia et al., 2010). Cell number was observed and counted every day using a haemocytometer under a microscope. Specific growth rate and doubling time will be calculated by using this formula:

$$= \frac{}{} \qquad (1)$$

$$_{d} = \frac{}{} \qquad (2)$$

where

μ_{max} =specific growth rate

x_2 =cell density at day 18

x_1 =cell density at day 4

t_d =doubling time

3.3.2 Measuring POME Characteristics

a) Heavy Metals

The concentration of heavy metals in the supernatant will be analyzed by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The adsorption capacity and the concentration measurement of heavy metal ion in the aqueous phase before and after algal sorption will be expressed according to:

$$Removal\ efficiency\ \ \% \ = \frac{Ai - Af}{Ai} \times 100 \ldots\ldots\ldots (3)$$

where

A_i =initial concentration

A_f =final concentration

b) Chemical Oxygen Demand (COD)

COD measurement will be carried out by using spectrophotometer DR5000 Reactor Digested Method according to the standard method provided by HACH (HACH, 2008). The DR5000-Reactor was preheated to 150^{o}C. 1ml of sample was diluted at ratio 1:50, 1:100, 1:250 of POME and distilled water, respectively. 2ml diluted POME of each standard was added to the corresponding high range COD Digestion Reagent vials. For "blank", 2ml of distilled water was added. Each vial was mixed well and placed in the reactor block. After two hours, the vials were removed and kept in a cooling rack for 20 min before reading. The stored HACH program 435 COD HR was recalled for COD test. The reading of COD in mg L-^{1} was displayed on the screen (HACH, USA 1997).

FIGURE 3.10 COD test by using DR 5000 Spectrophotometer

c) Biological Oxygen Demand (BOD)

Measurement of BOD with BOD track was carried out according to Standard Method provided by HACH. 1ml of sample was diluted at ratio 1:100 and 1:250 of POME and distilled water, respectively. The sample (95ml) was poured into the specialized 300 mL BOD track designed to allow full filling with no air space and sample bottle provided with an airtight seal. Four samples were prepared. BOD Nutrient Buffer Pillow was added to each sample and Lithium hydroxide Powder was added to the seal cup of each sample bottle. The instrument was placed in the incubator at 20^{o}C. The stored HACH program for 5.25 days and 0-700mg L^{-1} was selected for the BOD test. The reading was taken after 5 days with the reading BOD in mg L^{-1} displayed on the screen for each sample bottle (HACH, USA 1997).

FIGURE 3.11 BOD Track

d) Total Nitrogen (TN)

Measurement of TN was carried out by using equipment named TOC Analyzer and following the Standard Method provided by HACH.

FIGURE 3.12 TOC Analyzer

e) Lipid Extraction

Lipids were extracted from the algal cells by using a mixture of methanol, chloroform, and water. Algal sample was centrifuged at 3500 rpm for 10 mins and the pellet was mixed with water, methanol, and chloroform. After overnight stay, the mixture was re-centrifuged and the lower layer that contained lipid and chloroform was extracted and put into pre-weighed vials. All vials were placed in a water bath at 65 °C for 8 h or kept in an oven at 80 °C for 4 h to evaporate the chloroform and lipids, before weighing.

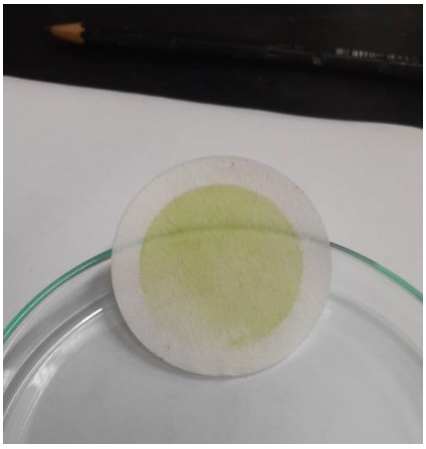


FIGURE 3.13 Filtered microalgae before putting in oven to be dried

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 Growth of Freely Suspended and Immobilized Cells

For *Nannochloropsis Oculata* , the highest cell density of 68.56 x 10^6 cells/bead was obtained at day 16 under 10% POME, which is slightly higher compared to freely suspended 65.52 x 10^6 cells/mL. As shown in the Table 4.7, this corresponds to the maximum specific cell growth rate (*μmax)* 0.108 d^{-1}, with doubling time 6.41 d^{-1} and lipids content of 31.45%. Although the cell growth was comparable to control at 68.93 x 10^6 cells at day 16 and lipids content was higher than with POME medium because the control medium does not consist of POME which may not provide the best condition for microalgae growth.

For *Chlorella* sp. the highest cell density of 31.93 x 10^6 cells/bead was obtained at day 14 under 10% POME, which is slightly higher compared to freely suspended 28.47 x 10^6 cells mL^{-1}. As shown in the Table 4.8, this corresponds to the maximum specific cell growth rate (*μmax)* 0.21 d^{-1}, with doubling time 3.96 d^{-1} and lipids content of 31.67%. Although the cell growth was comparable to control at 32.90 x 10^6 cells at day 14 and lipids content was higher than with POME medium because the control medium does not consist of POME which may not provide the best condition for microalgae growth.

Although cells growth of freely suspended and immobilized microalgae was recorded lower at lower or higher POME concentration, however it is reported that values of 0.27 g $L^{-1}d^{-1}$ and *μmax* of 0.49 d^{-1} with *Auxenochlorella protothecoides* MN 280. However, the lipid accumulations at 27−39% were relatively higher than the reported 28.9% total lipid content for A. protothecoides UMN 280 cultured in concentrated municipal wastewater (Zhou et al. 2012). Our study with *I. galbana* and

P. lutheri strains however achieved the highest cell density of 15.4 x 106 cells mL^{-1} and 14.2 x 106 cells mL^{-1}, with corresponding lipid content of 26.3 ± 0.31 % and 34.5 ± 0.82 %, respectively, at 15% POME level (Shah et al., 2014b).

A study on mixture of green algae and diatoms has the biomass concentrations increased from 0.5 g L^{-1} to 0.9 g L^{-1} and lipid content from 14% to 29% when the level of waste water is increased from 10% to 25% (Woertz et al 2009). A study on marine *Isochrysis* sp. utilizing 5% POME-fortified medium achieves maximum biomass of 91.7 mg m^{-2} day^{-1} and lipid content of 52.8 ± 2.4% under 10 L outdoor culture system (Vairappan & Yen 2008). Another report suggests optimal level at 14% POME, followed by 10%, 20% and 30% (Anton et al. 1994). High biomass accumulation has been reported for *Chlorella* sp. grown on concentrated municipal wastewater (Li et al 2011) and another study on *Scenedesmus* sp. shows 98% removal of inorganic nutrients from municipal wastewater with the highest biomass density of 0.11 gL^{-1} and lipid content increased from 14% to 31% (Xin et al. 2010).

4.1.1 Cell Number and Dry Weight

a) Control medium : *Nannochloropsis Oculata*

TABLE 4.1 The cell number and dry weight of *Nannochloropsis Oculata* with respect to time

Duration (day)	Cell Number ($10x10^6$ cell/ml)	Dry Weight (g)
0	4.16333	0.02
2	10.9333	0.08
4	20.5933	0.18667
6	28.1767	0.23333
8	34.9	0.30667
10	43.37	0.39
12	54.02	0.47333
14	61.8933	0.56667
16	68.93	0.65
18	60.3267	0.6

b) Control medium :*Chlorella sp*

TABLE 4.2 The cell number and dry weight of *Chlorella sp.* with respect to time

Duration (day)	Cell Number ($10x10^6$ cell/ml)	Dry Weight (g)
0	1.26	0.05
2	4.02667	0.08667
4	7.45333	0.14333
6	13.0267	0.26
8	18.6533	0.32333
10	21.9567	0.38333
12	26.49	0.43
14	32.8967	0.47
16	38.9867	0.53333
18	36.64	0.49

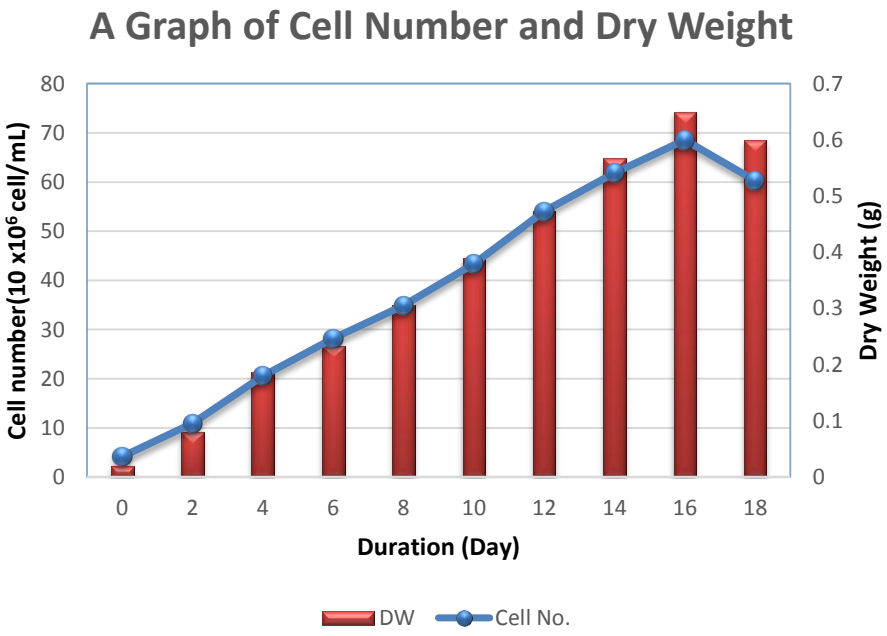


FIGURE 4.1 The cell number and dry weight of Nannochloropsis *Oculata* with respect to time.

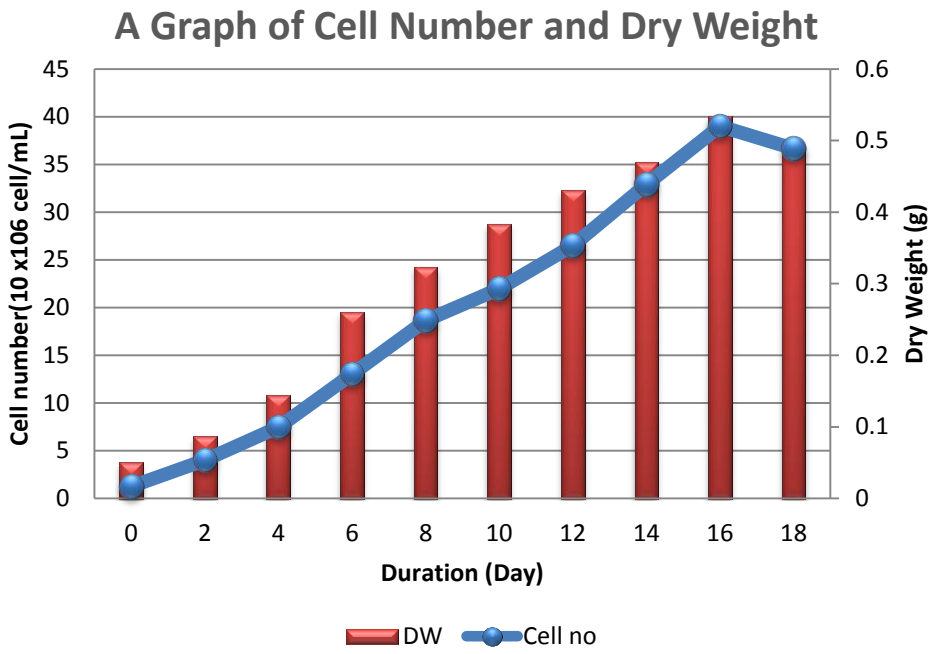


FIGURE 4.2 The cell number and dry weight of *Chlorella sp.* with respect to time

4.1.2 Cell Density

a) Freely Suspension: *Nannochloropsis Oculata*

TABLE 4.3 The effects of cell density for freely suspension *Nannochloropsis Oculata*. in different POME concentration with respect to time

Duration (day)	Cell Density(10 x10^6 cell/mL)					
	Control	1% POME	5% POME	10% POME	15% POME	20% POME
0	4.163333	2.384444	2.366667	2.366667	2.343333	2.6
2	10.93333	8.598778	10.87333	12.11333	10.39	8.873333
4	20.59333	12.66778	15.41	19.84333	17.24333	15.26333
6	28.17667	15.05222	21.09	28.42667	25.49667	23.45
8	34.9	19.07	25.45333	35.53667	33.16333	30.85333
10	43.37	23.36667	32.81	43.45333	40.55	36.38333
12	54.02	28.31667	36.53	53.30667	46.32333	42.75667
14	61.89333	32.38778	38.90333	58.55	52.49333	45.57
16	68.93	38.94	43.74	65.52	55.80667	48.17667
18	60.32667	34.54889	39.41333	59.07333	51.27667	44.72

b) Freely Suspension: *Chlorella sp.*

TABLE 4.4 The effects of cell density for freely suspension *Chlorella sp*. in different POME concentration with respect to time

Duration (day)	Cell Density(10 x10^6 cell/mL)					
	Control	1% POME	5% POME	10% POME	15% POME	20% POME
0	1.26	1.563333	1.366667	1.416667	1.343333	1.383333
2	4.026667	2.786667	2.673333	3.856667	3.62	3.52
4	7.453333	5.496667	5.373333	6.23	5.866667	5.393333
6	13.02667	8.683333	7.636667	9.94	9.886667	8.466667
8	18.65333	11.68333	10.79667	13.55	14.69667	13.78667
10	21.95667	14.53333	13.84333	19.06333	18.17	17.83
12	26.49	17.65333	16.25333	24.34	21.32667	20.63667
14	32.89667	21.24	23.07667	28.47	25.3	24.06667
16	38.98667	20.23	21.51333	27.82667	23.45333	22.36
18	36.64	17.34667	20.21333	25.96667	21.71333	21.08

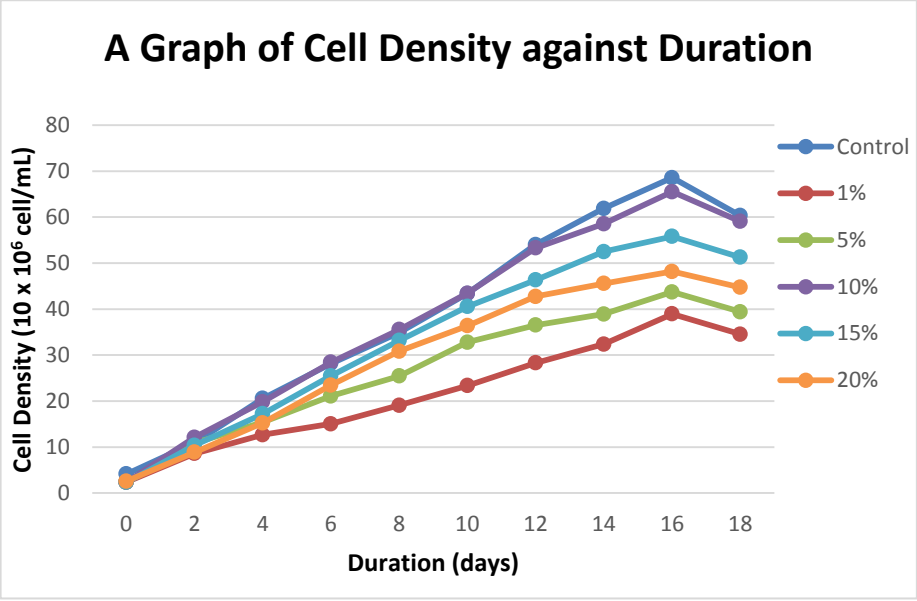


FIGURE 4.3 The effects of cell density for freely suspension *Nannochloropsis Oculata*. in different POME concentration with respect to time

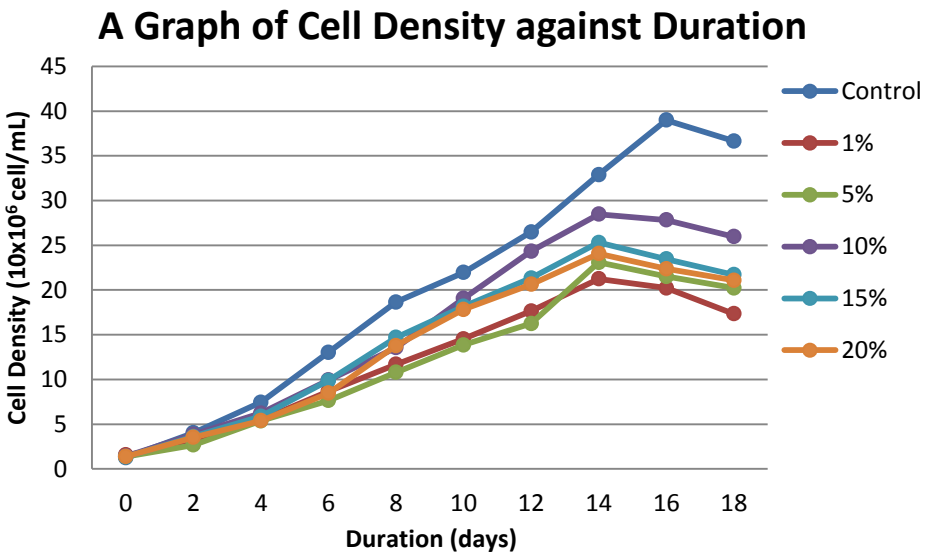


FIGURE 4.4 The effect of cell density for freely suspension *Chlorella sp.* in different POME concentration with respect to time

c) Immobilized Microalgae in Beads: *Nannochloropsis Oculata*

TABLE 4.5 The effects of cell density for immobilized *Nannochloropsis Oculata.* in different POME concentration with respect to time

Duration (day)	Cell Density(10 x10^6 cell/bead)					
	Control	1% POME	5% POME	10% POME	15% POME	20% POME
0	2.45	2.257778	2.62	2.406667	2.52	2.553333
2	16.86	9.68	12.69333	14.27667	15.53333	9.17
4	30.59	15.35667	18.64	28.37667	24.76	17.55
6	42.66	20.50667	23.74	39.01333	30.83667	25.50333
8	50.65	24.71333	27.73	47.25667	38.47	32.79333
10	56.76	28.76	34.47667	53.56333	45.52667	38.54333
12	59.98	32.68333	38.69	57.54667	52.65333	42.50333
14	64.01	36.38667	42.50667	62.76	58.60333	45.55
16	66.56	34.73667	46.57667	68.56	63.46333	50.34
18	63.33	32.30667	43.54333	62.11333	60.44667	46.59667

d) Immobilized Microalgae in Beads : C*hlorella sp.*

TABLE 4.6 The effects of cell density for immobilized *Chlorella sp.* in different POME concentration with respect to time

Duration (days)	Cell Density(10 x10^6 cell/bead)					
	Control	1% POME	5% POME	10% POME	15% POME	20% POME
0	1.26	1.563333	1.366667	1.416667	1.343333	1.383333
2	4.02666667	3.53	3.933333	6.566667	5.62	3.593333
4	8.45333333	6.786667	7.503333	9.37	6.78	6.573333
6	14.0266667	8.503333	10.63667	15.23333	12.79	8.646667
8	19.6533333	12.35667	14.37667	21.56333	15.07333	10.57333
10	22.9566667	16.01333	19.68667	25.82	16.54667	12.2
12	27.49	18.36333	23.46333	28.17	18.17	14.55333
14	33.8966667	22.01333	25.29667	31.92667	20.21667	18.12
16	39.9866667	20.32	24.19333	30.73333	19.66667	15.60667
18	38.64	19.43	23.84	29.35333	18.50667	13.77

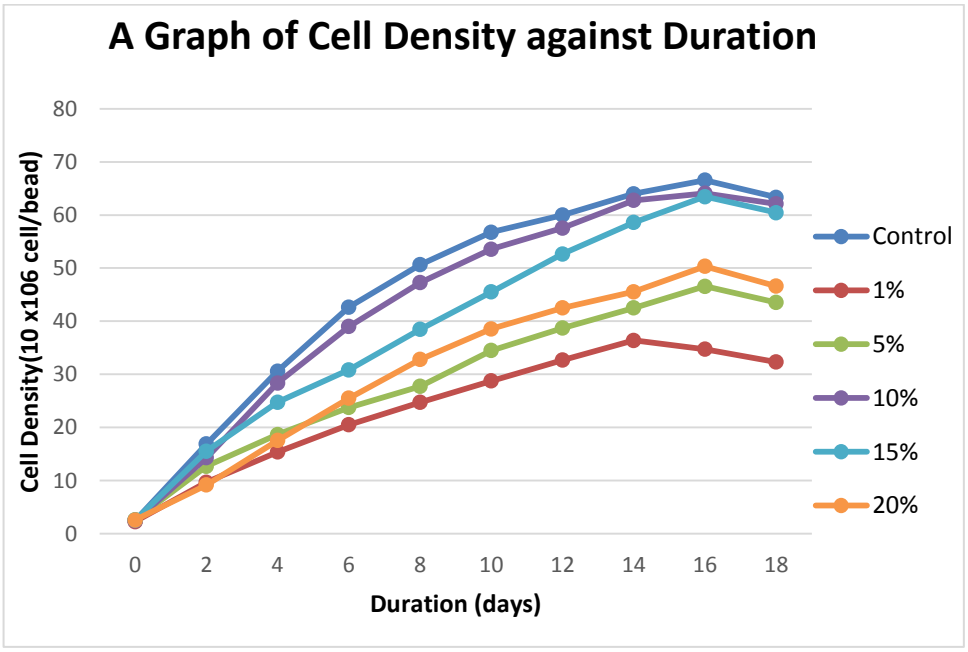


FIGURE 4.5 The effects of cell density for immobilized *Nannochloropsis Oculata.* in different POME concentration with respect to time

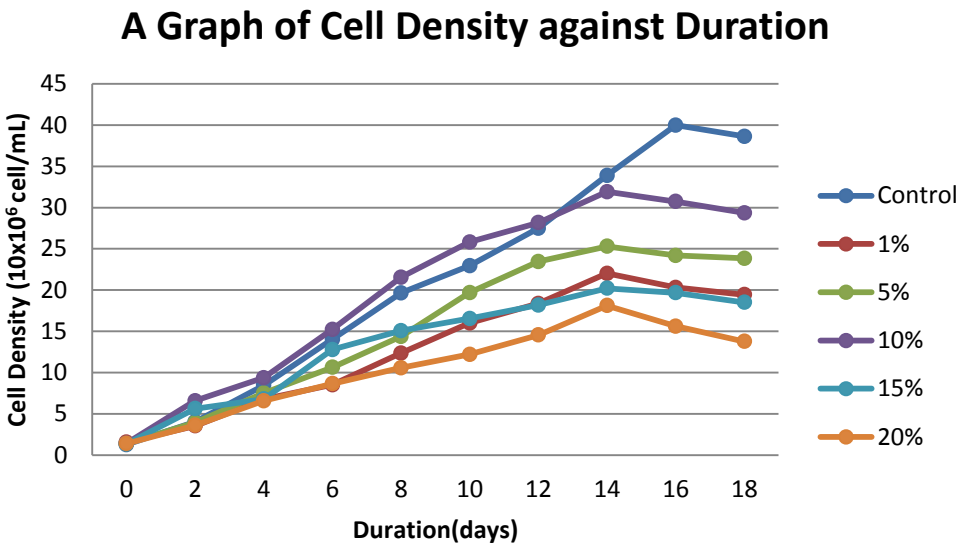


FIGURE 4.6 The effects of cell density for immobilized *Chlorella sp*. in different POME concentration with respect to time

4.1.3 Kinetic Of Cell Growth And Lipid Production

a) Nannochloropsis Oculata

TABLE 4.7 Kinetics of Cell Growth and Lipid Production of *Nannochloropsis Oculata* cultivated under control and different POME compositions in sea water

	MEDIA CONDITION	MAXIMUM SPECIFIC GROWTH RATE, μmax (d^{-1})	DOUBLING TIME, t_d (day)	LIPID CONTENT (%)
Freely Suspended	Control	0.11	6.3	30.54
	1%	0.093	7.49	23.87
	5%	0.092	7.53	25.87
	10%	0.108	6.41	31.45
	15%	0.111	6.24	28.34
	20%	0.163	4.25	22.87
Immobilized Cells	Control	0.073	9.49	31.32
	1%	0.086	8.05	24.87
	5%	0.082	8.45	26.34
	10%	0.079	8.77	32.45
	15%	0.086	8.05	28.76
	20%	0.095	7.27	23.76

b) *Chlorella sp.*

TABLE 4.8 Kinetics of Cell Growth and Lipid Production of *Chlorella sp.* cultivated under control and different POME compositions in sea water

	MEDIA CONDITION	MAXIMUM SPECIFIC GROWTH RATE, μmax (d^{-1})	DOUBLING TIME, t_d (day)	LIPID CONTENT (%)
Freely Suspended	Control	0.22	3.15	30.40
	1%	0.17	4.07	22.54
	5%	0.18	3.85	24.63
	10%	0.21	3.30	27.64
	15%	0.20	3.46	25.84
	20%	0.15	4.62	21.65
Immobilized Cells	Control	0.21	4.05	31.80
	1%	0.18	4.43	27.53
	5%	0.19	3.43	24.63
	10%	0.21	3.96	31.67
	15%	0.19	3.63	26.97
	20%	0.15	4.32	23.87

4.2 Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD)

For *Nannochloropsis Oculata*, the highest average BOD of 3153 mg L^{-1} and lowest average BOD of 193 mg L^{-1} was recorded at 20% and 1% POME, respectively, before inoculation of *Nannochloropsis Oculata*. The highest BOD removals of 82-90% were achieved for 1–20% POME at day 8 after the addition of immobilized *Nannochloropsis Oculata* which is superior to freely suspended microalgae. Higher removal of COD (91-95 %) were achieved for 1–20 % POME after addition of immobilized *Nannochloprosis Oculata* while lower removal of COD (40-54%) were achieved after addition of freely suspended *Nannochloropsis Oculata* at day 8. The COD and BOD removal was enhanced when the POME level (5-10 %) and incubation periods was increased.

For *Chlorella sp*., the highest average BOD of 2985 mg L^{-1} and lowest average BOD of 183.4 mg L^{-1} was recorded at 20% and 1% POME, respectively, before inoculation of *Chlorella* sp. The highest BOD removals of 84-90% were achieved for 1–20% POME at day 8 after the addition of immobilized *Chlorella* sp., which is superior to freely suspended microalgae. Higher removal of COD (90-96 %) were achieved for 1–20 % POME after addition of immobilized *Chlorella* sp., while lower removal of COD (42-56%) were achieved after addition of freely suspended *Chlorella* sp. at day 8. The COD and BOD removal was enhanced when the POME level (5-10 %) and incubation periods was increased.

In previous study, the BOD removal was lower at 68.3-82.5 % achieved with 1–20 % POME after microalgal addition. The removal of COD (74.5-77.4 %) and TOC (43.1-57.9 %) were also lower, achieved with addition of *I. galbana*, while removal of COD (77.6-80.1 %) and TOC (45.2-62.3 %) were achieved with *P. lutheri* (Shah et al., 2014b). This was in agreement with the 76% COD removal from piggery wastewater associated with microbe in high rate algal ponds (Godos et al. 2009) and the removal efficiency of 88% COD and 96% TOC with A. protothecoides UMN280 when the algae is grown in concentrated municipal wastewater (Zhou et al. 2012). Lower COD removal of 41.8% has been reported in the axenic culture condition of *Desmodesmus* sp. CHX1 (Cheng et al. 2013). Different algal strain could utilize 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 the organic material. *Synechocystis sp.* achieves the removal effiency of 98% BOD from treated wastewater under hydraulic residence time of 24h (Sekaran et al. 2013). The algae-based sewage treatment plant (STP) has reportedly achieved total BOD removal of 82% (Mahapatra et al. 2013). A three-stage aquaculture of certain macrophytes and algae such as *Eichhornia crassipes*, *Microcystis aeruginosa*, *Scenedesmus falcatus*, *Chlorella vulgaris* and *Chlamydomonas* mirabilis involving a water hyacinth culture in the first stage, followed by an algal culture, and finally a second water hyacinth also achieve BOD reductions around 96.9 % when tested in the laboratory conditions (Tripathi et al. 1991). Undigested dairy manure, diluted to 20 times, in a semi-continuous system increases *Chlorella vulgaris* biomass more

than twice in 4 days and higher nutrient and COD removal efficiency, than when the algae grown in the digested dairy manure (Wang et al., 2010). 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.

TABLE 4.9 Before and after treatment COD reading of *Nannochloropsis Oculata* cultivated under control and different POME compositions in sea water

	POME Concentration (%)	**Before Treatment (mg/L)**	**Removal Efficiency (%)**	
			After 8 Days	**After 16 Days**
Freely Suspension	1%	630	40	85
	5%	2978	51	90
	10%	5821	54	94
	15%	8853	46	90
	20%	11204	48	89
Immobilized Cells	1%	630	92	99.9
	5%	2978	94	99.9
	10%	5821	95	99.9
	15%	8853	93	99.9
	20%	11204	91	99.9

TABLE 4.10 Before and after treatment COD reading of *Chlorella sp.* cultivated under control and different POME compositions in sea water

	POME Concentration (%)	**Before Treatment (mg/L)**	**Removal Efficiency (%)**	
			After 8 Days	**After 16 Days**
Freely Suspension	1%	627	42	88
	5%	2974	53	91
	10%	5839	56	96
	15%	8947	51	92
	20%	1129	49	93
Immobilized Cells	1%	627	90	99.9
	5%	2974	93	99.9
	10%	5839	96	99.9
	15%	8947	92	99.9
	20%	11129	90	99.9

TABLE 4.11 Before and after treatment BOD reading of *Nannochloropsis Oculata* cultivated under control and different POME compositions in sea water

	POME Concentration (%)	Before Treatment (mg/L)	Removal Efficiency (%)	
			After 8 Days	After 16 Days
Freely Suspension	1%	193	42	81
	5%	864	45	94
	10%	1743	56	95
	15%	2594	47	95
	20%	3153	46	92
Immobilized Cells	1%	193	82	99.9
	5%	864	84	99.9
	10%	1743	90	99.9
	15%	2594	82	99.9
	20%	3153	88	99.9

TABLE 4.12 Before and after treatment BOD reading of *Chlorella sp.* cultivated under control and different POME compositions in sea water

	POME Concentration (%)	Before Treatment (mg/L)	Removal Efficiency (%)	
			After 8 Days	After 16 Days
Freely Suspension	1%	183	43	83
	5%	843	46	95
	10%	1642	54	98
	15%	2448	48	96
	20%	2985	50	95
Immobilized Cells	1%	183	84	99.9
	5%	843	86	99.9
	10%	1642	90	99.9
	15%	2448	83	99.9
	20%	2985	90	99.9

4.3 Total Nitrogen (TN)

For *Nannochloropsis Oculata*, the highest average TN of 104 mg L^{-1} and lowest average TN of 6.54 mg L^{-1} was recorded in 20% and 1% POME, respectively, before inoculation of immobilized *Nannochloropsis Oculata*. With short period of

time(after 8 days), the highest removal of TN (57%) was achieved in 1% POME, after the addition of immobilized *Chlorella* sp. (Table 4.14). The removal efficiency was increased to 97% after the 16 days treatment with immobilized microalgae higher compared to freely suspended microalgae.

For *Chlorella sp*., the highest average TN of 98.5 mg L^{-1} and lowest average TN of 5.43 mg L^{-1} was recorded in 15% and 1% POME, respectively, before inoculation of immobilized *Chlorella* sp.. With short period of time(after 8 days), the highest removal of TN (59%) was achieved in 10% POME, after the addition of immobilized *Chlorella* sp. (Table 4.14). The removal efficiency was increased to 98% after the 16 days treatment with immobilized microalgae higher compared to freely suspended microalgae.

TN is the sum of organic nitrogen, ammonia (NH_3), and ammonium (NH_4^+) in the chemical analysis of wastewater. Nitrogen is an essential ingredient for cell growth. The relative constancy of uptake, irrespective of nitrogen source, is due to the saturation of the assimilator to the production of amino groupings for entry into nitrogenous metabolism. Nitrite is generated in the process of nitrate being reduced to ammonium and it is possible that part of the nitrite produced is excreted into the media (Burhenne et al. 2000). The assimilation of either NO_3^- or NH_4^+ affects pH of the growth media. When ammonia is utilized, pH could decrease because of the release of H^+ ions while nitrate uptake increases pH because of OH^- release (Chevalier et al. 2000).

Another study with *Chlorella* sp. achieves the removal efficiency of 100% NH_4^+, 75.7-82.5% of TN, 62.5-74.7% of total phosphorus, and 27.4-38.4% of COD when the algae is grown in different dilutions (10, 15, 20, and 25 times) of digested dairy manure (Wang et al. 2010). The TN removal of 50.8–82.8 % is reported when *Chlorella* sp. is grown in different wastewaters from municipal wastewater treatment plant (Wang et al. 2010). In our study, both marine species grew effectively in polluted water with 78.8-90.8% TN removal from POME. Despite no drastic improvement in the amount of biomass obtained as compared to that from typical microalgae in the conventional conditions, the benefit of treatment of pollution water may make it a better alternative for economical large scale application.

TABLE 4.13 Before and after treatment TN reading of *Nannochloropsis Oculata* cultivated under control and different POME compositions in sea water

	POME Concentration (%)	Before Treatment (mg/L)	Removal Efficiency (%)	
			After 8 Days	After 16 Days
Freely Suspension	1%	6.54	44	71
	5%	29.4	38	67
	10%	65.6	46	73
	15%	87.2	53	70
	20%	104	40	70
Immobilized Cells	1%	6.54	57	95
	5%	29.4	54	93
	10%	65.6	53	97
	15%	87.2	51	89
	20%	104	49	91

TABLE 4.14 Before and after treatment TN reading of *Chlorella sp.* cultivated under control and different POME compositions in sea water

	POME Concentration (%)	Before Treatment (mg/L)	Removal Efficiency (%)	
			After 8 Days	After 16 Days
Freely Suspension	1%	5.43	44	68
	5%	28.6	37	70
	10%	56.7	50	78
	15%	80.3	45	73
	20%	98.5	42	74
Immobilized Cells	1%	5.43	58	98
	5%	28.6	54	95
	10%	56.7	59	98
	15%	80.3	53	92
	20%	98.5	50	90

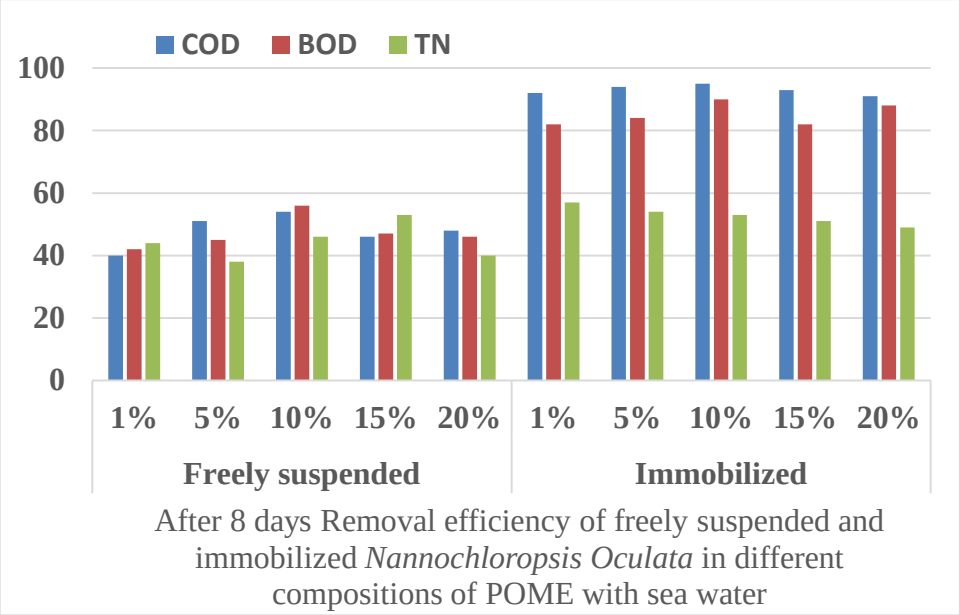


FIGURE 4.7 After 8 days COD, BOD and TN removal efficiency of freely suspended and immobilized *Nannochloropsis Oculata* in different compositions of POME with sea water

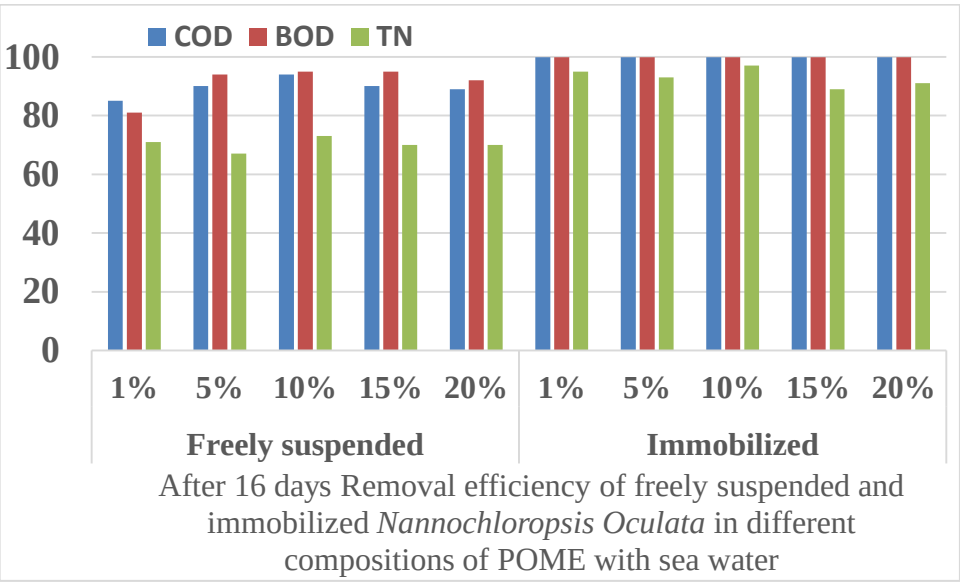
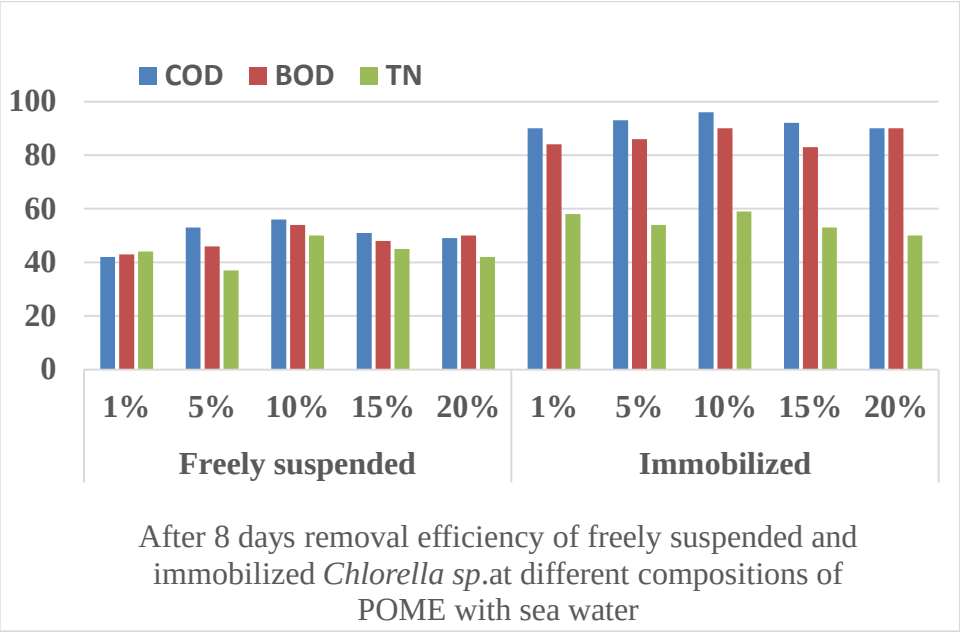
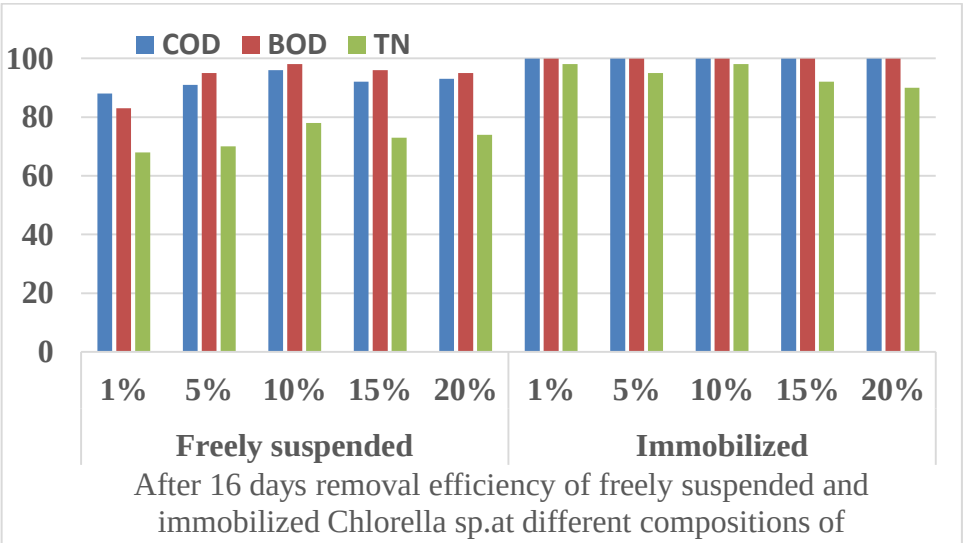


FIGURE 4.8 After 16 days COD, BOD and TN removal efficiency of freely suspended and immobilized *Nannochloropsis Oculata* in different compositions of POME with sea water



After 8 days removal efficiency of freely suspended and immobilized *Chlorella sp*.at different compositions of POME with sea water

FIGURE 4.9 After 8 days COD, BOD and TN removal efficiency of freely suspended and immobilized *Chlorella sp*.at different compositions of POME with sea water



After 16 days removal efficiency of freely suspended and immobilized Chlorella sp.at different compositions of POME with sea water

FIGURE 4.10 After 16 days COD, BOD and TN removal efficiency of freely suspended and immobilized *Chlorella sp*.at different compositions of POME with sea water

4.4 Heavy Metal Removal Fe(III) and Mn(II)

The bioremoval characteristics of *Chlorella* sp. were examined with regard to as Fe and Mn ions from POME. The residual concentrations of respective metals after 8 and 16 days of incubation period with freely suspended and immobilized *Nannochloropsis sp.* and *Chlorella* sp. are given in Table 4.15 and Table 4.16 respectively. Fe(III) and Mn(II) removal capacity by immobilized *Nannochloropsis Oculata* and *Chlorella* sp. was higher compared to freely suspended microalgae at different POME composition. The highest bioremoval capacity of Fe(III) (71-99.9%) and Mn(II) (98-99.9%) were achieved with immobilized *Nannochloropsis Oculata and Chlorella* sp. at different concentration of POME.

Alginate immobilized and free cells of *A. doliolum* and *C. vulgaris* showed higher uptake rates of copper and iron, suggesting that immobilization offers some protection against metal toxicity. Compared with free cells, immobilized cells showed greater efficiency for removing iron, even after three cycles, although there was a gradually decrease in efficiency in the second and third cycles (Rai and Mallick, 1992). The removal of Fe (99.73%), Mn (99.6%) and Zn (81.53%) from 5 mg L^{-1} initial metal concentration by using *Spirogyra* sp. and *Spirulina* sp. has been reported (Mane and Bhosle, 2012). Living algal biomass has been used for removal of heavy metal from contaminated wastewaters, due to its ability to remove such contaminants, either by adsorption onto the cell surface or by incorporation into the cells themselves (Rangsayatorn et al. 2002). The cell wall of microorganisms is the first barrier to the uptake of toxic metals, can play a crucial role as a defence mechanism (Zer et al. 1999). The used of metabolically active immobilized microalgae is a particularly attractive option in applications where extremely low levels of residual metal ions is necessary (Wilde and Benemann, 1993), for detoxification processes, and for metal recovery (Greene and Bedell, 1990).

Besides carbon, nitrogen and phosphorus, other macro-nutrients (e.g potassium, calcium and magnesium), micro-nutrients (manganese, molybdenum, copper iron, zinc, boron, chloride and nickel) and some trace elements are important for microalgal growth. Many of trace elements are important in enzyme reaction and

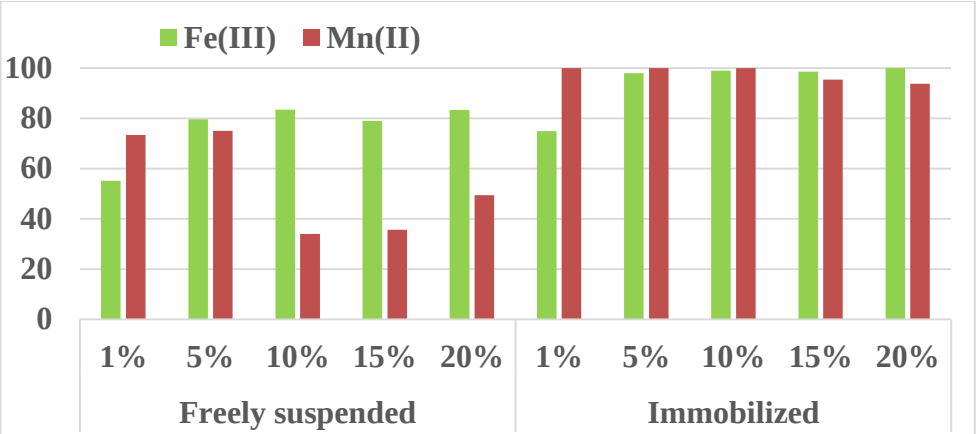
for biosynthesis of many compounds (Cavet et al. 2003). In the present study, both sea water and POME contain many natural macro and micro nutrients to fulfill microalgal growth requirements. Changes in all chemical parameters of the waste media after the culture of both microalgal species have paved the way for more environmentally-friendly approach to treat wastes whilst benefiting from harvesting the value-added products such as bioenergy and biocompounds.

TABLE 4.15 Before and after treatment Fe(III) and Mn(II) reading of *Nannochloropsis Oculata* cultivated under control and different POME compositions in sea water

		Fe(III)					Mn(II)				
	POME Concentration (%)	**Before Treatment (mg/L)**	**After 8 Days (mg/L)**	**Removal Efficiency (%)**	**After 16 Days (mg/L)**	**Removal Efficiency (%)**	**Before Treatment (mg/L)**	**After 8 Days (mg/L)**	**Removal Efficiency (%)**	**After 16 Days (mg/L)**	**Removal Efficiency (%)**
Freely Suspension	1%	3.43	1.54	55.1	0	100	0.6	0.16	73.3	0	100
	5%	3.83	0.78	79.63	0	100	0.52	0.13	75	0	100
	10%	11.9	1.98	83.36	0.76	93.61	1.06	0.7	33.96	0	100
	15%	14.2	2.98	79	0.55	96.13	1.32	0.85	35.61	0	100
	20%	15.2	2.54	83.29	0.94	93.82	1.74	0.88	49.43	0	100
Immobilized Cells	1%	3.43	0.86	74.93	0	100	0.6	0	100	0	100
	5%	3.83	0.08	97.91	0	100	0.52	0	100	0	100
	10%	11.9	0.12	98.99	0	100	1.06	0	100	0	100
	15%	14.2	0.2	98.59	0	100	1.32	0.06	95.45	0	100
	20%	15.2	0	100	0	100	1.74	0.11	93.68	0	100

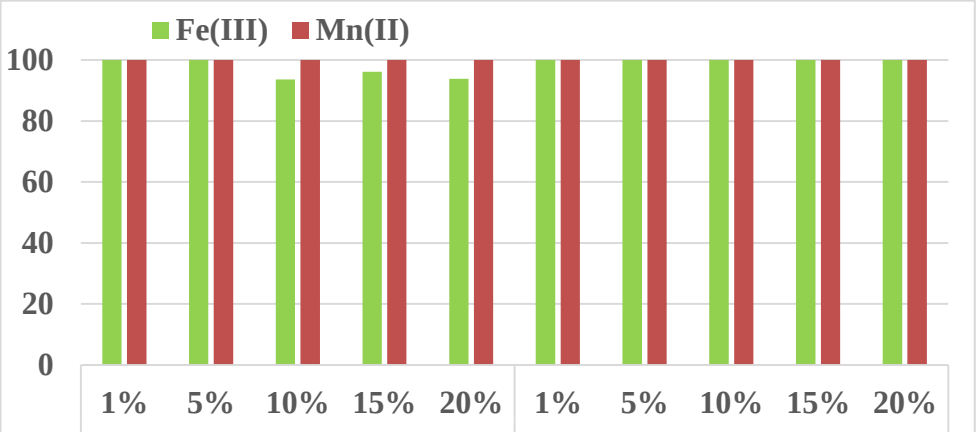
TABLE 4.16 Before and after treatment Fe(III) and Mn(II) reading of *Chlorella sp.* cultivated under control and different POME compositions in sea water

		Fe(III)					Mn(II)				
	POME Concentration (%)	**Before Treatment (mg/L)**	**After 8 Days (mg/L)**	**Removal Efficiency (%)**	**After 16 Days (mg/L)**	**Removal Efficiency (%)**	**Before Treatment (mg/L)**	**After 8 Days (mg/L)**	**Removal Efficiency (%)**	**After 16 Days (mg/L)**	**Removal Efficiency (%)**
Freely Suspension	1%	3.43	1.99	42	0.04	99.9	0.6	0.18	70	0	99.9
	5%	3.83	0.81	78	0.07	99.9	0.52	0.15	71	0	99.9
	10%	11.9	2.38	80	0.88	93.6	1.06	0.8	24	0	99.9
	15%	14.2	3.45	76	1.05	96.1	1.32	0.91	31	0	99.9
	20%	15.2	3.54	77	1.44	93.8	1.74	0.98	43	0	99.9
Immobilized Cells	1%	3.43	0.97	72	0	100	0.6	0.01	98	0	100
	5%	3.83	0.12	97	0	100	0.52	0.03	94	0	100
	10%	11.9	0.15	99	0	100	1.06	0.07	93	0	100
	15%	14.2	0.32	98	0	100	1.32	0.11	91	0	100
	20%	15.2	0.1	99	0	100	1.74	0.15	91	0	100



After 8 days Heavy metals removal efficiency of freely suspended and immobilized *Nannochloropsis Oculata* at different compositions of POME with sea water

FIGURE 4.11 After 8 days Fe(III) and Mn(II) removal efficiency of freely suspended and immobilized *Nannochloropsis Oculata* at different compositions of POME with sea water



After 16 days Heavy metals removal efficiency of freely suspended and immobilized *Nannochloropsis Oculata* at different compositions of POME with sea water

FIGURE 4.12 After 16 days Fe(III) and Mn(II) removal efficiency of freely suspended and immobilized *Nannochloropsis Oculata* at different compositions of POME with sea water

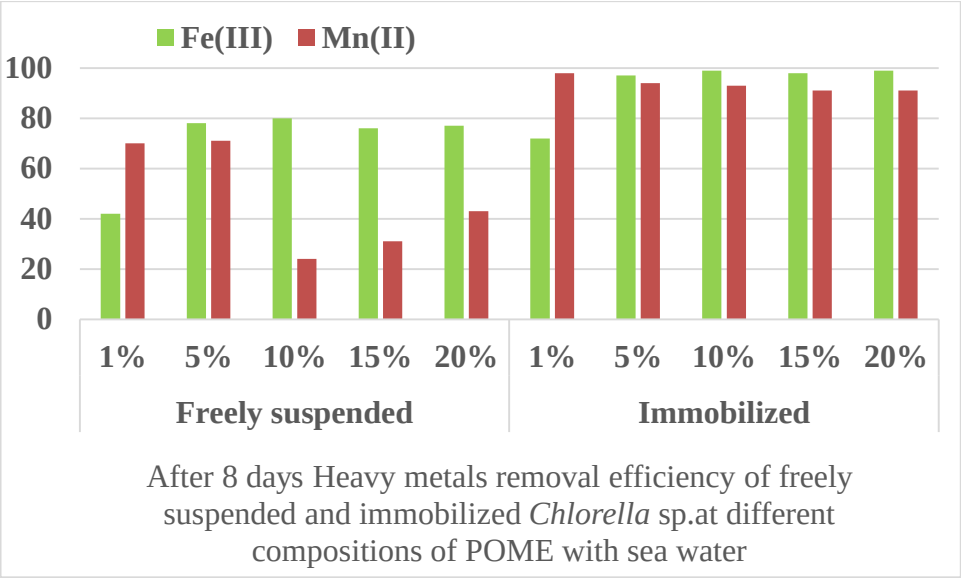


FIGURE 4.13 After 8 days Fe(III) and Mn(II) removal efficiency of freely suspended and immobilized *Chlorella sp*.at different compositions of POME with sea water

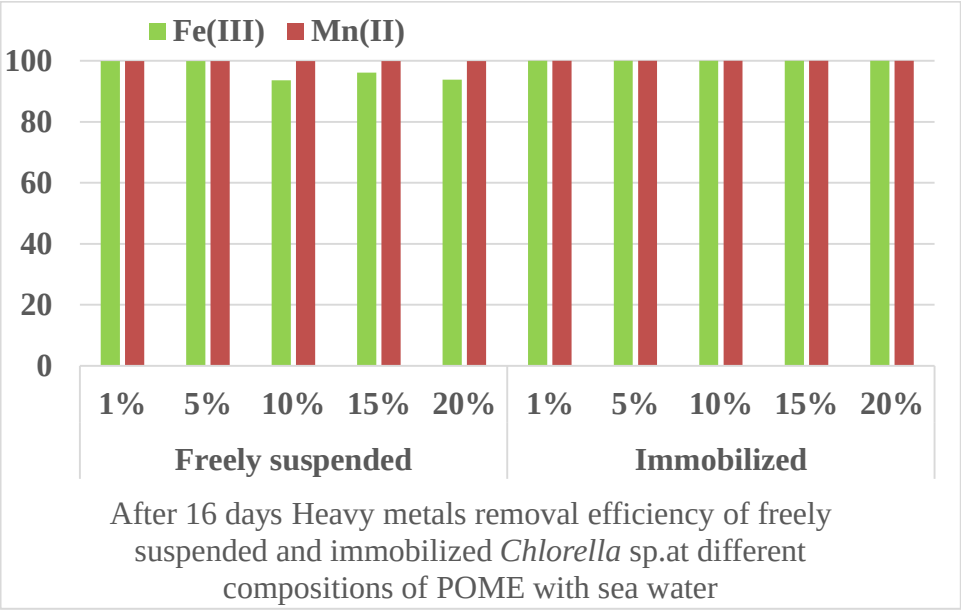


FIGURE 4.14 After 16 days Fe(III) and Mn(II) removal efficiency of freely suspended and immobilized *Chlorella sp*.at different compositions of POME with sea water.

CHAPTER 5
CONCLUSION AND FUTURE RECOMMENDATIONS

5.1 Conclusion

Wastewater is naturally abundance in nutrients that can be used for algal growth in wastewater treatment. By observing the properties and ability of the microalgae in the uptake of carbon, nitrogen, phosphorus and heavy metals, it is expected that microalgae show a potential in wastewater treatment for various types of effluents including POME. Immobilization of cells can be an alternative for cell harvesting as well as providing advantages in treating wastewater. Previous studies also proved that immobilized algae are probably a useful alternative for POME treatment, since it grew well under a different composition of POME in sea water.

It is also demonstrates that high lipid content for *Chlorella sp.* and *Nannochloropsis Oculata* were observed at immobilized microalgae in 10% POME. High removal efficiencies of heavy metals, COD, BOD and TN were achieved at different media concentrations of POME. After the treatment, the immobilized microalgae beads can be simply harvested through simple filtration method (sieving) without involving huge amount of energy input that will require more cost.

Therefore, this study is designed to achieve all the objectives mentioned previously. Particularly, the use of immobilized microalgae in these processes is very adequate and offers significant advantages in every effluent for wastewater treatment. The future of this area of algal cell technology is still considered.

5.2 Future Recommendations

a) Further studies can be conducted to analyze the feasibility of pollutants removal efficiency in POME treatment with different concentration of microalgae in beads.

b) Different type of effluent can be tested to analyzed whether immobilized microalgae is still efficient or not.

c) More time should be given so that more studies can be done such as biodiesel production from the microalgae prior wastewater treatment.

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