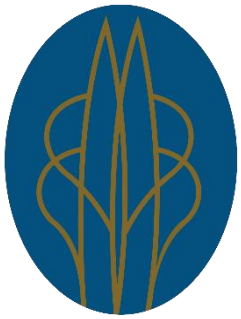

Final Year Project 2 Dissertation Report

Wastewater: Microalga Freely-Suspended Technique for Heavy Metal Removal

by

Syed Ahmad bin Syed Sheikh
14973

Dissertation report submitted in partial fulfilment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical Engineering)

JANUARY 2015

Universiti Teknologi PETRONAS
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CERTIFICATION OF APPROVAL

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Approved by,

_____

(Dr. Azizul bin Buang)

UNIVERSITI TEKNOLOGI PETRONAS

BANDAR SERI ISKANDAR, PERAK

January 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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SYED AHMAD BIN SYED SHEIKH

ABSTRACT

The hazardous mineral content such as nitrogen (N) and phosphorus (P), the existence of heavy metals in Palm Oil Mill Effluent, (POME) such as lead (Pb) and manganese (Mn) and having the characteristics of high chemical oxygen demand (COD) and biological oxygen demand (BOD) in the wastewater may lead to a serious pollution to the environment. Current methods in removing the heavy metals content in the wastewater have several limitations. POME remediation and removal of heavy metals in POME using microalgae is a sustainable and cost effective approach. Basically in this project, the purpose of the project is to study the efficiency of different types of microalga in removing the heavy metals content in POME. The project starts by collecting and preparing the raw samples of POME and proceeds with culturing of microalga, check the growth condition of microalga in POME environment, perform the treatment of heavy metals using microalga and lastly, analyse the result obtained from Atomic Absorption Spectroscopy and calculate the removal efficiency of each microalga for each type of heavy metals. The result expected for the project is that the microalga able and effective in removing the heavy metals in POME. The efficiency of the microalga will be discussed in the result and discussion section as well as in conclusion. One of the advantages of using microalgae is that, with their photosynthesis abilities, it is able to produce useful biomasses (Abdel-Raouf et al, 2012). Freely-suspended 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.

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Not to forget, to my family and friends, thank you for all the moral supports you have given me to get through the difficult times along the Final Year Project. Last but not least, to my beloved university, Universiti Teknologi PETRONAS (UTP), I am grateful to the management of the university for allowing me to have a chance in conducted such project in order for me to grab as much knowledge and experienced as I could from the project.

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CHAPTER 1: INTRODUCTION

1.1. Background of Study

Discharging wastewater to the environment such as rivers, lakes and seas from the industries are one of the recycling step of processing water. However, this wastewater must be initially treated since it contains organic materials and harmful heavy metals which could affect the human health and the environment especially to the aquatic lives. For example, those wastewater discharged from the manufacturing process of printed circuit board (PCB) and electroplating contains large amount of heavy metals which are copper (Cu) and nickel (Ni) (Lau et al, 1998). In sewage, three quarters of the organic carbon presents in proteins, amino acids, fats, carbohydrates and volatile acids while the inorganic constituents include high concentration of calcium (Ca), magnesium (Mg), chlorine (Cl), sulphur (S), phosphate and heavy metals (Abdel-Raouf et al, 2012). As for the Palm Oil Mill Effluent (POME), some of the wastewater discharged contains soluble materials, such as methane gas (CH_4), sulphur dioxide (SO_2), ammonia (NH_3) and halogens that are harmful to the environment. It also has high concentration value of chemical oxygen demand (COD) and biological oxygen demand (BOD). These contaminants presents in the wastewater would lead to water pollution if it is not meticulously treated.

Since this study focuses on the removal of heavy metals presents in wastewater, thus, only heavy metals removal methods are being discussed here. Currently, various methods are available in the world in treating the wastewater and removing the heavy metals. One of the methods available is the reverse osmosis method, where the heavy metals are separated by using a semi-permeable membrane where the pressure is greater than the osmotic pressure due to the dissolved solids in the wastewater. In most cases, the designed membrane will only allow the wastewater to pass through the dense layer while preventing the passage of the heavy metals. The next method is through electrodialysis. It is where the ionic components which is the heavy metals are separated through the semi-permeable ion selective membranes. The application of an electrical potential between two electrodes will cause migration of cations and anions towards respective electrodes. Due to the alternate spacing of cation and anion

permeable membranes, concentrated & diluted salts will formed. The third method used in the removal of heavy metals is through ultrafiltration. Ultrafiltration is pressure driven membrane operations that use porous membrane for the heavy metal removal (Rich and Cherry, 1987).

Another method of removing heavy metals in wastewater is through biosorption process. Biosorption process is the ability of the biological materials to accumulate or collect heavy metals through physico-chemical or metabolically mediated pathway of uptake from the wastewater. One of the potential heavy metal biosorbent is microalgae. In other words, this process uses microalgae as the adsorbent in order to adsorb the heavy metals. Microalgae is known to have high selectivity and capacity in the uptake of heavy metals. Based on the studies done by the previous researchers, averagely, the capacity uptake by the microalgae towards the heavy metals is up to 60%-100%. The capacity of the microalgae to uptake the heavy metals depends on the cell wall composition of the organism it is derived from the chemical composition of the heavy metals. In order to choose the most adequate microalgae for a certain type of microalgae, it is very essential to know what are the heavy metals presents in the wastewater and the concentration of heavy metals in it. It is an alternative method which has many advantages compared to the current conventional methods, however, up to now, only a few processes are established in the world. Adsorption of heavy metals by microalgae received an increased attentions only in the recent years though the process has been acknowledged a few decades. This is because of its potential for application in environmental protection or strategic or precious metals (Wilke et al, 2011).

In biosorption process, screened microalgae are used to reduce the concentration of the heavy metals presents in the wastewater effluent. By using microalgae-based treatment, it will interrupts the social-ecological principles to a degree lesser than other conventional methods (Kryder, 2007). In addition to that, by performing biological process for the treatment of heavy metals enriched wastewater, the microalgae can overcome some physical and chemical limitations and provide a cost-effective removal of the heavy metals as it is easily obtainable at the fishing industries. Besides that, the waste-grown microalgae has an added

value product where it can be utilized for biofuel production (Abdel-Raouf et al, 2012). Other major advantages of biosorption process using microalgae are as follows (Kratochvil and Volesky, 1998):-

- High efficiency
- Minimisation of chemical or biological sludge
- No additional nutrient requirements
- Regeneration of biosorbent
- Possibility of heavy metal recovery.

1.2. Problem Statement

The current conventional methods used in the industries in removing heavy metals have several limitations. For example, in the reverse osmosis method, the cost of operating such process is high. As for electrodialysis, due to the migration of cation and anion towards respective electrodes, metal hydroxides may formed which may lead the membrane to be clogged. For ultrafiltration method, sludge will generated (Rich and Cherry, 1987). Other than these three methods the chemical precipitation method, ion exchange and solvent extraction methods will also comprise a few disadvantages for example incomplete heavy metal removal, expensive equipment and monitoring system requirement, high reagent or energy requirements and generation of toxic sludge which require disposal (Wilke et al, 2011). This is why microalgae is used as the alternative method in removing heavy metals as its process has many advantages as mentioned earlier. In terms of oil palm industries, these industries produces palm oil mill effluent (POME) during the production of crude palm oil which it contains huge amount of chemical oxygen demand (COD) and biological oxygen demand (BOD) which may lead to water pollution.

1.3. Objectives and Scope of Study

The objective of this study is as follows:-

1. To study the effectiveness of using biosorption process (microalgal) in removing heavy metals contains in POME.
2. To compare the performance of seawater microalgae, *Nannochloropsis oculata* and fresh water microalgae, *Chlorella vulgaris* for heavy metal removal.

In terms of the selections of specific microalgae (*Nannochloropsis oculata* and *Chlorella vulgaris*), it will be evaluated based on the efficiency of heavy metal removal and high growth rates. Besides that, it is commonly used algae in water treatment plant to remove the heavy metals. Since the nearest wastewater to UTP that contains heavy metals is the FELCRA Nasaruddin, a palm oil mill in Bota, Perak, thus, the palm oil mill effluent (POME) will be collected there as the experiment samples. As for the microalga, it is obtained from the Fish Research Industries at Pulau Sayak, Kedah.

CHAPTER 2: LITERATURE REVIEW

2.1. Heavy Metal Pollution in Wastewater

Heavy metals referred to any metallic chemical element that has a relatively high density and toxic or poisonous at low concentrations. Basically, heavy metals are the natural components of the Earth's crust and it cannot be degraded nor destroyed. Poisoning due to heavy metals can be obtained through drinking contaminated water, high ambient of air concentration near to the emission sources and intake via food chain. In order to avoid metals accumulation in the food chain through the pollution of natural waters, heavy metal ions ought to be removed from the source (Wilke, Bunke and Buchholz, 2006). Heavy metals enter the environment through the wastewater from industrial processes such as electroplating, crude palm oil production, mining and metallurgical processes (Yu and Kaewsam, 1999).

In the petrol-based materials and other industrial facilities, lead (Pb) can be presented in the wastewater of these industries. In the chrome plating industries, petroleum refining, leather tanning, wood preserving, textile manufacturing and pulp processing, chromium (Cr), could exist in the wastewater. In the electroplating industries, zinc (Zn) and iron (Fe) metals will flow within the wastewater and into the river. As for palm oil mill effluent (POME), heavy metals contains in the effluent are cadmium (Cd), copper (Cu), chromium (Cr) and iron (Fe) (Ohimain et al., 2012). These heavy metals will affect the human health and the environment if the wastewater is not treated. A few examples of health risks done by the heavy metals are:-

- Iron (Fe): Fatigue, constipation, Tinnitus, gastrointestinal complaints and Jaundice
- Chromium (Cr): Nausea and vomiting. May lead to carcinogen (cancer), kidney and liver damage if exposed in long term.
- Zinc (Zn) – Nausea and vomiting
- Lead (Pb) – Damage to nervous system, circulatory system, reproductive system and gastrointestinal tract and kidney

2.2. Heavy Metal Removal using Microalga

In the year 1997, Lau A., Wong Y.S., T. Zhang and F. Y. T. Nora have conducted a study in heavy metal removal specifically for copper (Cu) and nickel (Ni) in an immobilized microalga reactor. The objective of the study was to know the efficiency of copper (Cu) and nickel (Ni) removal with alginate-algal beads through column reactor packed. The microalga used were *Chlorella vulgaris* which is a unicellular green alga with a cell diameter of 5µm. The algal cells were immobilized together with sodium alginate, which was a polysaccharide gel matrix in the form of spherical beads with a diameter of 3 to 4mm. The immobilization of the spherical algal beads with 4% gel concentration of sodium alginate was obtained by extruding the alginate-algal mixture. Then, the 75mL alginate-algal beads was packed within the column reactor. Initially, the reactor was fed with 4L, 30mg/L of copper (Cu) from copper (ii) sulphate ($CuSO_4$) metal solution in up-flow direction. At the end of the feeding, the algal column was regenerated with dilute nitric acid (HNO_3) solution. Once it is completed the copper was replaced with nickel (Ni) from nickel (ii) chloride ($NiCl_2$) and the experiment was repeated.

The result obtained from the experiment was 97% of copper (Cu) and 91% of nickel (Ni) was taken up by the algal beads from 4L, 30mg/L metals with a residual of 1.76mg/L Cu and 8.0mg/L Ni. The results showed that algal beads had stronger binding affinity for copper (Cu) than nickel (Ni). This is probably due to the fact that copper (Cu) was an essential element for normal algal growth, thus the cell surface possesses ligands or specific groups in holding copper (Cu) for assimilation. In conclusion for the experiment, the immobilized *Chlorella vulgaris* microalgae has demonstrated to be good adsorbent and has high capacity and efficiency in adsorbing the heavy metals. Even if the microalgae is being regenerated, the microalga can be reused without dropping its metal removal efficiency.

From the research done by King Saud University, Riyadh, Saudi Arabia and Beni-Suef University, Eqypt, microalga and metal sequestering processes can occur from different mechanism. It depends on the microalga itself, species of metal ions, condition of the solution and whether the microalga cells are living or non-

living. In living, the microalga cells trace nutrient metals such as cobalt (Co), molybdenum (Mo), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), chromium (Cr), lead (Pb) and selenium (Se) are accumulated intracellularly by active biological transport. From experiment conducted by Gale (1986), live photosynthetic microalga have effective role in heavy metal detoxification for mine wastewater. It showed that 99% of dissolved and particulate heavy metals could be removed by using cyanobacteria in the artificial pools system (Abdel Raouf, Al-Homaidan and Ibraheem, 2012).

In another study done by Soeder et al. (1978), *Coelastrum proboscideum* microalgae managed to absorb 100% of lead, Pb from a 1.0 ppm solution at 23^0C for 20 hours and about 90% of it after one and half hours at 30^0C. As for cadmium (Cd), the heavy metal was absorbed a little less efficiently which is only about 60% from 40 ppb solution after 24 hours. According to studies done by McHardy and George (1990), in artificial freshwater, *Cladophora glomerata* was found to be an excellent microalgae in accumulating zinc (Zn). Lastly, in the year 1990 by Baeza-Squiban et al. and in 1991 by Schimdt, the green microalgae type named *Dunaliella bioculata* produced an extracellular esterase which degrades the pyrethroid insecticide Deltamethrin. Microalgal also found to be able to degrade a range of hydrocarbon as those existing in oily wastes (Cerbniglia et. Al., 1980; Carpenter et al., 1989).

CHAPTER 3: METHODOLOGY

3.1. Preparation of Palm Oil Mill Effluent (POME) Medium

Fresh POME sample will be collected from FELCRA Nasaruddin, a palm oil mill in Bota, Perak. The sample must be kept cool in a refrigerator at 4^0C in order to avoid microbial contamination activity and change of sample composition. Next, the sample will be filtered to remove sand and dust particles and then centrifuged using Avanti J-251 Centrifuge. The supernatant of the effluent which contains nutrient will be taken for algal culture while the pellet formed in the effluent will be removed for other uses. It will be diluted with sea water to various range of POME composition, which are 1%, 5%, 10%, 15% and 20%. Once the sample with various compositions is done, the sample will be heated to a temperature of 121^0C for 30 minutes. This is to eliminate the presents of bacterial and other contaminations. The pH value of the sample will be adjusted to a range of pH 7–8 and will be re-filtered upon use.

3.2. Culturing of Microalgae

The one seawater type and one freshwater type species of microalga that use in this project, *Nannochloropsis oculata* and *Chlorella vulgaris* were collected from the Fisheries Research Institute (FRI), located in Pulau Sayak, Sg. Petani, Kedah. The culturing method for both types of microalga were same except in terms of the salinity. The salinity of seawater type microalgae, *Nannochloropsis oculata* is 30ppt while the freshwater type microalgae, *Chlorella vulgaris* is 7ppt. The stock culture (with density of 50.6 x 10_6 cells mL^{-1}) was inoculated into each 250 mL Erlenmeyer culture flask to get 10% (v/v) inoculum density. Conway media was used for control culture and maintenance. Filtered sea water was obtained from FRI. The standard conditions for control culture were 30ppt NaCl and initial pH 8, under 24 h 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 16 days. The culture flasks were grown on an orbital shaker at 80 rpm, at 28 ± 2 ^{0}C. All the glass-wares used in the experiment were sterilized by autoclaving at 121^0C for 20 minutes, and all media

constituents were added aseptically a laminar flow cabinet. Three replications were used both for the culture and control media.

3.3. Concentration of Heavy Metal Analysis

In terms of analysing the concentration of heavy metals in the supernatant, an Atomic Absorption Spectroscopy (AAS) will be used. In essence, the flame in AAS involves generating a gaseous population of free atoms by heating a sample in a flame and then passing narrow bandwidth light at a certain wavelength through the atoms in the flame. These conditions result in absorption of radiation that is selective for a particular element. 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:

$$Q = \frac{C_i V_i - C_f V_f}{m}$$

where Q = metal uptake capacity (mg/g),

C_i = initial metal concentration (mg/l),

V_i = initial volume (l),

C_f = final metal concentration (mg/l),

V_f = final volume (l),

m = initial biosorbent loading (g).

3.4. Chemical Oxygen Demand (COD) Analysis of POME

Chemical Oxygen Demand, also known as COD, is a test commonly used to indirectly measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water. For COD measurement, it will be carried out according to the Standard Method provided by HACH (HACH, 2008) by using DR2800 and 5000-Reactor Digested Method. The DR5000-Reactor will be pre-heated to the temperature of 150°C. 1ml of the sample of POME will be diluted with distilled water into 3 ratios, which are 1:50, 1:100 and 1:250, respectively. 2ml of each standard of diluted POME will be added to the corresponding high range COD Digestion Reagent vials. As for "blank" sample, 2ml of distilled water will be added. Each of the vials will be mixed well and positioned in the reactor block. After 2 hours, the vials will be removed and kept in a cooling for 20 minutes before taking the reading. The HACH program 435 COD HR was recalled for COD test. The COD reading of the sample, in mg L^{-1} will be displayed on the screen (HACH, USA 1997).

3.5. Biological Oxygen Demand (BOD) Analysis of POME

Biological Oxygen Demand or BOD is the amount of dissolved oxygen needed by aerobic 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. For BOD measurement with BOD track, it will be carried out according to Standard Method provided by HACH (HACH, 2008). 1ml of the sample of POME will be diluted with distilled water into 2 ratios, which are 1:100 and 1:250, respectively. 95ml of the sample will be poured into the specialized 300mL BOD trak designed to full-filled the sample bottle provided with no air space by using an airtight seal. Next, 4 POME-to-distilled water samples will be prepared (1:99, 5:95, 10:90 and 15:85) and 3.8cm of magnetic stir bar will be placed in each sample bottle. BOD Nutrient Buffer Pillow will be added to each of the samples and Lithium Hydroxide, LiOH powder will be added to each seal cups of the sample bottles. The instrument will then be placed in the incubator at 20°C. The HACH program for 5.25 days and 0-700mg L^{-1} will be selected for the BOD test. The reading will be collected after 5 days with the BOD reading, in mg L^{-1}, displays on the screen of each sample bottle (HACH, USA 1997).

3.6. Total Organic Carbon, Total Nitrogen (TOC & TN) and Oil and Grease of POME

Measurement of TOC and TN will be carried out by using TOC Analyzer (TOC-VCSH SHIMADZU) according to the APHA Standard Method (APHA, 2005). The sample will be diluted at the ratio of 1:50, 1:100 and 1:250. As for oil and grease, it will be measured by using oil and grease analyser (InfraCal TOG Model HATR-T2). The samples of POME will be analysed by adding hexane into bottles containing POME and vigorously shaken for 2 minutes for complete mixing. Once the two layers separated, 50µl will be extracted from the top layer by using syringe and deposited in the center of sample crystal. Oil concentration displayed will be recorded.

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

$$\text{Removal efficiency (\%)} = \frac{A_i - A_f}{A_i} \times 100$$

where Ai = initial parameter concentration

Af = final parameter concentration

3.7. Determination of Cell Density

Cell density is determined to measure the growth of microalgae by counting the number of cells using haemocytometer. On fixed days of alga growth, by using the capillary dropper, approximately 10µL sample will be removed. Later, the sample will be transferred to the filling slide chamber and examined under high power microscope (10 x 40 MAG).

3.8 Gantt Chart

No.	Details	2015													
		January				February				March				April	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.	Microalgae Culturing Activity														
2.	POME Characterization														
3.	Cell Density Counting														
4.	Treatment of Heavy Metals using *Nannochloropsis oculata*														
5.	Analysis of *Nannochloropsis oculata* in Removal of Heavy Metals in Wastewater														
6.	Submission of Progress Report														
7.	Treatment of Heavy Metals using *Chlorella vulgaris*														
8.	Analysis of *Chlorella vulgaris* in Removal of Heavy Metals in Wastewater														
9.	Pre-Sedex Presentation														
10.	Submission of Technical and Dissertation Report														

CHAPTER 4: RESULTS AND DISCUSSIONS

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 (TS), Total Suspended Solids (TSS) and Total Volatile Solids (TVS). The analysed results are shown in Table 4.1.

Table 4.1: POME Characterization.

Parameters	Literature (mg/L)	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. Cell Density Count of Microalgae

As mentioned earlier in section 3.8.1, the cell density is determined to measure the growth of microalgae by counting the number of cells using haemocytometer. A haemocytometer is a microscope chamber slide with a small (3mm x 3mm) square etched onto the surface. The slide has a coverslip which rests exactly 0.1mm above the slide. Cells in suspension are introduced into this area and then counted (Creighton University, 2013). Below is the schematic diagram of the haemocytometer under the microscope.

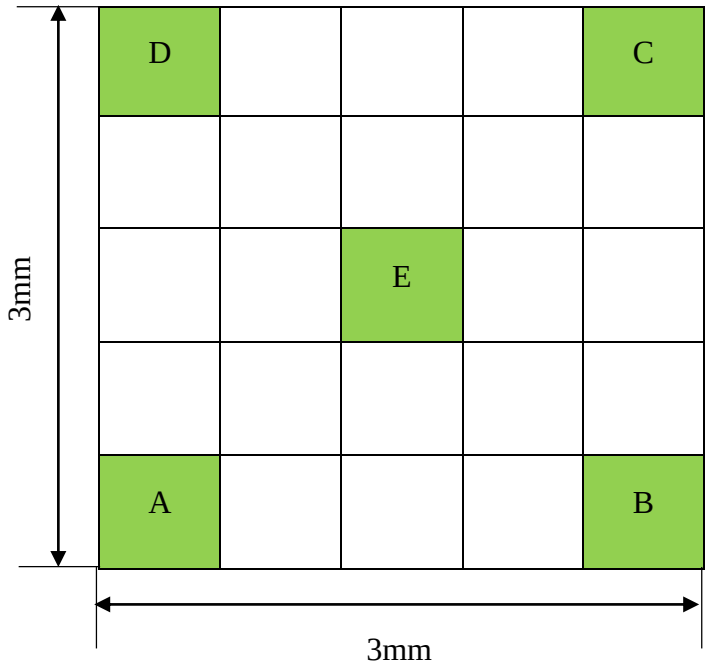

Figure 4.2(a): Schematic Diagram of Haemocytometer

To count for cell density growth of the algae, 5 areas were chosen which labelled with area A, B, C, D and E. The cells existing in each area were counted and calculated using the following equation to get the correct amount of cell growth in 1 mL. The results of the cell counting in 3 days are shown in Table 4.2(a), Table 4.2(b) and Table 4.2(c).

$$Cell\ Density = \frac{A + B + C + D + E}{5}\ X\ \frac{1}{4}$$

Table 4.2(a): Cell Density Count Day 1

Type	Day 1 - 06/02/2015						
	A	B	C	D	E	Total	Cell Density ($x10^6$), cells/mL
Control	4	2	4	3	9	22	1.10
1%	5	14	1	7	9	36	1.80
5%	1	1	0	3	3	8	0.40
10%	3	0	4	3	1	11	0.55
15%	4	2	2	3	6	17	0.85
20%	1	3	2	4	1	11	0.55

Table 4.2(b): Cell Density Count Day 2

Type	Day 2 - 10/02/2015						
	A	B	C	D	E	Total	Cell Density ($x10^6$), cells/mL
Control	8	10	5	4	11	38	1.90
1%	3	5	13	9	10	40	2.00
5%	5	2	9	6	3	25	1.25
10%	5	2	6	3	3	19	0.95
15%	2	7	1	3	7	20	1.00
20%	13	9	7	0	0	29	1.45

Table 4.2(c): Cell Density Count Day 3

Type	Day 3 - 11/02/2015						
	A	B	C	D	E	Total	Cell Density ($x10^6$), cells/mL
Control	15	10	15	8	13	61	3.05
1%	12	8	6	9	10	45	2.25
5%	6	7	8	5	10	36	1.80
10%	7	8	2	7	8	32	1.60
15%	5	3	5	23	11	47	2.35
20%	13	9	5	7	6	40	2.00

Table 4.2(d): Summary of Cell Density Count in 3 Days

Type	Cell Density ($x10^6$), cells/mL		
	Day 1 06/02/2015	Day 2 10/02/2015	Day 3 11/02/2015
Control	1.10	1.90	3.05
1%	1.80	2.00	2.25
5%	0.40	1.25	1.80
10%	0.55	0.95	1.60
15%	0.85	1.00	2.35
20%	0.55	1.45	2.00

The summary of the cell density count is then presented in graph form in order to see clearly the trend of cell growing per day as illustrated in Figure 4.2(b).

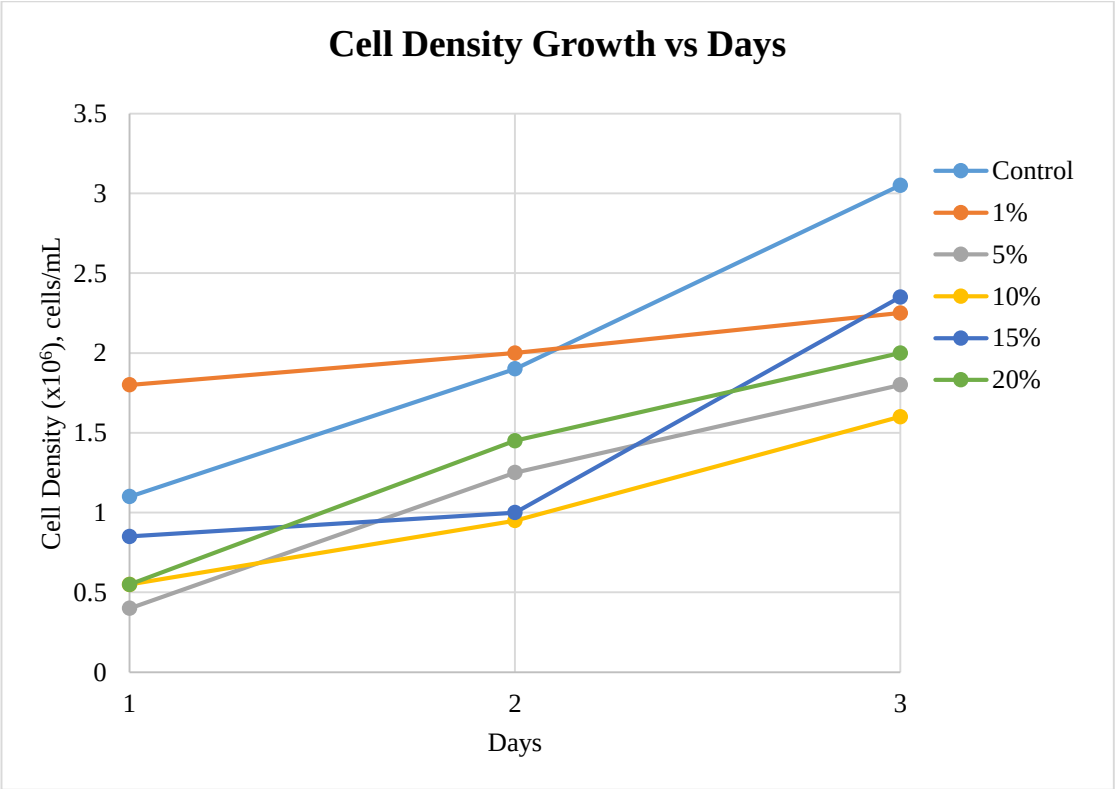

Figure 4.2(b): Summary of Cell Density Count in 3 Days

From the graph, it can be seen that, at any concentration of POME, the cell of the microalgae grows from day to day, indicated that the cells are living and reproducing itself in the POME so that the microalgae can treat the heavy metals present in it. It is expected for the control sample to have the highest amount of cell density as it is not exposed to POME, thus making it easier to reproducing itself without the need to treat the heavy metals. This result indicated that it is possible for microalgae to be living and reproducing itself though there is presents of heavy metals in it.

Prior to the treatment of heavy metals using microalgae, it is critical to assure the presents and types of heavy metals existing in POME. Based on the literature review, heavy metals existing in POME are many. For this project, only three types of heavy metals are identified and will be focussed on which are iron (Fe), zinc (Zn) and magnesium (Mg) as it is the highest concentration available in the POME sample compared to other heavy metals. To know the concentration of the heavy metals iron (Fe), zinc (Zn) and magnesium (Mg) presents in raw POME, 15 samples, in total, of raw POME was tested using Atomic Absorption Spectroscopy (AAS). The result of the tests are as follows:

4.3. Results of Heavy Metals Presents in Raw POME

a. Iron (Fe)

Concentration of standard solution prepared:

i. 0.00 ppm
ii. 0.50 ppm
iii. 1.00 ppm
iv. 2.00 ppm

Table 4.3(a): Sample Result of Heavy Metal Iron (Fe)

Sample No.	Concentration of Heavy Metal Iron (Fe), ppm
1	4.33
2	4.53
3	4.37
4	4.50
5	4.43
Average Concentration	4.43

b. Zinc (Zn)

Concentration of standard solution prepared:

i. 0.00 ppm
ii. 1.00 ppm
iii. 2.00 ppm
iv. 4.00 ppm

Table 4.3(b): Sample Result of Heavy Metal Zinc (Zn)

Sample No.	Concentration of Heavy Metal Zinc (Zn), ppm
1	0.18
2	0.15
3	0.17
4	0.17
5	0.18
Average Concentration	0.17

c. Magnesium (Mg)

Concentration of standard solution prepared:

i. 0.00 ppm
ii. 0.50 ppm
iii. 1.00 ppm
iv. 2.00 ppm

Table 4.3(c): Sample Result of Heavy Metal Magnesium (Mg)

Sample No.	Concentration of Heavy Metal Magnesium (Mg), ppm
1	1.65
2	1.67
3	1.64
4	1.65
5	1.64
Average Concentration	1.65

From these results, it is confirmed that heavy metals iron (Fe), zinc (Zn) and magnesium (Mg) do exist in POME with iron (Fe) has the highest concentration with the average concentration of 4.43ppm, followed by magnesium (Mg), 1.65ppm and lastly zinc (Zn) with the average concentration of 0.17ppm. Once the heavy metals presents in POME has been confirmed, the treatment of the heavy metals using microalgae can be initiated. The results of the treatment using the seawater type microalgae, *Nannochloropsis oculata* and freshwater type microalgae, *Chlorella vulgaris* are as follows using Atomic Absorption Spectroscopy (AAS):

4.4. Result of Heavy Metals Presents after Treatment using *Nannochloropsis oculata*

a. Iron (Fe)

Concentration of standard solution prepared:

i. 0.00 ppm
ii. 1.50 ppm
iii. 3.00 ppm
iv. 6.00 ppm

Table 4.4(a): Sample Result of Heavy Metal Iron (Fe) after Treatment using *Nannochloropsis oculata*

Sample Type	Concentration of Heavy Metal Iron (Fe), ppm	Average Concentration, ppm
1%	0.50	0.495
	0.49	
5%	1.29	1.280
	1.27	
10%	2.07	2.080
	2.09	
15%	2.38	2.395
	2.41	
20%	3.52	3.510
	3.50	

b. Zinc (Zn)

Concentration of standard solution prepared:

i. 0.00 ppm
ii. 1.00 ppm
iii. 2.00 ppm
iv. 4.00 ppm

Table 4.4(b): Sample Result of Heavy Metal Zinc (Zn) after Treatment using *Nannochloropsis oculata*

Sample Type	Concentration of Heavy Metal Iron (Fe), ppm	Average Concentration, ppm
1%	-0.12	-0.115
	-0.11	
5%	-0.04	-0.045
	-0.05	
10%	0.01	0.005
	0.00	
15%	0.05	0.050
	0.05	
20%	0.13	0.120
	0.11	

c. Magnesium (Mg)

Concentration of standard solution prepared:

i. 0.00 ppm
ii. 0.50 ppm
iii. 1.00 ppm
iv. 2.00 ppm

Table 4.4(c): Sample Result of Heavy Metal Magnesium (Mg) after Treatment using *Nannochloropsis oculata*

Sample Type	Concentration of Heavy Metal Iron (Fe), ppm	Average Concentration, ppm
1%	-0.27	-0.270
	-0.27	
5%	-0.27	-0.270
	-0.27	
10%	-0.27	-0.275
	-0.28	
15%	-0.27	-0.270
	-0.27	
20%	-0.28	-0.280
	-0.28	

4.5 Result of Heavy Metals Presents after Treatment using *Chlorella vulgaris*

a. Iron (Fe)

Concentration of standard solution prepared:

i. 0.00 ppm
ii. 1.50 ppm
iii. 3.00 ppm
iv. 6.00 ppm

Table 4.5(a): Sample Result of Heavy Metal Iron (Fe) after Treatment using *Chlorella vulgaris*

Sample Type	Concentration of Heavy Metal Iron (Fe), ppm	Average Concentration, ppm
1%	0.51	0.500
	0.49	
5%	0.77	0.765
	0.76	
10%	1.87	1.860
	1.85	
15%	3.60	3.600
	3.60	
20%	4.49	4.495
	4.50	

b. Zinc (Zn)

Concentration of standard solution prepared:

i. 0.00 ppm
ii. 1.00 ppm
iii. 2.00 ppm
iv. 4.00 ppm

Table 4.5(b): Sample Result of Heavy Metal Zinc (Zn) after Treatment using *Chlorella vulgaris*

Sample Type	Concentration of Heavy Metal Iron (Fe), ppm	Average Concentration, ppm
1%	-0.11	-0.110
	-0.11	
5%	-0.06	-0.060
	-0.06	
10%	0.02	0.020
	0.02	
15%	0.06	0.060
	0.06	
20%	0.10	0.105
	0.11	

c. Magnesium (Mg)

Concentration of standard solution prepared:

i. 0.00 ppm
ii. 0.50 ppm
iii. 1.00 ppm
iv. 2.00 ppm

Table 4.5(c): Sample Result of Heavy Metal Magnesium (Mg) after Treatment using *Chlorella vulgaris*

Sample Type	Concentration of Heavy Metal Iron (Fe), ppm	Average Concentration, ppm
1%	-0.27	-0.270
	-0.27	
5%	-0.29	-0.290
	-0.29	
10%	-0.29	-0.285
	-0.28	
15%	-0.29	-0.285
	-0.28	
20%	-0.29	-0.285
	-0.28	

4.6 Comparison Results between *Nannochloropsis oculata* and *Chlorella vulgaris*

Table 4.6: Comparison Results between *Nannochloropsis oculata* and *Chlorella vulgaris*

Before Treatment			**After Treatment**						
Raw Samples			**Sample (%)**	***Nannochloropsis oculata***			***Chlorella vulgaris***		
Fe (ppm)	**Zn (ppm)**	**Mg (ppm)**		**Fe (ppm)**	**Zn (ppm)**	**Mg (ppm)**	**Fe (ppm)**	**Zn (ppm)**	**Mg (ppm)**
4.43	0.17	1.65	1	0.495	-0.115	-0.270	0.500	-0.110	-0.270
4.43	0.17	1.65	5	1.280	-0.045	-0.270	0.765	-0.060	-0.290
4.43	0.17	1.65	10	2.080	0.005	-0.275	1.860	0.020	-0.285
4.43	0.17	1.65	15	2.395	0.050	-0.270	3.600	0.060	-0.285
4.43	0.17	1.65	20	3.510	0.120	-0.280	4.495	0.105	-0.285

4.7 Comparison Efficiency Results between *Nannochloropsis oculata* and *Chlorella vulgaris*

Table 4.7: Comparison Efficiency between *Nannochloropsis oculata* and *Chlorella vulgaris*

Microalga Species	Sample (%)	Efficiency (%)		
		Iron, (Fe)	Magnesium, (Mg)	Zinc, (Zn)
Nannochloropsis oculata	1	88.83	167.65	116.36
	5	71.11	126.47	116.36
	10	53.05	97.06	116.67
	15	45.94	70.59	116.36
	20	20.77	29.41	116.97
Chlorella vulgaris	1	88.71	164.71	116.36
	5	82.73	135.29	117.58
	10	58.01	88.24	117.27
	15	18.74	64.71	117.27
	20	-1.47	38.24	117.27

Based on the results obtained, it is proven that microalgae can remove the heavy metals. It can be seen clearly in Table 4.7 where the efficiency of each type of micaroalga in removing the heavy metals. For heavy metal iron (Fe), *Chlorella vulgaris* shows a higher efficiency in removing the heavy metal at the concentration of 1% to 10% POME. However, the efficiency abruptly dropped from 58.01% to 18.74% when the concentration reached to 15% POME. The efficiency keeps on dropping until a negative value was shown when the concentration of POME increased to 20%. This indicates that *Chlorella vulgaris* cannot withstand and no longer effective when the microalgae is exposed to a high level concentration of the heavy metal. This does not happened to

Nannochloropsis oculata. The efficiency results shown by *Nannochloropsis oculata* is much better even when the POME concentration reached to 20% which is 20.77%. Thus, for removing the heavy metal iron (Fe), at low concentration, *Chlorella vulgaris* gives a better result. As for high concentration, *Nannochloropsis oculata* is much more suitable in removing the heavy metal.

Different result was shown for heavy metal zinc (Zn). Both *Nannochloropsis oculata* and *Chlorella vulgaris* shows a good result in removing the heavy metal. From 1% to 10% POME concentration, the efficiency range of removing the heavy metals is from 88.24% to 167.65%. This shows that in low concentration of POME, both microalga is very effective in removing the heavy metal. However, when the concentration of POME increased to 15% and 20%, the efficiency dropped gradually as the microalga cannot withstand the toxicity level in the POME and started to die. Despite that happened, the results still give a good reading at 20% POME concentration which is 29.41% for *Nannochloropsis oculata*, and 38.24% for *Chlorella vulgaris*.

The best result obtained in removing the heavy metals using microalga is when removing the heavy metal magnesium (Mg). At any concentration, the efficiency of removing the heavy metal is more than 100% for both microalga. This shows that, both microalga, *Nannochloropsis oculata* and *Chlorella vulgaris* are very effective in the most suitable and efficient in removing magnesium content in the wastewater. From the result, it can be said that this heavy metal has the highest tendency for both microalga to remove it. More studies must be done in order to further understand such case occurred.

CHAPTER 5: CONCLUSION AND RECOMMENDATION

5.1. Conclusion

In removing heavy metals contents in the wastewater, the usage of microalgae the best and most effective way in doing it. From the results obtained, it can be concluded that in removing heavy metal iron (Fe), at low concentration, freshwater type microalgae is more suitable and effective to remove it but in high concentration, seawater type microalgae is much more effective as it can withstand higher iron content in wastewater. For heavy metal zinc (Zn), both types of microalga can remove the heavy metal entirely when it is in low concentration. However at high concentration, the efficiency of both microalga reduced steadily but still have a good percentage of removing the heavy metal. As for heavy metal magnesium (Mg), both microalga have the ability to remove the heavy metal 100%.

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.

One of the most promising areas of research is using this technology to reduce environmental pollutions through biodegradation of many harmful compounds. The application of immobilized technology to environmental area is in its preliminary stages, but the results seen so far are promising. Immobilization of algae can solve the problem of POME remediation and bioenergy cogeneration. After the immobilization microalgae beads have grown to stationary phase, the beads can be easily harvested through sieving without involving huge amount of energy input.

5.2. Recommendation

Using microalga for biosorption process in heavy metal removal is in developmental stages as the process industries are in the initial stages of familiarizing with the process. Thus, further improvement in both performance and costs can be expected in future once the industries had the clear picture of it. Further analysis on the POME treatment using different immobilization techniques can be tested using different microalga strains. To attract more usage of immobilization technology, some strategies have to be developed to solve microalga harvesting problem and to convert harvested biomass into biofuel production.

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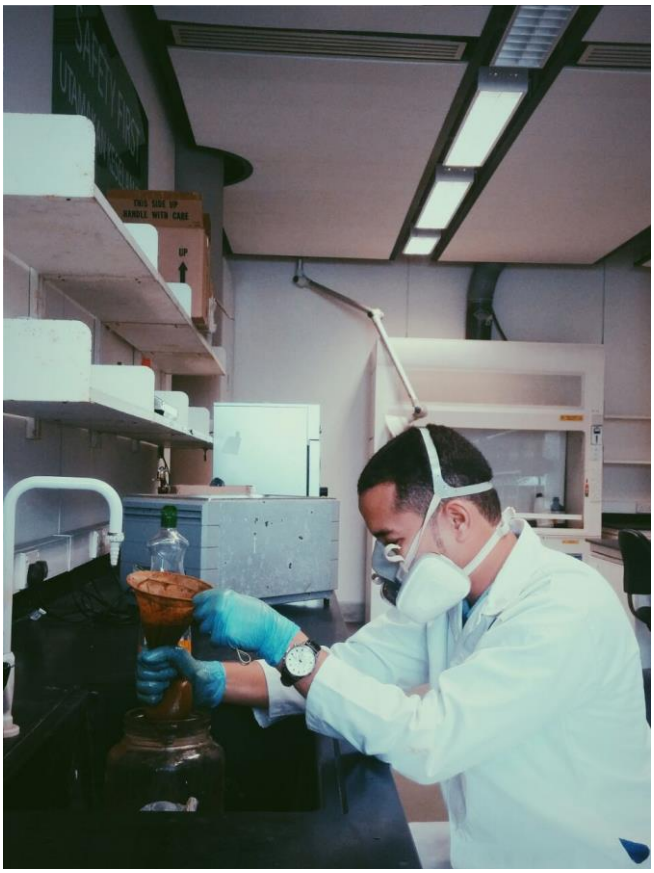
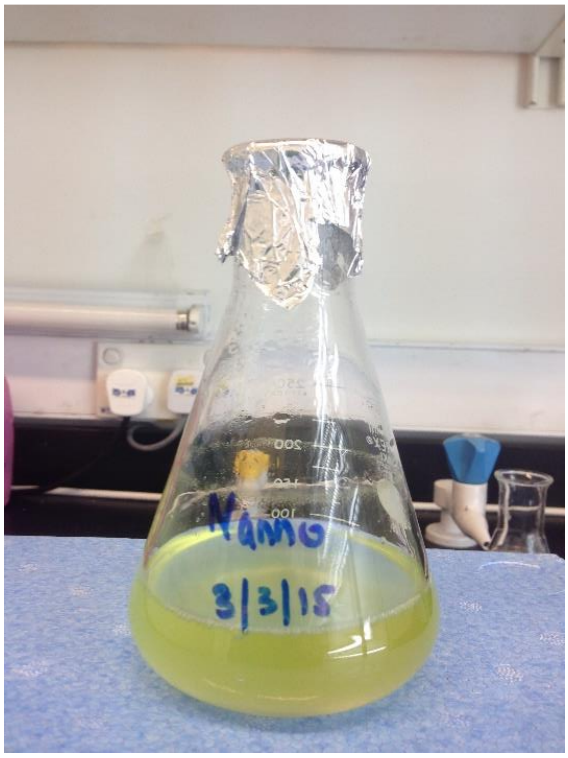
APPENDICES

Appendix 1: Collection of Raw POME at FELCRA Nasaruddin, Bota, Perak

Appendix 2: Filtering Raw POME using Coffee Sock



Appendix 3: Sample of Microalga (*Chlorella vulgaris* & *Nannochloropsis oculata*)



Appendix 4: Sample of Treatment of POME using Microalga