# USE OF MARINE MICROALGAE IN BIOREMEDIATION OF PALM OIL MILL EFFLUENT

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# ABSTRACT

## USE OF MARINE MICROALGAE IN BIOREMEDIATION OF PALM OIL MILL EFFLUENT

Malaysia is the largest producer and exporter of palm oil products in the world for the last ten years. The state of Sabah is the leading producer in Malaysia, with a total production capacity of 24 million tones of Fresh Fruit Branch (FFB) per annum. Despite the fact that this industry does contribute to the development of the country, it also generates huge amount of agro-industry wastes that endangers the environment. General treatment often involved biological degradation which is not efficient in reducing the inorganic pollutants. It is possible to utilize marine microalgae to further treat secondary treated Palm Oil Mill Effluent (POME) by using the inorganic pollutants as a nutrient source to produce valuable live feed for aquaculture. Preliminary study on effects of temperature and salinity on growth of three important aquaculture microalgae; proved *Isochrysis* sp. as the best species as it has the highest specific growth rate, $\mu = 0.84$ day$^{-1}$ (at 23°C, 30 ppt). This species is known for their suitability for continuous culture. This species was cultured in f/2 in a photo-bioreactor in two different photoperiods (12:12 h and 24:0 h) with the light intensity of 200 µmol$^{-2}$sec$^{-1}$. It was observed that the growth rate and crude protein was lower in the 12:12 h photoperiod while there was an increase of lipid by 49%. The overall total fatty acid per gram of sample was doubled in 12h photo-period with a 40.2 % increase of Docosahexaenoic Acid (DHA) and traces of Eicosapentaenoic Acid (EPA) that was not detected in the 24:0 h culture. Based on this finding, 12:0 h photo-period was deduced as the suitable photo-period to produce quality Isochrysis sp. Secondary treated POME was collected and pre-digested anaerobic and aerobically separately before formulation of alternative media. 5% of aerobically treated POME with 0.075% of inorganic NPK fertilizer in seawater gives optimum growth with similar growth with that of standard f/2 in the photo-bioreactor. The harvested biomass showed promising increase of lipid (19.1%) and fatty acids (91%). The pollutants were also successfully reduced; orthophosphate (87%), nitrate (38%), total nitrogen (39%) and BOD (21.3%). The outdoor 10L culture was also promising as it provides a cheaper means for bioremediation and production of quality biomass. Reductions in pollutants were similar with the photo-bioreactor. The outdoor culture Isochrysis sp. had a gross biomass production of 91.7mg/m$^2$/day. The produced biomass had a slight increase in lipid while individual fatty acids concentrations only differ about 30% from the indoor photo-bioreactor. The feeding of rotifer with a combination of alternatively outdoor grown *Isochrysis* sp. with *Nanochloropsis* sp. improves and helps prevent the rotifer cultures from crashing.

## ***ABSTRAK***

*Sepuluh tahun ini, Malaysia merupakan pengeluar dan pengeksport produk-produk kelapa sawit yang utama di dunia. Negeri Sabah adalah pengeluar kelapa sawit terbesar di Malaysia dengan jumlah kapasiti pengeluaran sebanyak 24 juta tan FFB setahun. Walaupun industri ini menyumbang kepada pembangunan negara, ia juga boleh memudaratkan alam sekitar dengan menghasilkan kuantiti efluen agro-industri yang tinggi. Rawatan degradasi biologikal yang biasanya digunakan kurang berkesan dalam mengurangkan pollutan-pollutan inorganik. Oleh itu, mikroalga marin dapat dicadangkan sebagai rawatan lanjutan bagi efluen kelapa sawit (POME) yang telah melalui rawatan biologikal. Pollutan-pollutan inorganik digunakan oleh mikroalga sebagai sumber nutiren dan biomas yang dihasilkan dijadikan makanan hidup dalam ternakan akuakultur. Kajian asas merangkumi pengaruh suhu dan saliniti terhadap pertumbuhan tiga spesis mikroalga akuakultur; telah mengenalpastikan yang Isochrysis sp. adalah species optimum kerana mempunyai kadar pertumbuhan spesifik,* $\mu = 0.84\ day^{-1}$ *(pada 23C, 30ppt) yang paling tinggi. Spesies microalgae in terkenal untuk penyesuaiannya dalam pengkulturan berterusan. Ianya dikultur menggunakan f/2 dalam 'foto-bioreaktor' pada suhu 23°C, intensiti cahaya 200* $\mu mol^{-2}s^{-1}$ *dan saliniti 30ppt dengan waktu-foto yang berbeza (12:12 jam dan 24:0 jam). Kadar pertumbuhan dan protein bagi Isochrysis sp. didapati lebih tinggi dalam waktu-foto 24:0 jam manakala komposisi lipid adalah 49% lebih tinggi dalam waktu-foto 12:12 jam. Secara keseluruhan, jumlah asid lemak per gram sampel adalah dua kali ganda dalam waktu-foto 12:12 jam di mana terdapatnya peningkatan sebanyak 40.2% DHA dan EPA yang tidak dapat dikesan dalam waktu-foto 24:0 jam. Berdasarkan profil asid lemak ini, waktu-foto 12:12 jam ditentukan sebagai waktu-foto yang sesuai dalam menghasilkan Isochrysis sp. yang berkualiti. POME yang telah menjalankan rawatan sekunder dikumpulkan dan dikenakan rawatan anaerobik dan aerobik secara berasingan sebelum digunakan untuk formulasi media alternatif. Formulasi 5% POME (rawatan aerobik) dengan penambahan 0.075% baja inorganic NPK dengan air laut didapati menghasilkan kadar pertumbuhan yang agak sama dengan kadar pertumbuhan Isochrysis sp. dalam media f/2 menggunakan 'foto-bioreaktor'. Biomas yang diperolehi menunjukkan kenaikan lipid sebanyak 19% manakala asid lemak meningkat sebanyak 91%. Pollutan-pollutan juga telah berjaya dikurangkan; ortofosfat (87%), nitrat (38%), nitrogen (39%) dan BOD (21.3%). System pengkulturan luar (10L) juga memanfaatkan kerana ianya dapat merendahkan kos bioremedasi dan menghasilkan biomas yang berkualiti. Pengurangan pollutant adalah sama dengan foto-bioreaktor dan produksi biomass adalah 91.7* $mg/m^2$*/hari. Biomass yang dihasilkan terdapat peningkatan dalam peratusan lipid; manakala kandungan individual asid lemak hanya berbeza sebanyak 30% daripada sample foto-reaktor. Kultur rotifer yang diberi makanan kombinasi Isochrysis sp.ini dengan Nanochloropsis sp. didapati meningkatkan lagi mutu dan kestabilan kultur-kultur rotifer.*

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| | |
|---|---|
| FFB/annum | Fresh Fruit Branch per annum. |
| µ | Specific growth rate. |
| f/2 | Marine microalgae seawater medium by Guillard & Ryther (1962) |
| POME | Palm Oil Mill Effluent Mill |
| AVONA | Univariate Analysis of Variance |
| $BOD_3$ | Biochemical Oxygen Demand (3 days) |
| TDS | total dissolved solids |
| TSS | total suspended solids |
| PUFA | polyunsaturated fatty acids |
| SFA | saturated fatty acids |
| MUFA | monounsaturated fatty acids |
| DHA | docosahexaenoic acid |
| EPA | eicosapentaenoic acid |
| ARA | arachidonic Acid |
| PFD | photon flux density |
| ºC | Celsius |
| ppt | part per thousand (unit for salinity) |
| h | hour |
| % | percentage |
| $mg/m^2/day$ | milligram per metre square per day |
| mg/L | milligram per litre |
| g | gram |
| ml | millilitre |
| g/ml | gram per millilitre |
| r.p.m | rotation per minute. |
| min | minutes |
| cell/ml | cell per millilitres |
| $\mu mol^{-2} sec^{-1}$ | micro per mole square per second |
| mm | millimetre |
| µm | micrometre |
| µl | microlitre |
| L | litre |
| mg/g | milligram per gram |

# CHAPTER 1

# INTRODUCTION

## 1.1 PALM OIL INDUSTRY IN SABAH, MALAYSIA

Palm oil has been regarded as one of the country's lucrative industries for the last 30 years. This 'wonder oil' is naturally free of cholesterol, rich in unsaturated fatty acids, vitamins and palmitic acids. The oil palm (*Elaeis guineensis* Jacquin) is said to have originated from the Guinea coast of West Africa. It was first introduced into other regions of Africa, South East Asia and Latin America as early as the 15$^{th}$ century and was often used as ornamental plants before its commercial value was known.

Malaysia is the world's leading producer and exporter of palm oil products for the last ten years, contributing 45% of the global palm oil demand, which is about 14,962 000 metric tonnes (Malaysian Palm Oil Statistics, 2005). This was followed by Indonesia with 41% of the world production and the rest is contributed by other countries throughout the world in smaller quantities (Figure 1).



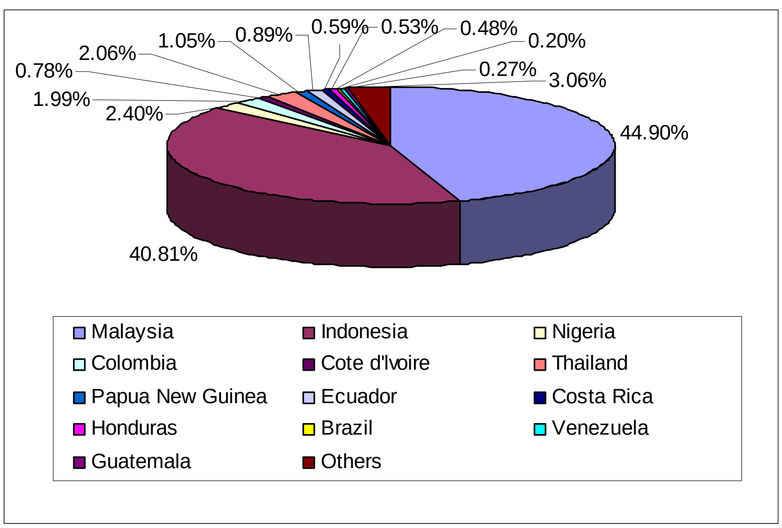


Figure 1: World major producer of palm oil in 2005.
Source: Malaysia Palm Oil Statistics (2005)

Oil palm has been grown in Malaysia since the early 1920s; initial plantations were relatively small covering only 400 hectares (Industrial Process & The Environment, 1999). This number has grown though the years and by the year 2005, the plantation area has increased to 4,051,374 hectares which supports about 423 palm oil mills throughout the country that has the production capacity of approximately 89 million tonnes of fresh fruit branch (FFB)/year (Malaysian Palm Oil Statistics, 2005)

The rapid growth of plantation areas throughout Malaysia also increased the numbers of manufacturing sectors like milling, refining and oleo-chemical. To date there are about 423 mills, 55 crushing factories, 64 refineries and 20 oleo-chemical factories around Malaysia. These industries produce crude palm oil, processed palm oil, palm oil, crude palm kernel oil, processed palm kernel oil, palm kernel oil and palm kernel cake. Among these products, the most exported is the processed palm oil (Malaysian Palm Oil Statistics, 2005).

In Malaysia, the state of Sabah is the largest producer of palm oil. The oil palm plantation itself was an astounding 1,209,368 hectares of the total plantation areas in this country. The existing palm oil mills in Sabah in 2005 were 109 mills with total production capacity of 25,484,200 tonnes fresh fruit branch per annum (FFB per annum). The area with the most palm oil industries is Lahad Datu; followed closely by Sandakan (Malaysian Palm Oil Statistics, 2005).



Revenue from this industry has contributed significantly to the growth and development of the country. The total export revenue from the palm oil industry was about RM 29 billion at the end of 2005 (Malaysian Palm Oil Statistics, 2005). While rapid growth of these industries benefit the country, the waste generated causes environmental problems that need to be solved.

## 1.2 WASTE GENERATED AND ITS EFFECTS ON THE ENVIRONMENT

The production of palm oil products consists of mainly two main processes. The first being the extraction of crude palm oil from the fruit branch in mills followed by the refinery process in the refinery plant where the crude oil is further purified into final products. Both of these processing plants generate waste that have the potential to endanger the aquatic environment. These two wastes are different in their compositions and amount of organic matter produced (Ma, 1999a; Ma, 1999b). In comparison, the effluent generated by palm oil mills has more damaging effects on the environment than that of the refinery plant as the effluent from mills have a higher content of organic matter. Generally, the effluents from palm oil mills are known as Palm Oil Mill Effluent (POME). POME is a thick brownish liquid that has a very rich content of organic matter and essential plant nutrients. Its main characteristics are the high Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD).

In 2005 itself, approximately 19 million tonnes of POME was generated in Sabah alone. Due to the high levels of organic matters and nutrient elements like nitrogen and phosphorus, the effluents produced are highly polluting. These pollutants have a very high potential to deteriorate the aquatic environment if not handled properly. Most of the palm oil mills in Sabah are situated near rivers which provide the daily water requirement for the processes. Therefore most of the effluents are generally either disposed (after secondary treatment) into these waterways or in land as composites (Industrial Process & The Environment, 1999).

Effluent discharge in its raw or partially treated form into waterways undergoes degradation that depletes dissolved oxygen in these waterways. The oil and grease in these effluents also form a thin layer of film that prevents oxygen from dissolving into the waters. This leads to total depletion of dissolved oxygen which causes 'suffocation' of aquatic organisms (Industrial Process & The Environment, 1999).

The discharge of organic waste into waterways also causes anthropogenic acceleration of eutrophication known as *Cultural eutrophication* (Laws, 1993). This condition will eventually lead to massive algae bloom due to abundance of nutrients. These blooms cause oxygen depletion in the waterways which endangers aquatic lives, destruction of coral reefs and reduce the value of the body of water as a recreational resource (Laws, 1993).

Therefore it is very important to treat the effluent by reducing the organic and plant nutrients to a much lower level before it is discharged into waterways.

## 1.3 TECHNOLOGY CURRENTLY USED IN TREATING PALM OIL MILL EFFLUENT (POME)

Conventional procedures used to treat POME consist of bioremediation using active non marine microbes by anaerobic and aerobic digestion that utilizes the organic matter to produce biogas and a mixture of biosolids (Ma, 1999a). Bioremediation refers to biodegradation process that remove the contaminants that have the ability to endanger human health and environment either in the aquatic or terrestrial system (Crawford, 1996). When managed at an optimal level, these treatments are able to meet the regulations in the Environmental Quality Act 1974 and the specific regulations of palm oil mill effluent 1977 (Industrial Process & The Environment, 1999).

But in reality, effluents are produced at an alarming rate and overloading often happens at treatment plants. Most of the palm oil mills in Malaysia use the traditional grid, anaerobic and aerobic pond as means of primarily and secondary treatments. These ponds are often expensive, require intensive land space and lack efficiency, where it may allow seepage and accidental release into the waterways. A tertiary treatment of the effluent is required at this stage to further prepare the effluent for discharge into waterways (Kennedy & Hishamuddin, 2001). Therefore there is a need to further look into the general treatment technologies that are currently used in this country.



It is a well studied fact that microalgae are important organisms in bioremediation of waste waters and eutroficated water bodies (Travieso *et al*., 1996; Villasclaras *et al*., 1996; Craggs, Mcauley, & Smith, 1997; González *et al*., 1997; Craggs *et al*., 1997; Lau *et al*., 1995; Ogbonna *et al*., 2000). Most studies that involves usage of microalgae in tertiary treatment of POME, uses freshwater microalgae. In Malaysia, species such as *Chlorella, Spirulina* and *Scenedesmus* are often used in the field of bioremediation, particularly in biodegradation of palm oil mill effluents and sago starch mill effluents in Peninsula Malaysia (Sivalingam, 1978;

Sivalingam, 1983; Phang, & Ong, 1988; Phang *et al.*, 2000; Kennedy, & Hishamuddin, 2001).

To date, there is no reported study on the use of marine microalgae in POME treatment. Based on ongoing investigation using marine microalgae for bioremediation and aquaculture life-feed; it was noted that these organisms have the characteristics and potential as a bioremediation agent. Besides this, similar studies by other researchers had also reported on marine microalgae ability to digest organic waste (Paniagau-Michel *et al.*, 1987; Matsunaga *et al.*, 1999; Vílchez et al., 1997; Craggs *et al.*, 1997).

In Sabah, the palm oil mills are located close to the coastal waters, and often effluents are discharged into the sea. Hence, pollution of coastal waters occurs due the over-loading of nutrients. By utilizing marine microalgae as means of bioremediation, these pollutants can be assimilated by the marine microalgae to produce valuable biomass. Therefore by incorporating marine microalgae based bioremediation of POME; the bloom in marine aquaculture can be encourage by producing quality economical live feeds

## 1.4 OBJECTIVES

There are five main objectives throughout this study:

a) To perform preliminary growth studies of *Isochrysis* sp., *Chaetoceros* sp. and *Tetraselmis* sp.

b) To determine suitable photoperiod for semi-continuous culture of *Isochrysis* sp.

c) To select suitable media formulation of biodegraded POME.

d) To study the growth, biochemical contents and bioremediation potential of *Isochrysis* sp. grown in digested secondary treated POME in both photo-bioreactor and outdoor system.

e) To asses the potential of biomass of *Isochrysis* sp. grown using the fortified effluent enriched seawater as a source of quality nutrient feeds in aquaculture.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 PALM OIL MILL EFFLUENT: PROPERTIES AND MANAGEMENT

Palm oil mill effluent (POME) is an agro-industrial based wastewater that is generated during the production of crude palm oil. For every tonne of fresh fruit bunches (FFB), 1.5 cubic meters of water is used and 50 % from this results in POME (Industrial Process & The Environment, 1999). Therefore, it can summarised that for every tonne of FFB processed, 0.75 tonnes of effluent is generated.

POME is a combination of wastewater generated from the various processes in the palm oil mill. About 60 % of POME is generated from the oil clarification process while the sterilization process contributes 36 %. The remaining 4 % is contributed by the hydrocyclone separation of cracked mixture of kernel and shell (Industrial Process & The Environment, 1999). Wastewaters from the oil clarification and sterilization processes are the most polluting effluents.



Raw POME is often described as non toxic brownish colloidal sludge like liquid that has a very high content of organic matter with high Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). It is often hot (80-90 °C) and has a pH value less than 5 (Palprasert, 1989; Ma, 1999a). Table 2.1 shows the typical characteristics of the raw POME.

Other than organic mattes, raw POME also has substantial amount of inorganic nutrients and trace metals that encourages plant and alga growth (Ma, 1999a). Due to it unique contents, improper discharge into waterways leads to aquatic pollution.

Table 2.1: Characteristics of raw POME.

| No | Parameters | Measurement (mg/L) |
|---|---|---|
| 1 | Oil and Grease | 4,000 |
| 2 | $BOD_3$ | 25000 |
| 3 | COD | 50,000 |
| 4 | Total Solid | 40,500 |
| 5 | Suspended Solid | 18,000 |
| 6 | Total Volatile Solid | 34,000 |
| 7 | Total Nitrogen | 750 |
| 8 | Ammoniacal Nitrogen | 35 |
| 9 | Phosphorous | 18 |
| 10 | Potassium | 2,270 |
| 11 | Magnesium | 615 |
| 12 | Calcium | 439 |
| 13 | Boron | 7.6 |
| 14 | Iron | 46.5 |
| 15 | Manganese | 2.0 |
| 16 | Copper | 0.89 |
| 17 | Zinc | 2.3 |

Source: Ma (1999a)



In the 1960s and 1970s, POME was observed to be the largest source of water pollution. Therefore to manage and control the environmental deteriorations due to palm oil related industries, the Environmental Quality act 1974 and the specific regulations of palm oil mill effluent 1977 was enforced (Abdul Rani, 1995). Based on this act, the palm oil industries must first treat the raw POME before it can be discharged into waterways or on land as composite. In cases where the effluent is to be discharged onto land, the only parameter imposed is BOD, where the limit is 5, 000 mg/L. While, for waterway discharge, the limits are; BOD (100 mg/L), suspended solids (400 mg/L), oil and grease (50 mg/L), ammoniacal-nitrogen (filtered sample)(150 mg/L), total nitrogen (200 mg/L), pH (5.0-9.0) and temperature (45 °C) (Legal Research Board, 2003).

POME is conventionally bio-degraded using active microbes either aerobically or anaerobically. In reality these conventional treatments lack efficiency and commitment from the management of the mills. Treatments of effluents are often view as financial burden without any return.

Therefore to reach a balance between environmental conservation with that of economic development, research are being carried out on new methods to not only treat POME but also to produce valuable by-products. Some of these treatments involve the membrane technology where the water recovered from treating POME can be recycled (Abdul Latif *et al.*, 2003; Abdul Latif *et al.*, 2005). The anaerobic digestion has also being studied by various researchers in order to perfect the treatment and its potential use in production of biogases and fertilizer (Borja, *et al.*, 1996; Ugoli, 1997; Najafpour *et al.*, 2005).

Other than these primary and secondary treatments, tertiary treatments using freshwater organism to further treat POME have also been studied (Kennedy & Hishamuddin, 2001; Habib *et al.*, 1997; Phang & Ong, 1988; Sivalingam, 1983; Sivalingam, 1978). Most of these investigations centred on first using treatments like anaerobic and aerobic digestion to further degrade the waste before it was used as a medium to culture mircoalgae. These cultures were successful in further reducing the pollutant levels. Due to the non-toxic characteristic of POME, the biomass harvested was of high commercial value.

Reports by Sivalingam (1978 & 1983) proved the potential use of *Chlorella* isolated from palm oil effluent sludge as means of reducing the pollutant levels and provide good quality human/animal feeds as a by-product. Phang and Ong (1988) studied the production of algal in digested POME effluent in the pond systems. The dominant species in the various pond groups were *Chlorella, Merismopedia, Monoraphidium griffithii, Euglena sp.1, Phacus sp. 1* and *Euglena sanguinea*. These studies proved that POME can be used as alternative media source and as a potential organic carbon in aquaculture. While in 2001, Kennedy and Hishamuddin successfully cultured uniagal species of *Spirulina platensis* in both raw and aerobic digested POME.

In 1997, Habib and fellow researchers also studied the potential of POME as carbon source for chironomid larvae or better known as ‘bloodworm”. The production and nutritional value of bloodworm grown in POME was observed to be higher than that of the bloodworm grown using the microalgae diet. These studies showed that it was feasible to reduce pollutant levels and at the same time produce commercially valuable aquatic organism.

There were also studies that reported the potential use of marine microalgae in bioremediation of wastewater (Craggs *et al*., 1997; Paniagua-Michel *et al*., 1987; Valenzuela-Espinoza *et al*., 1999; Wang, 2003); but none involved bioremediation of POME. Due to the non toxic characteristics of POME and the ability of microalgae to utilize bio-digested organic waste to produce organic biomass (Paniagua-Michel *et al*., 1987), the application of certain valuable marine microalgae in bioremediation of secondary treated POME to produced quality biomass is a reasonable idea to be delved upon.

## 2.2 MARINE MICROALGAE: GROWTH AND POTENTIAL

Marine microalgae are often regarded as heterogeneous group of organisms that are microscopic, unicellular and photoautotrophic. They can generally be divided into three main categories: Naked flagellates, non-motile unicells and centric diatoms. Most of these marine microalgae have a wide application in industries like aquaculture, chemical production and bioremediation (Víchez *et al*., 1997; Pauw & Persoone, 1988; Regan, 1988).



The marine microalgae that are often used in the field of aquaculture are important as a source of essential fatty acids to marine organisms like bivalve molluscs, larvae of gastropods and shrimps, and various zooplanktons. The latter in turn serves as an important live food supply for rearing larvae of numerous marine fish and crustaceans (Pauw & Persoone, 1988).

These marine organisms have limited ability to produce certain polyunsaturated fatty acids (PUFA) like eicosapentaenoic acid (EPA (22:5n-3) and docosahexaenoic acid (DHA (20:6n-3) from precursor fatty acids like linolenic acid. Therefore to improve the growth rates and larval survival these PUFA are supplemented by diet of microalgae (Volkman *et al*., 1989). Some of the most frequent used species as live feeds are *Tetraselmis suecica, Tetraselmis chui, Isochrysis galbana, Isochrysis sp. (T.ISO), Chaetoceros muelleri, Chaetoceros*