MICROALGAE FOR TERTIARY TREATMENT OF PALM OIL MILL EFFLUENT (POME)- EFFECT OF LIGHT PENETRATION AND KINETIC STUDY

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ABSTRACT

Microalgae was found to have a high potential to reduce biochemical oxygen demand (BOD) level and colour of black-coloured wastewater, Palm Oil Mill Effluent (POME) in palm oil industry. The major factor that will influent the effectiveness of microalgae in tertiary treatment of POME was light penetration. This thesis presents the effect of light penetration and kinetic study in tertiary treatment of Palm Oil Mill Effluent (POME) by utilizing microalgae. 10% v/v of microalgae was cultured in 10 % v/v to 100 % v/v autoclaved and centrifuged POME in 250 ml conical flask. Sampling was done at the beginning and the end of seven days to observe the microalgae growth under light irradiance of 6000 Lux. Kinetic study was then carried out on the sample with the highest growth rate to observe growth profile of microalgae mix culture within seven days at interval of 24 hours. The BOD and colour of initial sample (autoclaved and centrifuged POME without microalgae) and final sample (at t = 7 days) was determined by applying dilution method (Standard Method 5210B) and ADMI weighted ordinate method respectively. Results revealed that microalgae experienced the highest growth rate at 30 % v/v POME. The minimum light penetration was approximately 2000 Lux corresponding to the light penetrated in 30 %v/v of POME. The microalgae cultured in 30 % v/v of POME had the highest specific growth rate (1.39 d^{-1}) and biomass productivity (0.61 g/L.d). The ratio of POME concentration and the respective growth profile of microalgae led to the similar removal efficiency of BOD. While the low decolourisation yield (colour removal efficiency less than 20%) proved that microalgae was not effective in removing coloured compound.

ABSTRAK

Mikroalga didapati mempunyai potensi yang tinggi untuk merendahkan tahap permintaan oksigen biokimia (BOD) dan keamatan warna air sisa, POME yang berwarna hitam dalam industri minyak sawit. Faktor utama yang akan mempengaruhi keberkesanan mikroalga dalam rawatan POME adalah penembusan cahaya. Tesis ini membentangkan kesan penembusan cahaya dan kajian kinetik dalam rawatan air sisa POME dengan menggunakan Mikroalga. 10 % v/v mikroalga ditumbuhkan dalam 10 % v/v - 100 % v/v POME yang telah diautoklaf dan dipusat dalam 250 ml kelalang kon. Persampelan telah dilakukan pada hari pertama dan hari terakhir dalam tempoh tujuh hari untuk memerhatikan pertumbuhan mikroalga di bawah sinaran cahaya sebanyak 6000 Lux. Kajian kinetik kemudian dijalankan ke atas sampel dengan kadar pertumbuhan yang paling tinggi untuk melihat profil pertumbuhan budaya campuran mikroalga dalam tempoh tujuh hari setiap selang 24 jam. BOD dan warna sampel terawal (sample POME yang telah diautoklaf and dipusat tanpa penubuhan mikroalga) dan sampel terakhir (pada t = 7 hari) ditentukan dengan menggunakan kaedah pencairan (Kaedah Standard 5210B) dan kaedah ADMI. Keputusan menunjukkan bahawa mikroalga mengalami kadar pertumbuhan yang paling tinggi pada 30 % v/v POME. Penembusan cahaya minimum adalah dalam anggaran 2000 Lux selari dengan keamatan cahaya yang berjaya menembusi 30 % v/v POME. Mikroalga yang diternak dalam 30 % v/v POME mempunyai kadar penumbuhan (1.39 d^{-1}) dan produktiviti biomas (0.61 g/L.D) yang tertinggi. Nisbah kepekatan POME dan profil pertumbuhan mikroalga yang selari dengan nisbah telah membawa penurunan BOD yang hamper sama. Kecekapan pengurangan warna yang kurang daripada 20 % membuktikan bahawa mikroalga tidak berkesan dalam mengurangkan sebatian berwarna.

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μ	Specific growth rate
μ_{max}	Maximum growth rate
A	Area of culture
a_c	Averaged spectral absorption coefficient
A_t	Light Attenuation
b	Experimental rig light path
BOD	Biochemical Oxygen Demand
C_f	Final concentration
C_o	Initial concentration
C_{POME}	Concentration of POME
C_x	Biomass concentration
DO	Dissolved Oxygen
I	Light irradiation at outlet
I_{avg}	Average light intensity
I_{in}	Incident irradiation
I_o	Light irradiation at inlet
K_L	Saturation constant for light intensity
L	Light intensity in Monod Model
P	Decimal volumetric fraction of sample used
PFD_{in}	Photon flux density at inlet
POME	Palm Oil Mill Effluent
t	time
V	Volume of culture
x	Cell density

1 INTRODUCTION

1.1 Background Study

According to Malaysian Palm Oil Council (MPOC), the palm oil industry of Malaysia has developed rapidly and accounted for 39 % of world palm oil production and 44% of world exports. In 2010, Malaysia had produced 22.89 million tonnes of crude palm oil (CPO) and increase to 24.97 million tonnes in 2011. This was a huge success to palm oil industry and it continues to grow annually. To extract the palm oil from fresh fruit bunch (FFB), wet palm oil milling process is the most favourable way. As stated by Lam and Lee (2011), big amount of water and steam was used to wash and sterilize FFB in the wet palm oil milling process. Approximate 50% of the water together with the oil and fine cellulosic fruit residues will form thick and brownish wastewater that is called Palm Oil Mill Effluent (POME).

In industry, open ponding system was applied mostly. The waste stabilization ponds include anaerobic, aerobic and facultative ponds. However, primary and secondary treatment by using ponding system sparks controversy. Based on the industrial standard, the average amount of POME harvested while producing per tonnes of CPO is approximately 3.5 m^3 (Zaini *et al.*, 2009). For 24.97 million tonnes of CPO produced in 2011, an estimated amount of 87 million m^3 of POME will be generated. Zaini *et al.* (2009) also reported that a tonne of wastewater from the process carried 27 kg of BOD, 62 kg of COD, 35 kg of suspended solid and 6 kg of oil and grease. The high value of BOD and COD eventually trigger water pollution. Besides that, the current conventional open ponding treatment system of POME produced a highly colored effluent which will cause reduction in photosynthesis and toxic to aquatic biota.

Indeed, the effluent quality fails to meet the standard discharge limit set by Department of Environment (DOE). As the environmental awareness rising among the public, the environment sustainability of the process would be doubted. Therefore, POME treatment is crucial to curtail environmental ramifications.

Microalgae had emerged as the potential source for treating the POME. Its characteristics such as rapid growth rate, low cost and consumption of nitrogen and phosphorus as the food for growth make it a favorable element to treat POME. Therefore, microalgae can be used as an advanced tertiary treatment to improve the effluent quality.

1.2 Motivation and Significant of Study

According to the MPOB, BOD was the key parameter in the standards. The BOD_5 value for the untreated POME was 25,000 ppm and currently the Department of Environment had proposed a tightening of the discharged BOD level to 20 ppm. In spite of that, the current BOD discharged load in the industry which utilised open ponding system was 100 ppm.

Moreover, the removal efficiency of colour and phenolic group was challenged. Before treatment, the raw POME is the thick liquor with dark-brown colour. The pigment and phenolic content of POME induce the reduction in photosynthesis process, create carcinogenic substances in drinking water and toxicity to aquatic lives. Neoh *et al.* (2012) further clarified that ponding system applied by the 85% of the local palm oil mills is inherently inefficient to remove the dark brown colour in POME. This was due to POME is treated solely on the existing of the indigeneous microorganisms but not any biological agent or chemicals.

Microalgae present itself as solution to remove colour and reduce BOD level of POME in a preliminary study. Thus, it has been proposed to be used for tertiary treatment of POME. The maximum depth of the pond to let the microalgae exposed to sufficient sunlight is 0.2 to 0.5 m (Lam and Lee, 2011). Despite with the ideal depth, yet the sunlight cannot penetrate easily through thick and brownish POME and support the growth of microalgae. To maximize the efficiency of the treatment by using microalgae, the problem of light penetration must be investigated. While the BOD and colour removal efficiency will be the additive support to the research.

Most significantly, minimum requirement for the microalgae to survive in POME can be identified. Microalgae as a viable advanced treatment of POME was expected to refine the quality of POME that can satisfy the DOE standards. Particularly, the colour removal efficiency is counted to experience a breakthrough. As demanded by the industry, microalgae hence expected to maintain the consistency of effluent quality (BOD level less than 20 ppm).

1.3 Objective

The main objective of the experiment is to determine minimum light penetration of POME for microalgae growth and develop kinetic study of microalgae for the tertiary treatment of POME waste.

1.4 Scopes

In the experiment, a mix culture of microalgae was used. The POME was diluted to different concentration (from 0 to 100%) and the quality of untreated POME such as the BOD level and colour will be determined. Mix microalgae culture was grew in different POME concentration for seven days. The minimum light penetrations that enable microalgae grow in different POME concentration was necessary to be identified by justifying the highest cell number from the samples after seven days. The second part was conducted by culturing microalgae in various dilutions of POME and monitoring the cell number up to at least seven days at constant light intensity (6000 Lux). The kinetic study was done based on the growth rate and the yield.

2 LITERATURE REVIEW

2.1 *POME and Current Treatment Process*

POME which produced by wet palm oil milling process, was considered as severe wastewater source that affecting the environmental balance. The thick brownish liquor of POME had been categorized as the most significant pollutant as it contained high BOD and COD level together with the suspended solid, organic particles, oil and grease. The common ways that the palm oil millers in Malaysia usually adopted to treat the POME were ponding system, anaerobic digestion and aerobic treatment. Nevertheless, there were pros and cons by using the systems.

2.1.1 Open Ponding System

Ponding system which was applied approximately by 85% of local palm oil mills, containing anaerobic, facultative and aerobic ponds for treating the POME (Neoh *et al.*, 2012). Figure 2.1 illustrates the flow process of ponding system in Malaysia. To further curtail the organic content in POME, facultative pond and aerobic ponds are necessary before discharging the POME into the river. The extensive usage of ponding system was due to less energy is required to operate the system and technology requirement was unsophisticated, therefore low cost of operation. Moreover, it was reliable and stable.

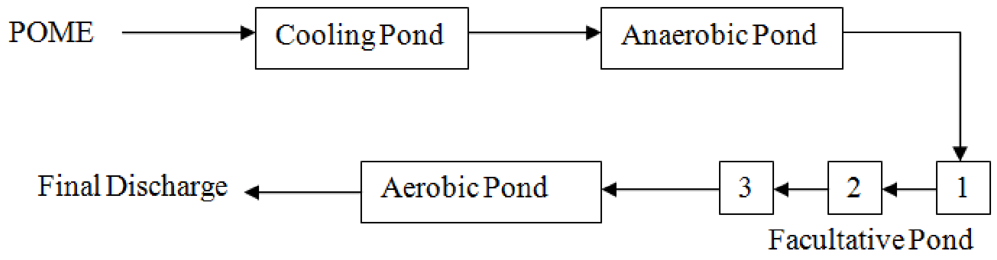

Figure 2.1: The ponding system (Wong, 1980).

Yet, Lam and Lee (2011) pointed out the main disadvantage of using the ponding system. At once, the sludge will accumulate at the bottom of the pond and scum will form on the surface of POME, hence bringing dissatisfaction of the effluent quality. Building of the ponding system usually require large areas of land, thus become the obstacle for the factories located near to urban or developed areas. Another environment issue that was bringing up in the POME treatment using ponding system was that the releasing of greenhouse gases, Methane gas (CH_4) and Carbon dioxide gas (CO_2) was uncontrollable and cause air pollution and global warming.

The depth of the pond in the ponding system is vital as stated by Lam and Lee (2011). For instance, the depth of anaerobic pond, facultative pond and aerobic pond were 5-7 m, 1-1.5 m and 0.5-1 m respectively. According to Wong (1980), the efficiency of BOD removing by adapting the ponding system was 74-81%. In term of amount of BOD discharged, less than 50 mg/L or 50 ppm of BOD level can be achieved.

2.1.2 Open Digesting Tank

One of the factors that will greatly determine the efficiency of ponding system was the land area. Open digesting tank was applicable for treating the POME while the land area was limited. Lam and Lee (2011) declared that the advantage of applying open digesting tank was effective in removal of solids or sludge that was accumulated at the bottom of the ponds. The sludge was a potential fertilizer source. Yacob *et al.* (2005) reported that 34.9 kg of COD per 1 m^3 of POME was removed by using the open digesting tank system. The result also denoted that approximately 80.7% of COD was removed before the treated POME being channeled into the facultative ponds for further treatment.

Considering the disadvantage, the open digesting tank tends to expose to hydrogen sulfide for a long period, lead to the corrosion of the steel structure and thus easier to collapse.

2.1.3 Anaerobic Digestion

Another system that was applied was anaerobic digestion. Under anaerobic digestion, the complex organic matter will degrade by microorganism in the absence of oxygen. The main benefit that using the system was the production of sludge was low (Wu *et al.*, 2010). Coupling with the generation of renewable energy, biogas promotes circulation and mixing in the system and thus produces less sludge. However, the methane emission from the anaerobic digestion system was very high and cause air pollution. Wu *et al.* (2010) also stated that the colour removal efficiency is low and the amount of nutrient removal such as nitrate and ortho-phosphate is not significance.

In addition, Abdurahman *et al.* (2012) had compared the COD removal efficiency of POME by using several anaerobic treatment systems: anaerobic filtration, fluidized bed reactor and upflow anaerobic sludge blanket (UASB). For anaerobic filtration, small reactor volume was required to produce high quality effluent. However, clogging might occurred at high organic loading rate (OLR), thus it was not suitable for high suspended solid wastewater. For fluidized bed reactor, it provides well-mixed condition as well as large surface area for biomass attachment. In spite of that, high cost was needed for the bed fluidization. While for UASB, a typical UASB reactor was shown as in Figure 2.2. Though it was beneficial for high suspended solid wastewater, its performance was depending on sludge settleability and sludge floatation at high OLRs. Table 2.1 summarized the efficiency of COD removal by applying different anaerobic treatment system. As observed, the anaerobic filtration had the highest COD removal efficiency which was 94%.

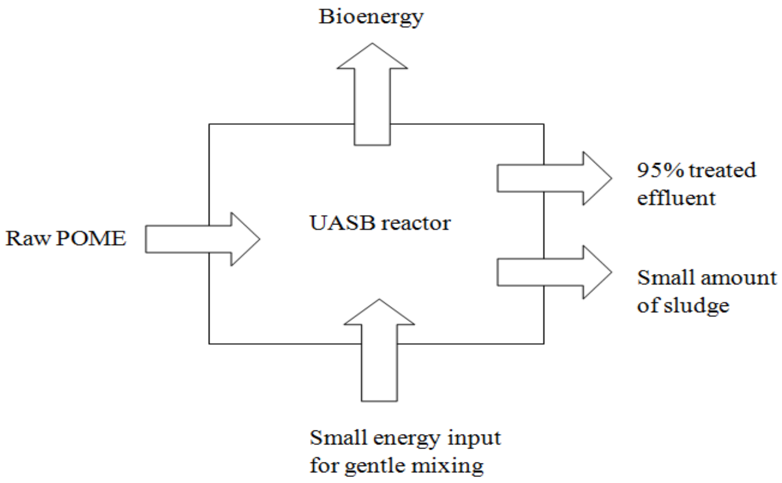

Figure 2.2: The anaerobic wastewater treatment process by using UASB reactor (Badroldin, 2010).

Table 2.1: Summary of COD removal efficiency for POME in Different Anaerobic Treatment System.

No	Anaerobic Treatment System	COD removal efficiency
1	Anaerobic filtration	94%
2	Fluidized Bed Reactor	78.0-94.0%
3	UASB	63.0-81.0%

2.1.4 Aerobic Treatment

The employment of aerobic treatment is also common in industry. Aerobic treatment unit was preferable when limited land area was available for the POME treatment. Aeration system was implemented in the aerobic treatment of POME. It thus encourages the growth of naturally-occurring aerobic microbes and renovating the quality of the wastewater. In the system, POME will enter to the aeration unit then followed by mixing with dissolved oxygen and suspended microbes (John and Robert, 2004). The aerobic microbes will then convert the organic compounds in POME into energy, new cells and residual matter. The main issue in the aerobic treatment was the maintenance of steady-state condition where the oxygen transfer rate will be equal to the rate of oxygen consumption by the microorganisms.

Vijayaraghavan *et al.* (2007) proposed the method of aerobic oxidation based on activated sludge process to treat the POME. Activated sludge is a process for treating sewage and industrial wastewaters using air and a biological floc composed of bacteria and protozoa. The slurry of biological floc together with the POME was known as mixed liquor.

As illustrated in Figure 2.3, the suspended biological floc adsorbs the organic solids and soluble organic compounds as the wastewater pumped into the aeration chamber. Biochemical oxidation was occurred to oxidize the soluble organics. Once POME had received sufficient treatment, the mixed liquor will be discharged into settling chamber. John and Robert (2004) justified that the biological solids which also known as activated sludge were reactivated and return to the aeration system in order to re-seed POME that was entering to the tank.

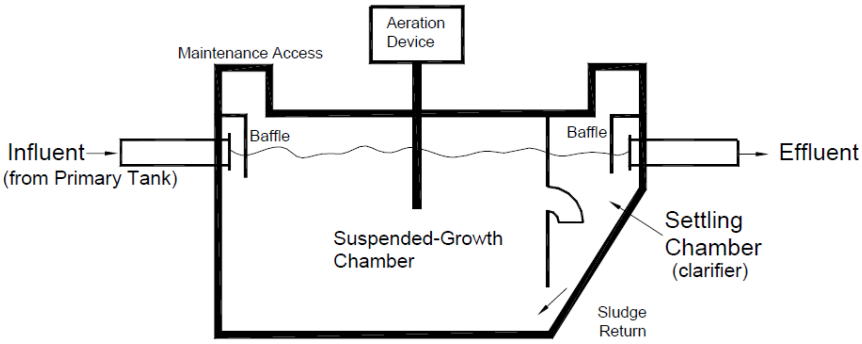

Figure 2.3: The suspended-growth aerobic treatment unit (John and Robert, 2004).

Vijayaraghavan *et al.* (2007) reported the efficiency of the aerobic treatment based on activated sludge process on BOD level of anaerobically digested POME and raw POME. After 60 hours of the treatment process, the BOD_5 removal percentages for anaerobically digested POME and raw POME were 93% and 82% respectively. Based on Chan *et al.* (2010), the removal efficiencies of COD, BOD and TSS were ranging from 91-96%, 92-99% and 94-99% respectively. These results had shown that the activated sludge system is very effective in treating the POME.

The bulking of sludge in POME can be prevented if suitable level of dissolved oxygen was provided. Vijayaraghavan *et al.* (2007) mentioned that although the effectiveness and efficiency is better than anaerobic treatment system due to low hydraulic retention time (HRT), high energy is needed to operate the aeration system, thus it is not economic. Similar to the anaerobic system, the colour removal efficiency was unsatisfied.

Treatment by biological means is highly effective and sustainable to the environment. Although various systems had existed, advanced treatment is essential to improve the quality of the POME, further miniaturize the environmental impacts.

2.2 POME Treatment Using Microalgae

Tertiary treatment process was aimed to remove all organic ions. It can be executed either biologically or chemically. The biological tertiary treatment performed better compared to the chemical processes which were generally too costly to be implemented and which may cause secondary pollution. Bio-treatment of POME by utilizing microalgae is desirable. According to Abdel-Raouf *et al.* (2012), their photosynthetic capabilities are able to convert the solar energy into useful biomass. At the same time, nutrients such as nitrogen and phosphorus which usher the eutrophication will be assimilated. The overall photosynthetic stoichiometric formula was shown as below:

$$6\ H_2O + 6\ CO_2 + \text{Light} \longrightarrow C_6H_{12}O_6 + 6\ O_2$$

Microalgae rely on the nutrients to survive and replicate. BOD and COD reduction of POME was possible by using microalgae. Oxygen that was produced by microalgae contributes to the oxidation of organic matters (waste), hence reducing the BOD and COD level (Mata *et al.*, 2012). The efficiency of treatment was elevated due to reduction of BOD and COD level. This statement was supported by the results of Mata *et al.* (2012), where under optimum condition (sufficient light exposure and aeration rate), the COD reduction improved day by day and the highest reduction percentage was up to 70%.

In addition, microalgae rose as potential element was due to its rapid growth rate (Lam and Lee, 2011). This can be strengthening by the fact that microalgae is able to produce 10 to 100 times more fuel compare to the others biofuel producer as abundance of microalgae can be produced in short period. Mata *et al.* (2012) showed that the production of biodiesel by microalgae was 51927 kg biodiesel/year which was approximately 100 times more than biodiesel production by soybean (562 kg biodiesel/year). However, its growth rate is limited by several factors such as light intensity, availability of nutrients, aeration rate, organic loading rate, amount of carbon dioxide and so on.

2.2.1 Microalgae

Microalgae were the unicellular species which exist individually, chain or groups found in freshwater and marine systems. According to Lam and Lee (2011), microalgae species can be divided into four groups: diatoms (*Bacillariophyceae*), green algae (*Chlorophyceae*), blue-green algae (*Cyanophyceae*) and golden algae (*Chrysophyceae*). The eight most tolerant to organic pollutants genera were found to be *Euglena*, *Oscillatoria*, *Chlamydomonas*, *Scenedesmus*, *Chlorella*, *Nitzschia*, *Navicula* and *Stigeoclonium*. The major constituents of microalgae were carbohydrates, proteins, nucleic acids and lipids (typically phospholipid and glycolipids).

The growth of microalgae characterized into five stages. The time taken for lag phase to occur was relatively long. During the little increase in cell density happened, an algal culture was transferred from a plate to liquid culture. Generally, under similar growth conditions of light, temperature and salinity, an inoculum taken from a healthy exponentially growing culture was unlikely to have any lag phase when transferred to fresh medium. The upscaling time hence shorten. The lag phase in growth was attributed to the acclimatization of the microalgae, such as the increase of the levels of enzymes and metabolites involved in cell division and carbon fixation.

During the second phase which was the exponential phase, the cell density increases as a function of time. Growth rate was one important way of expressing the relative ecological success of a species or strain in adapting to its natural environment or the experimental environment imposed upon it.

In the phase of declining growth rate, cell division decelerates. The nutrients, light, pH, carbon dioxide or other physical and chemical factors started to limit microalgae growth. In this phase of growth, biomass is often very high. At low cell densities, large amount of CO_2 may lower the pH and curtail the growth. However, CO_2 limitation at high cell densities causes any further biomass increase linearly rather than exponential (with respect to time).

Light limitation usually occurred at high biomass when the cells absorbed most of the incoming irradiation and individual cells shade each other. Growth in most phytoplankton was saturated at relatively low irradiances of 50-200 μmol. photons m^{-2} s^{-1}. However, microalgae generally well adapted to surviving conditions of low incident light and may survive for extended periods under these conditions. This was further proven by the studies of Suzana *et al.* (2013), the cell densities of microalgae *Nannochloropsis sp.* were increasing in the period of 8 days under the light intensity of 50-200 μmol. photons m^{-2} s^{-1} (3700-14,800 Lux).

Microalgae enter stationary phase when net growth was zero. In the fourth stage the limiting factor and the growth rate were balanced, lead to a relatively constant cell density. Nitrogen limitation may result in the reduction in protein, lipid and carbohydrate content. Light limitation will result in increasing pigment content of most species and shifts in fatty acid composition. Light intensities that were previously sufficient or optimal for growth in the first three phases can now become stressful and lead to a condition known as photo-inhibition. If the incident illumination was maintained relatively high then a large proportion of cells may become stressed, photo-inhibit and the culture can be pushed into the death phase. This is especially the case if the culture is also nutrient stressed.

When cultures enter stationary phase, it is generally preferable for many species to halve or further reduce the incident light intensity to avoid photo-inhibition. Some green algae may survive under very low illumination. Lowering temperature combined with lowering irradiance can further reduce stress. Survival is inversely proportional to temperature. The shutting down of many biochemical pathways as stationary phase proceeds means that the longer the cells are held in this condition the longer the lag phase will be when cells are returned to good growth conditions. When cell metabolism can no longer be maintained, the death phase of a culture is generally very rapid.

There were several factors of growth limitation. The total yield or biomass of microalgae will be determined by the nutrient present in the lowest concentration in relation to the microalgae's requirement.

2.2.2 Mechanisms of POME Treatment using Microalgae

POME treatment using microalgae was reliable because it can be adapted to the adsorption technique. Dotto and Pinto (2012) proposed microalgae *Spirulina plantensis* as an alternative biosorbent to remove dyes from the wastewater.

Generally, there were two types of adsorption in wastewater treatment: one involving non-biomass materials and another one involve the biomass (Neoh *et al.*, 2012). The non-biomass adsorption was utilizing materials such as macro-composite and bottom ash in the removal of dye. In this case, the biomass adsorption which was also known as biosorption was concerned due to the usage of living biomass, microalgae.

Biosorption by utilizing the biomass was found to have the great potential on removing the impurities. It also provides an alternative technology to improve the colour removal efficiency.

In the study, microalgae was grown in the POME. Some of the previous studies reveal that the biosorption of the colour pigment was using the dead cells. However, Neoh *et al.* (2012) supported the method of growing the microalgae in the wastewater (POME). In fact, the rapid growth rate will subsequently increase the amount of biomass thus increase in colour adsorption and removal of other compound in POME. Indirectly, it also enhances the efficiency of the treatment process by removing the BOD and COD level as well as the total phenolic compound.

Dotto and Pinto (2012) further clarified the biosorption systems which is the fundamental study of mass transfer kinetics and the rate controlling steps. The three main steps must be considered: external mass transfer, intra-particle diffusion and molecule uptake by the active sites. The external mass transfer was the limiting steps and pH was one of the factor will affect the value of external mass transfer coefficient, thus will affect the efficiency of biosorption system. In the study of Dotto and Pinto (2012), decreasing of pH value will eventually increase the biosorption rate. In low pH value, the sulfonated groups of the dyes were dissociated rapidly and at instance, the *S. platensis* surface was protonated. Thus, the electrostatic attraction was increased, aiding the mass transfer in the external layer.

In the similar findings of Neoh *et al.* (2012) by using bacteria *Aspergillus niger*, low pH served as the best condition for fungi to adsorb humic acid and lignin thus reducing the amount of coloured compound in treated POME. Production of citric acid, oxalic acid, gluconic acid and others might occurred during the fermentation of POME by fungus *Aspergillus niger*, resulting to an increase of acidity of the POME. High concentration of protons cause the adsorption rate to be increase as the repulsive forces between microbes with humic acid and lignin were reduced. In addition, the ionization of humic acid and lignin decrease and self aggregation took place. As a result, the microbes trap the humic acid and lignin easily.

Size of the cells also played an important role in the adsorption process. Smaller cells are generally better adapted to coloured compound, because their higher surface/volume ratio provides more surface area per volume for colour compound uptake at the cell surface.

2.3 Colour and Phenolic Compound Removal

As mentioned previously, the colour removal efficiency after the primary and secondary treatment was not satisfied. The coloured compound of POME was due to the presence of lignin, degraded product, tannin and humic acids (Neoh *et al.*, 2012). Discharge of the coloured compound will eventually lead to limitation of light penetration and reduce the photosynthesis activity of the water lives. Besides, Neoh *et al.* (2012) further explained that when the coloured compound reacted with the metal ions, the discharge wastewater will become toxic. Humic acids together with the chlorine in drinking water treatment were the sources for the formation of carcinogenic compound.

Augustin *et al.* (2008) proposed the method of electrocoagulation to remove the colour of the POME. Results showed that the dark brown colour, opaque POME successfully reduced to pale yellow solution after applying the electrocoagulation method. However, Neoh *et al.* (2012) pointed out that even the method was efficient in colour removal, electrode passivation which means the blockage of the electrode surface might occurred and lead to high maintenance costs. The betterment of colour removal efficiency is relying upon the advanced treatment of POME by microalgae. According to Daneshvar *et al.* (2007), the microalgae, *Cosmarium sp.* able to biodegrade and decolourize the dye solution containing Malachite Green (MG). The result was proved by the decreased of the MG absorbance peak. The removal efficiency of colour by live microalgae can reach 74%. Using aerobic granular sludge in a batch reactor for removing the coloured compound produce an efficiency of 38% which considered as low.

Phenolic compound that was found in POME will eventually cause phytotoxicity of POME. Zulkarnain *et al.* (2012) stated that the biological conversion of phenolic compound in POME can be related to the decrease of COD level and colour in POME. Microalgae is having the potential to reduce the phenolic content of POME.

Similar finding of Zulkarnain *et al.* (2012) showed that after 48 hours of fermentation period by using fungus *Aspergillus niger*, the total phenolic content can be reduced from initial value of 967.09 ± 5.03 GAE mg/L to 935.93 ± 4.65 GAE mg/L (GAE stands for mg of gallic acid equivalent per liter).

The study of Hirooka *et al.* (2003) screened the ability of various algae for their ability to deplete the concentration of hazardous phenol, 2, 4-dinitrophenol (DNP) under photoautotrophic conditions. At a concentration range of 5-40 µM of DNP, microalgae *Chlorella fusca* and *Anabaena variabilis* grew well and showed high DNP removal ability. Moreover, the microalgae's abilities to remove various phenols were studied. During the cultivation period of 5 days, more than 90% of 40 µM o- and m-nitrophenol and DNP was removed. Hirooka *et al.* (2003) also found that microalgae would be applicable to the removal of hazardous phenols without the addition of any organic carbon sources.

2.4 Removal of Nitrogen and Phosphorus Compound

POME contains inorganic compounds such as nitrate, ammonium and phosphate ions, which leads to eutrophication. Microalgae cultures act as an alternative biological treatment for POME due to its proficiency to use the inorganic nitrogen and phosphorus for their growth. Nitrogen accumulation brings harms to the environment. Particularly, nitrogen contributes to the formation of nitrite where nitrite was the precursors of N-nitroso compounds, mainly nitrosamine. They are the possible sources of carcinogenic, tetratogenic and mutagenic properties.

Microalgae able to fix nitrogen into ammonia (NH_3), nitrites (NO^{-2}) or nitrates (NO^{-3}) which can be absorbed and converted to protein and nucleic acids. According to Larsdotter (2006), organic phosphates tend to converted to orthophosphates by phosphatises at the surface of the cell especially when there was a shortage of inorganic phosphate. Besides, microalgae are able to assimilate the excess phosphorus and stored in the cell in the polyphosphate (volutin) granules.

Concentration of the microalgae species will affect the removal efficiency of organic compounds. In the study of Choi and Lee (2012), the higher the concentration of microalgae, *Chlorella vulgaris*, the better the removal efficiency of nitrogen and phosphorus. The highest removal efficiency of total nitrogen compound in the wastewater was 84.81% by using 6 g/L of *Chlorella vulgaris* after 8 days. Whereas for other runs using 1 g/L, 2 g/L and 4 g/L, the removal efficiency obtained was 81.04%, 83.42% and 84.32% respectively. Similar trend was observed in phosphorus removal. 6 g/L of *Chlorella vulgaris* was able to remove 36.12% of total phosphorus compound.

2.5 Environmental Variables Affecting POME treatment by Microalgae

In tertiary treatment of POME, the efficiency of BOD, COD, organic matters (nitrogen and phosphorus), colour and phenolic compound removal were the main concerns. Utilising microalgae in tertiary treatment of POME will be reliable only if the environmental factors that will affect the microalgae growth are considered. Microalgae growth and nutrient uptake are not only affected by the availability of nutrients, they also depend on complex interactions among physical factors such as pH, light intensity, temperature and biotic factors. The biotic factor significantly influencing algal growth is the initial density, it is expected that the higher the algal density, the better the growth and the higher the nutrient removal efficiency (Abdel-Raouf *et al.*, 2012).

2.5.1 Light Penetration

In terms of light penetration, as a photosynthetic organism, microalgae need light to grow and convert the food to energy. Thus, it is an important parameter. The climate in Malaysia needs to take in consideration. For instance, good microalgae growth rates have been reported under a light intensity of 4000 µmol/m^2.s (216,000 Lux). According to Kumar *et al.* (2010), the intensity was twice the solar flux at midday of summer in a medium latitude spot. Besides, the influence of the light cycles has been revealed as the dominant factor in photosynthetic activity and microalgae growth rate.

Eduardo *et al.* (2009) clarified that light was a limiting substrate in a photobioreactor, which were affected by light/dark zones. Previous study revealed that both sunlight and artificial light had been used via outer surface exposure as well as inner volume exposure, through the placement of lighting devices (e.g. LEDs or optical fibers) inside the reactor. Lighting design specifically to control the ratio of light and dark period can be achieved via artificial light, such as hybrid lighting systems. In addition, cell concentration is another limitation which determined the light availability in photobioreactor. Mutual shading often occurred at high cell densities. As a result, the cells are exposed to different light intensities, with a considerable effect on system performance.

According to Mata *et al.* (2012), approximately 3000-4100 Lux of fluorescent illumination needed for *C. vulgaris* in closed area whereas 20000-75000 Lux needed in open air cultures. At the same time, Mata *et al.* (2012) also showed that at the longest light exposure time (24 hours) and highest light intensity (12000 Lux or 162 µmol/m^2.s), 0.80 g/L of dry biomass of microalgae can be produced in 4 days. With the same light intensity, however, the production of 0.90 g/L biomass can be achieved in 9 days with 12 hours of light exposure. This proved that microalgae needs sufficient light exposure and light intensity to promote to maximum growth rate.

As observed from the study of Eduardo *et al.* (2009), the maximum cell productivity of unialgae cultures *Aphanothece microscopic Nageli* (5.100 ± 0.255 g/L) can be achieved in 0: 24 h night/day photoperiod. The cell productivity will decrease proportionally with the fraction of time that the microalgae was exposed to intermittent light conditions (photoperiod of 2:22 h to 10:14 h). An exception behaviour was observed in photoperiod of 12: 12h, the cell productivity slightly increases (0.301 ± 0.016 g/L) than photoperiod with 14 h and 16h. Acclimatisation of the cultures was determinant in the photosynthetic rates of the microalgae.

Moreover, Kitaya *et al.* (2005) had determined the optimum light intensity for the growth microalgae, *Euglena gracilis*. The specific growth rate of *Euglena gracilis* can reach a peak of 0.046 h^{-1} when photosynthetic photons flux of 100 µmol m^{-2}. s^{-1} (7400 Lux) was applied. After the microalgae replicates and form high amount of biomass, the high algal density would lead to self-shading, an accumulation of auto-inhibitors, and a reduction in photosynthetic efficiency (Abdel-Raouf *et al.*, 2012). Based on the study of Benjamas and Salwa (2012), at a light intensity of less than 8000 lux (108 µmol m^{-2}. s^{-1}), marine microalgae *Chlorella sp.* grew better and gave higher biomass.