TERTIARY TREATMENT OF PALM OIL MILL EFFLUENT (POME) BY THE COMBINATION OF MICROALGAE AND ACTIVATED SLUDGE USING SEQUENTIAL BATCH REACTOR

(RAWATAN TAHAP TINGGI BUANGAN KILANG MINYAK SAWIT MENGGUNAKAN GABUNGAN MIKROALGA DAN ENAPCEMAR DIAKTIFKAN DALAM REAKTOR BERJUJUKAN KELOMPOK)

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ABSTRACT

TERTIARY TREATMENT OF PALM OIL MILL EFFLUENT (POME) BY THE COMBINATION OF MICROALGAE AND ACTIVATED SLUDGE USING SEQUENTIAL BATCH REACTOR

(Keywords: Palm oil mill effluent, microalgae, activated sludge, sequential batch reactor, tertiary treatment)

Microalgae was found to have potential to reduce biochemical oxygen demand (BOD) level and colour of black-coloured wastewater like 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 project presents the effect of light penetration and kinetic study in tertiary treatment of Palm Oil Mill Effluent (POME) by utilizing microalgae. An alternative option of using activated sludge system was also examined. 10% v/v of microalgae was cultured in serially diluted (10 % to 100 % v/v), autoclaved and centrifuged POME. 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. In a separate study, the effect of initial pH of POME (4 -10), hydraulic retention time (12 – 72 h), organic loading rate (200 – 1000 mg/L), mixed liquor suspended solids (1000 – 10000 mg/L), solid retention time (2 – 20 days) and addition of molasses as external carbon source (10 – 100 mg/L) into the activated sludge treatment system using sequential batch reactor and microorganism from palm oil refinery were investigated. Initial pH at 6.5, hydraulic retention time of 48 h, solid retention time of 10 days, initial MLVSS value at 2000

mg/L and organic loading rate of 650 mg/L, together with the feeding of 60 mg/L of molasses at the beginning of treatment, enable a BOD reduction of up to 65%. The new discharge requirement, however, could not be met. In short, it is possible that the non-degradable or slow-degradable organic compounds existed in the POME hinder the complete treatment of the waste.

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ABSTRAK

RAWATAN TAHAP TINGGI BUANGAN KILANG MINYAK SAWIT MENGGUNAKAN GABUNGAN MIKROALGA DAN ENAPCEMAR DIAKTIFKAN DALAM REAKTOR BERJUJUKAN KELOMPOK

(*Kata kunci:* Buangan kilang minyak sawit, mikroalga, enapcemar diaktifkan, reactor berjujukan kelompok, rawatan tahap tinggi)

Mikroalga didapati berpotensi untuk merendahkan tahap permintaan oksigen biokimia (BOD) dan keamatan warna air sisa, POME yang berwarna hitam dalam industri minyak sawit. Faktor utama yang mempengaruhi keberkesanan mikroalga dalam rawatan POME adalah penembusan cahaya. Projek ini membentangkan kesan penembusan cahaya dan kajian kinetik dalam rawatan air sisa POME dengan menggunakan Mikroalga. 10 % v/v mikroalga ditumbuhkan dalam POME yang telah dicairkan secara bersiri (10 % - 100 % v/v), diautoklaf dan diempar. Persampelan dilakukan pada awal dan terakhir 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 campuran mikroalga dalam tempoh tujuh hari dengan selang 24 jam. BOD dan warna sampel terawal (sample POME yang telah diautoklaf dan diempar tanpa 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 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 tumbuh 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 hampir sama. Kecekapan pengurangan warna yang kurang daripada 20% membuktikan bahawa mikroalga tidak berkesan dalam mengurangkan sebatian berwarna. Dalam satu kajian yang berasingan, kesan pH awal POME (4-10), masa tahanan hidraulik (12-72 h), kadar muatan organik (200-1000 mg/L), MLVSS (1000-10000 mg/L), masa pengekalan pepejal (2-20 hari) dan penambahan molases sebagai sumber karbon luaran (10-100 mg/L) ke dalam sistem rawatan enapcemar diaktifkan menggunakan reaktor kelompok berurutan dan mikroorganisma dari kilang penapisan

minyak sawit telah disiasat. pH awal POME pada 6.5, masa tahanan hidraulik 48 h, masa pengekalan pepejal 10 hari, nilai awal MLVSS pada 2000 mg/L dan kadar muatan organik 650 mg/L, bersama-sama dengan 60 mg/L molasses yang dimasukkan pada permulaan rawatan, membolehkan pengurangan BOD sehingga 65%. Pendek kata, ketidakcapaian pelepasan baru mungkin disebabkan oleh sebatian organik bukan terurai atau perlahan-terurai wujud dalam POME yang menghalang rawatan lengkap sisa.

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LIST OF ABBREVIATIONS

BBM	Bold's basal medium
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
CPO	Crude palm oil
DNP	2,4-dinitrophenol
DO	Dissolved oxygen
DOE	Department of Environment
F/M	Food to microorganism ratio
FFB	Fresh fruit bunch
GAE	Gallic acid equivalent
HRT	Hydraulic retention time
MG	Malachite Green
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
MPOC	Malaysian Palm Oil Council
N/P	Nitrogen to phosphate ratio
OLR	Organic loading rate
POME	Palm oil mill effluent
SRT	Solid retention time
TSS	Total suspended solids
UASB	Upflow anaerobic sludge blanket

CHAPTER 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 created while producing per tonnes of CPO is approximately 3.5 m^3 (Zaini *et al.*, 2010). 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.* (2010) 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 coloured 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 favourable 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 indigenous 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 objectives of the study are:

1. To determine minimum light penetration of POME for microalgae growth.
2. To investigate the kinetic of microalgae growth for the tertiary treatment of POME waste.
3. To investigate the effect of important operating parameters of activated sludge treatment system for tertiary treatment of POME waste.

1.5 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 (6,000 Lux). The kinetic study was done based on the growth rate and the yield.

CHAPTER 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.

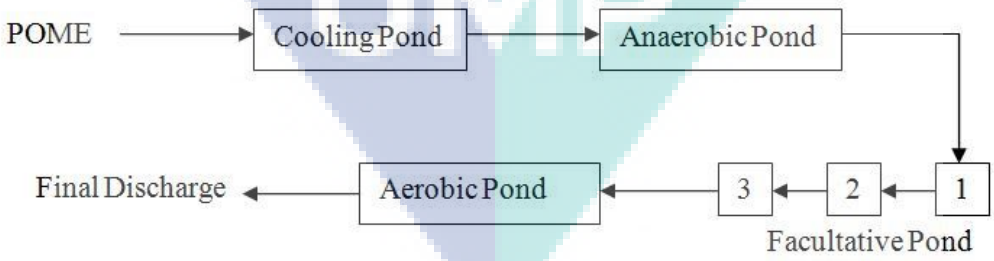

Figure 2.1: The ponding system (Wong, 1980).

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 color 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%.

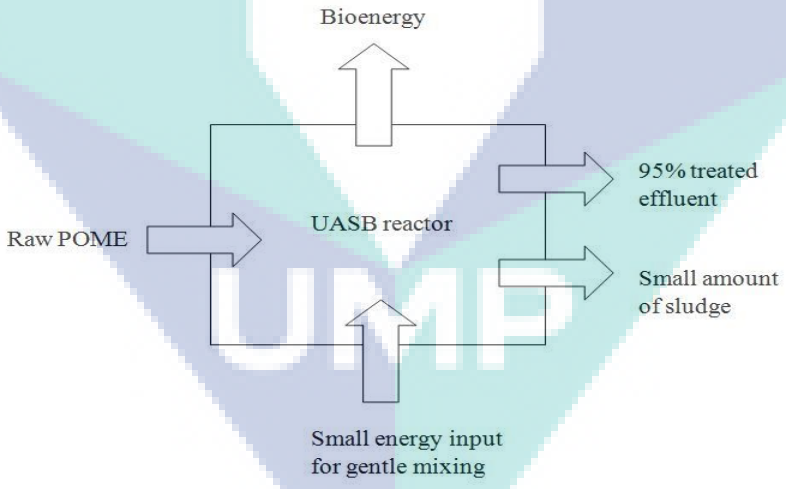

Figure 2.2: The anaerobic wastewater treatment process by using UASB reactor (Badroldin, 2010).

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.

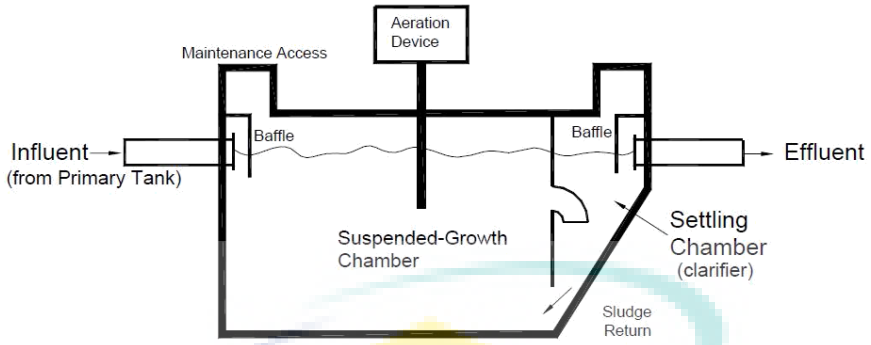

Figure 2.3: The suspended-growth aerobic treatment unit (John and Robert, 2004).

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 color 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:

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

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. 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 (3,700-14,800 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 color removal efficiency.

In the study, microalgae was grown in the POME. Some of the previous studies reveal that the biosorption of the color 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 color 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 colored 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 colored compound, because their higher surface/volume ratio provides more surface area per volume for color compound uptake at the cell surface.

2.3 Color and Phenolic Compound Removal

As mentioned previously, the color removal efficiency after the primary and secondary treatment was not satisfied. The colored compound of POME was due to the presence of lignin, degraded product, tannin and humic acids (Neoh *et al.*, 2012). Discharge of the colored 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 colored 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 color of the POME. Results showed that the dark brown color, 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 color removal, electrode passivation which means the blockage of the electrode surface might occurred and lead to high maintenance costs. The betterment of color 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 decolorize the dye solution containing Malachite Green (MG). The result was proved by the decreased of the MG absorbance peak. The removal efficiency of color by live microalgae can reach 74%. Using aerobic granular sludge in a batch reactor for removing the colored 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 color 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 40µM 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. 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), color and phenolic compound removal were the main concerns. Utilizing 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 into 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 3,000-4,100 Lux of fluorescent illumination needed for *C. vulgaris* in closed area whereas 20,000-75,000 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 (12,000 Lux or 162 $\mu mol/m^2.s$), 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 behavior was observed in photoperiod of 12:12 h, the cell productivity slightly increases (0.301 ± 0.016 g/L) than photoperiod with 14 h and 16h. Acclimatization 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 $\mu mol/m^2.s$ (7,400 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 8,000 Lux (108 $\mu mol/m^2.s$), marine microalgae *Chlorella sp*. grew better and gave higher biomass.

Eventually its growth declined when light intensity greater than 8,000 Lux. This might be regard as photo-inhibition of microalgae. Thus, in this research, the light intensity provided for a closed system was 6,000 Lux for a photoperiod of 12:12 hours due to prevention of photo-inhibition scenario and this degree of light irradiance approached the range of light intensity suggested by Mata *et al.* (2012) and Suzana *et al.* (2013).

2.5.2 pH

Several studies had shown that biosorption process using microalgae were highly pH dependent and is the most vital parameter to be considered. According to Kumar *et al.* (2010), most microalgae species are favored by neutral pH, whereas some species are tolerant to higher pH (e.g. *Spirulina platensis* at pH 9) or lower pH (e.g. *Chlorococcum littorale* at pH 4). There is a complex relationship between CO_2 concentration and pH in microalgal bioreactor systems, owing to the underlying chemical equilibrium among such species as CO_2, H_2CO_3, HCO_3^-, and CO_3^{2-}.

Increasing carbon dioxide concentration can lead to higher biomass productivity, but will also decrease pH, which can have an adverse effect upon microalgae physiology. By contrast, CO_2 uptake by microalgae has been shown to cause a rise in pH to 10-11 in open ponds. Although the pH increment can be beneficial for inactivation of pathogens in microalgae wastewater treatment, it can also inhibit microalgae growth.

Nitrogen absorption by microalgae will affect the pH values. The pH tends to increase due to assimilation of nitrate ions, but if ammonia was used as the nitrogen source, the pH values might drop to 3.0. Besides, high pH value can lead to precipitation of phosphate by formation of calcium phosphate but may re-dissolve during night time due to the decrement of pH (Larsdotter, 2006).

Daneshvar *et al.* (2007) showed that the dye removal percentage of dye solution containing Malachite Green by microalgae *Cosmarium sp*. was 92.4% when pH value was 9. The isoelectric point for algae species would be at a pH of 3.0. At a lower pH which was less than pH 3, the H^+ cations compete with the dye cations which lead to reduction of the biosorption system's efficiency. At a higher pH value which greater

than pH 3, the surface of biomass was negatively charged and the electrostatic force of attraction between biomass and dye cations will be improved. In addition to that, Daneshvar *et al.* (2007) also showed that the decolorization rate experience tremendous increment when the pH increase from 4.0 to 6.0. The color removal efficiency was rose from 26.3% to 68.4% for a pH increment from 3.0 to 4.0.

2.5.3 Temperature

According to Daneshvar *et al.* (2007), temperature effect on the decolorization rate was significant. The dye removal efficiency was determined from 5°C to 45°C. The decolorization rate was increased with the increment of the temperature. Further increase in temperature might lead to rapid decline of microalgae growth rate. Larsdotter (2006) stated that in humid climates where the evaporation process was inhibited, overheating of the algae culture will be a serious issue. As mentioned, microalgae easily get photoinhibited by high light intensity. Dauta *et al.* (1990) pointed out that at optimum temperature, microalgae can better tolerate to higher light intensity. Generally, temperature in the range of 15°C to 25°C is suitable for most algal species.

2.5.4 Nutrient Availability

Microalgae was autotroph where they can synthesize organic molecules from inorganic nutrients. According to Larsdotter (2006), the rate at which a microalgae cell absorbs specific nutrients depend on the difference between the concentration inside and outside of the cell as well as the diffusion rates through the cell wall.

Microalgae species grown under nutrient replete conditions exhibited higher growth rates and shorter doubling time. The biomass for microalgae *Dunaliella tertiolecta* was doubled in approximately 21.6 hours (Roleda *et al.*, 2013). Low to negative growth rate would be observed under nutrient-depleted conditions. Emma and Rosalam (2012) clarified that under nutrient-depleted condition, the intracellular chlorophyll and chloroplast numbers were decreased where a big amount of phospholipid and glycolipid lie within the chloroplast.

The nutrients in urban wastewater could be almost 100% removed by microalgae consortium at any N/P ratio according to Samori *et al.* (2013). The Stumm empirical formula for microalgae is $C_{106}H_{263}O_{110}N_{16}P$ (N/P ratio of 16:1). A potential limitation of any assimilative nutrient removal process would be probably caused by an imbalance of N/P in the wastewater compared to the N/P ratio in the cell tissues.

2.5.5 Carbon dioxide supply

Larsdotter (2006) mentioned that aeration can provide the atmospheric carbon dioxide to the microalgae culture. In contrast, since the ambient atmospheric concentration (0.033%) is much lower than the optimum to support the microalgae growth, extra carbon dioxide supply was necessary. This can be accomplished by providing the cultures an ideal amount of 1 to 5% of carbon dioxide. Emma and Rosalam (2012) proposed that microalgae specific growth rate of up to 0.66 per day under the aeration of 2–15% carbon dioxide.

The study of Emma and Rosalam (2012) also revealed the effect of the carbon dioxide concentration on the microalgae biomass. The biomass productivity was 0.376 g/L/d when microalgae, *Chlorella sp.* was cultivated under 6–8% carbon dioxide concentration. In the presence of 9–10% carbon dioxide concentrations, lower biomass yield at 0.15 g/L/d was resulted. In addition, carbon dioxide concentration is key parameter to increase the lipid content, lipid composition and biomass yield. Under an aeration rate of 1 L/min with a 5% CO_2 supplementation and illumination of 25 µmol/m^2.s at the surface of the reactor, a production of 63% lipid content of *C. emersonii* was observed.

The amount of carbon dioxide in the treatment greatly affects the efficiency since it varied the pH values. For instance, addition of carbon dioxide will upshot the decrement of pH. At a higher pH value (pH greater than 9), the inorganic carbon will be in the form of carbonate ion (CO_3^{2-}) which was impossible to be assimilated by microalgae.

CHAPTER 3
METHODOLOGY

3.1 Materials

3.1.1 Bold's Basal Medium

The materials in Table 3.1 are needed for preparation of Bold's Basal Medium (BBM).

Table 3.1: The Chemicals Needed For Preparation Of Different Stock Solution (Richard, 2007).

Stock Solution No.	Chemical Name	Formula	Weight (g)	Distilled Water (mL)
1	di-potassium hydrogen orthophosphate	K_2HPO_4	1.875	250
	Potassium di-hydrogen orthophosphate	KH_2PO_4	4.375	
	Magnesium sulphate	$MgSO_4.7H_2O$	1.875	
	Sodium Nitrate	$NaNO_3$	6.250	
	Calcium chloride	$CaCl_2.2H_2O$	0.625	
	Sodium chloride	NaCl	0.625	
2	EDTA tetrasodium salt	$EDTA\text{-}Na_4$	5.000	100
	Potassium hydroxide	KOH	3.100	
	Ferrous sulphate	$FeSO_4.7H_2O$	0.498	
	Sulphuric acid conc. (wt per mL= 1.84g)	H_2SO_4	0.184	
	Boric acid	H_3BO_3	1.142	
3	Zinc sulphate	$ZnSO_4.7H_2O$	0.353	25
	Manganese chloride	$MnCl_2.4H_2O$	0.058	
	Cupric sulphate	$CuSO_4.5H_2O$	0.063	
	Cobaltous nitrate	$Co(NO_3)_2.6H_2O$	0.020	
	Sodium molybdate	$Na_2MoO_4.2H_2O$	0.048	

3.1.2 POME collection

The POME sample was collected from Facultative Pond 3 in Sri Senggora Palm Oil Mill which located in Sri Jaya, Pahang. After the collection of POME, it was stored in the refrigerator at 4°C. Before using, the POME was autoclaved at 121°C and centrifuged at 10,000 rpm (Eppendorf 5810R, Germany) for 15 minutes to eliminate indigenous microbes and suspended solid material.

3.2 Preparation of Culture Medium (Bold's Basal Medium) for Microalgae

In making Bold's Basal Medium (BBM), several stock solutions need to be prepared according to Table 3.1. To prepare 1 L of BBM, 10 mL of stock solution No.1, 1 mL of stock solution No.2 and 0.1 mL of stock solution No.3 were added to volumetric flask and top up to 1 L. The BBM medium solution had been prepared using ultra-pure water with resistivity of 18 MΩ/cm at 25°C and autoclaved for 20 min at 121°C (Hirayama HVP-50, USA). All the cultivation was performed under sterile condition in Biohazard Safety Hood (Herasafe™ KS (NSF) Class II, Type A2, USA) which was sterilized with UV Light for 30 minutes and cleaned with 70% ethanol before use. Bunsen burner had been used to sterilize all the non-sterile metal instruments. Mix culture of microalgae were stored on nutrient agar in petri dish. It was then transferred to the seed culture medium by punching out 5 mm of the agar plate culture with a sterilized cutter. The seed culture was grown in 250 mL conical flask containing 100 mL of Bold's Basal Medium at 25°C on a rotary shaker incubator (PROTECH 722, USA).

3.3 Determination of the Effect of Light Penetration

A microalgae cell density of 1 x 10^5 cells/ml was cultured in 0 to 100 % v/v POME and ultrapure water in 250 ml conical flask. In order to prepare microalgae mix culture with 1 x 10^5 cells/ml cell density, firstly the average cell number for original microalgae culture was determined. The cell number obtained can be substituted in subscript M_1 in the Equation (3.1). While the final cell number, M_2 should be 1 x 10^5 cells/ ml and the total volume, V_2 was 250 ml.

$$M_1V_1 = M_2V_2 \qquad \text{Eq. (3.1)}$$

The volume of mix culture of microalgae, V_1 that was needed to achieve cell concentration of 1 x 10^5 cells/ml can be determined by using equation above. For 10% v/v POME sample, 25 ml of POME was needed while the rest of volume made up of microalgae mix culture and ultrapure water. The ratio changes according to the concentration of POME (10% to 100%). For each samples, light penetration analysis was conducted using experiment rig for light penetration illustrated in Figure 3.1 to determine the light irradiance degree after transmitted through the microalgae culture chamber at the beginning of experiment.

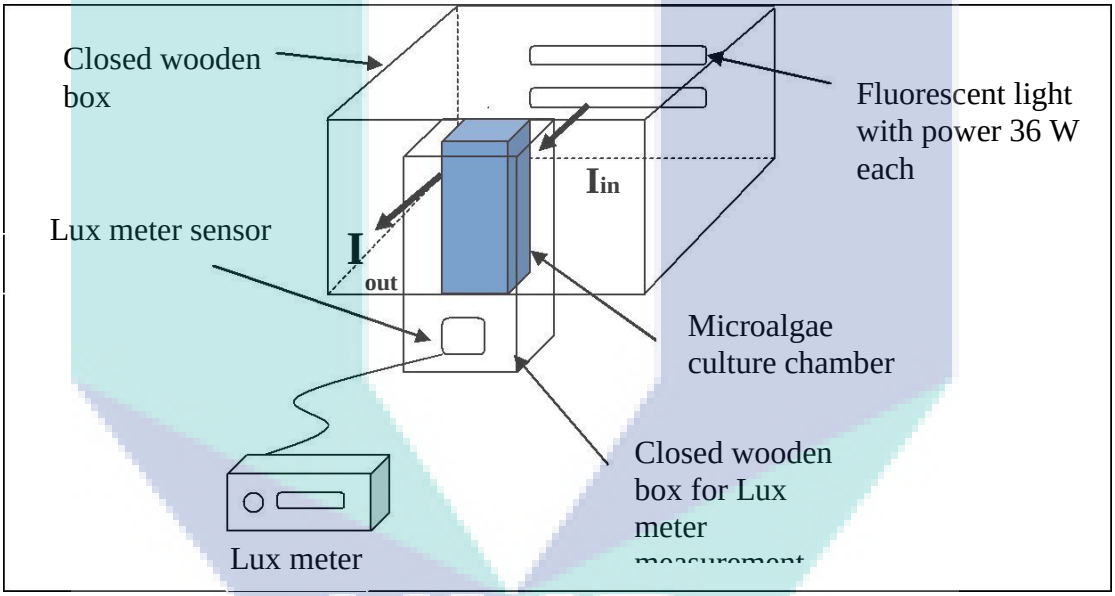

Figure 3.1: Experiment Rig for Light Penetration

Then, the microalgae cultures were grown in conical flask with silicone stopper, sparging with Air Pump Zone Z-80 in the wooden incubator box with fluorescent lighting source with light intensity at approximately 81 µmol^{-2}sec^{-1} (6,000 Lux), and photoperiod 12:12 hour light/dark cycle. The microalgae growth was observed for 7 days. Sampling was done at the beginning and the end of 7 days.

3.4 Kinetic Study on Microalgae Growth Rate

Kinetic study was carried out on the sample with the highest growth rate in the previous

section to observe growth profile of microalgae mix culture within seven days at time interval of 24 hours. For every 24 hours, 1 ml sample was used for the determination of cell density using Neubauer Improved Haemocytometer. The cell suspension was loaded on filling notch of haemocytometer and the haemocytometer was placed on the inverted microscope stage (Carl Zeiss Axiovert 40, Germany) before cell counting was started. At seventh day, 35 mL of sample was centrifuged at 10,000 rpm, 25°C for 15 minutes. The cell pellet was dried at 100°C for two days to obtain cell dry weight while the supernatant were used to carry out BOD_5 and colour analysis. Figure 3.2 summarizes the procedure of the experiment.

Microalgae Culture in POME

- 1 ml of sample for cell count by haemocytometer every 24 hours
- 35 ml of sample was centrifuged by using Centrifuge at the end of seventh day (Temperature: 25^{o}C, Speed: 10000 rpm, Time: 15 min)

Cell pellet was dried at 100˚C to obtain cell dry weight

Supernatant Analysis for:
- BOD_5
- Colour

Figure 3.2: The flow chart for microalgae growth rate kinetic study.

3.5 Analytical Method

3.5.1 BOD_5 Analysis

Analysis of BOD_5 for supernatant was done according to Standard Method, Section

5210B (APHA, 1992). 1 L of dilution water had been prepared by adding 1 mL of phosphate buffer, magnesium sulfate, calcium chloride, ferric chloride solution into 1 L volumetric flask and top up with deionised water to total volume 1 L. 10 mL of supernatant sample was added into 500 ml beaker and diluted with distilled water up to 300 ml. The pH was adjusted within 6.5 to 7.5 by adding acid/alkali. At the same time, 300 ml dilution water as mentioned above was prepared as control in another 500 ml beaker. Then, all prepared sample and control were filled into 300 ml incubation bottle separately. Dissolved Oxygen (DO) concentration, D_1, for each sample was measured and recorded using Dissolved Oxygen meter. After that, the flared mouth of bottle had been covered by aluminium foil and put into BOD incubator for five days at temperature of 20°C. After five days, final DO, D_2, of all samples were measured and BOD_5 value was calculated according to the formula below and the analysis was carried out in duplicates:

$$BOD_5 = (D_1 - D_2)/P \qquad \text{Eq. (3.2)}$$

Where;

D_1 = DO value in initial sample

D_2 = DO value in final sample

P = Decimal volumetric fraction of sample used = 0.3

3.5.2 Colour Analysis

For the colour analysis, ADMI weighted ordinate method was applied. In the method, the sample of POME need to be filtered by using 0.45 µm membrane filter until the sample is not turbid and pH adjusted to 7.6. The sample cell was filled with the pH adjusted sample while the blank was filled with deionized water. Then, the colour of the blank and pH adjusted sample were measured by using HACH DR2010 spectrophotometer at 725 nm.

3.6 Kinetic Model

The Monod kinetic model was used to describe the effect of light penetration on the microalgae growth rate. For light limited growth kinetic model, the maximum microalgae growth rate (μ_{max}) was expressed as below:

$$\mu = \frac{\mu_{max} L}{K_L + L} \quad \text{Eq. (3.3)}$$

where L is the light intensity that passed through the microalgae medium and K_L is the saturation constant for light intensity (Hermanto, 2009). For POME limited growth model, the light intensity was replaced by POME concentration.

The calculation of specific growth rate (μ) (Hadiyanto and Nur, 2012) is shown as below where x_0 and x_t are the initial and final cell density measured at the period where microalgae cell experienced exponential phase by using Neubauer Improved Haemocytometer:

$$\mu = \frac{ln\, x_t - ln\, x_0}{t - t_0} \quad \text{Eq. (3.4)}$$

The biomass yield based on POME and light intensity (quantum yield) can be calculated using Eq. (3.5) and (3.6) (Zijffers *et al.*, 2010).

$$Biomass\ Yield = \frac{Biomass\ Concentration\ (\frac{g}{L})}{POME\ Concentration\ (\frac{g}{L})} \quad \text{Eq. (3.5)}$$

$$Quantum\ Yield = \frac{C_x \times \mu \times V}{PFD_{in} \times A \times 3600 \times 10^{-6}\ (\frac{gmol}{photons})} \quad \text{Eq. (3.6)}$$

where C_X is the biomass concentration, V is the volume of culture, PFD_{in} is the photon fLux density at inlet and A represent the area of culture. The upper term also represented the volumetric biomass productivity.

The BOD and colour removal efficiency were calculated by using the initial (C_0) and final concentration (C_f) of dissolved oxygen and pigment in POME as shown in Equation (3.7).

$$Efficiency = \frac{C_0 - C_f}{C_0} \times 100\% \quad \text{Eq. (3.7)}$$

3.7 Effect of operating parameters on the performance of activated sludge process

3.7.1 Effect of pH

10 liter of POME sample had been taken from Neram Palm Oil Mill, Kemaman. pH, BOD_5, COD and TSS of POME sample were measured. 13 sets of 2 liter conical flask completed with aeration system by using SOBO aquarium air pump with air flow output of 4 L/min and pressure of 2 MPa. 5.5 liter of acclimated activated sludge was obtained from acclimatization activated sludge reactor, and added into 2 liter conical flask for 0.5 liter each. Volume of POME and acclimated activated sludge were placed into conical flask at F/M ratio = 0.3 kg BOD/kg MLVSS.day. Eq. (3.8) below was used to calculate the volume required and tabulated in Table 3.2.

Table 3.2: Volume of POME and activated sludge samples required at F/M ratio of 0.3 kg BOD/kg MLVSS.day

POME Sample		**Activated Sludge Sample**	
BOD_5	650 mg/L	MLVSS	2,000 mg/L
Volume of POME	0.46 L	Volume of Activated Sludge	0.50L

$$F/M\ Ratio = \frac{BOD\ of\ POME,mg/L\ X\ Volume\ of\ POME,L}{MLVSS\ of\ Activated\ Sludge,mg/L\ X\ Volume\ of\ Activated\ Sudge,L} \quad \text{Eq. (3.8)}$$

Initial pH in conical flask had been adjusted to 4.0 ± 0.1 to 10.0 ± 0.1 with interval of pH 0.5 ± 0.1 by using hydrochloric acid or sodium hydroxide (0.1 M). Aeration had been carried out for 24 hours. Then, 300 ml of mixed liquor sample for each conical flask were taken for Dissolved Oxygen (DO) test. Once DO test were completed, 100 ml of mixed liquor sample were taken for MLSS and MLVSS test; while the balance mixed liquor sample were transferred into 250 ml conical flask for 1 hour settlement. Then, the supernatant was measured for its pH, BOD, COD and TSS values, while the activated sludge were collected for MLSS and MLVSS analysis.

3.7.2 Effect of Hydraulic Retention Time (HRT)

The set up was similar as in Section 3.7.1, except that the initial pH in conical flask had been adjusted to 6.5 ± 0.1. A total of 6 sets apparatus were set up, aeration had been carried out for 12 hours to 72 hours with the interval of 12 hours for HRT ranging from

12 to 72 hours. Samples were withdrawn for each HRT and analyzed as per Section 3.7.2.

3.7.3 Effect of Organic Loading Rate (OLR)

In this study, 9 sets of 0.5 L acclimated activated sludge with MLVSS concentration 2,000 mg/L was used. Palm oil mill effluent sample with BOD level from 200 mg/L to 1,000 mg/L were prepared by using distilled water to dilute the POME sample at an interval of 100 mg/L. Volume of POME was adjusted so that together with acclimated activated sludge maintaining F/M ratio of 0.3 kg BOD/kg MLVSS.day. Initial pH had been adjusted to 6.5 ± 0.1 and the aeration was carried out for 48 hours. Similar analyses were done for the samples in this study.

3.7.4 Effect Initial Mixed Liquor Volatile Suspended Solids (MLVSS)

10 sets of acclimated activated sludge with MLVSS concentration 1,000 mg/L to 10,000 mg/L at an interval of 1,000 mg/L were prepared from settled activated sludge and added into 2 liter conical flask at F/M Ratio = 0.3 kg BOD/kg MLVSS.day. POME sample with BOD concentration 650 mg/L were added. Initial pH was adjusted to 6.5 ± 0.1 and the aeration was carried out for 48 hours. Samples were withdrawn and analyzed as described in the previous section.

3.7.5 Effect of Solid Retention Time (SRT)

10 sets of acclimated activated sludge with MLVSS concentration 2,000 mg/L were prepared, followed by POME sample with BOD concentration 650 mg/L where the volume of POME sample were calculated based on F/M ratio of 0.3 kg BOD/kg MLVSS.day. Initial pH was adjusted to 6.5 ± 0.1 and aeration was carried out for SRT from 2 days to 20 days at an interval of 2 days. Similar analyses were carried as described previously.

3.7.6 Effect of Molasses concentration as external carbon source

In this study, 11 sets of acclimated activated sludge with MLVSS concentration 2,000 mg/L were added into POME sample with BOD concentration 650 mg/L where the

volume of POME sample were calculated based on the F/M ratio of 0.3 kg BOD/kg MLVSS.day. Initial pH was set at 6.5 ± 0.1 with 48 hours aeration. During the start-up of aeration process, molasses with concentration of 10 ± 0.5 mg/L to 100 ± 0.5 mg/L were added into the respective flask as external carbon source. Sampling was done at the end of the experiment and analyses were done as per description in the previous section.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Determination of the Effect of Light Penetration

Light irradiance, an important determinant of microalgae's life, was the main focus in current work. To carry out the experiment, POME samples with different concentration (0-100 % v/v) was prepared and used for culturing the microalgae. It should be noted that the seeding density used was 1 x 10^5 cells/ml. As observed in Figure 4.1, the average light penetration value was decreasing with the increasing of POME concentration. The R^2 value obtained was 0.987, representing the data fit the parabola curve well. However, slight deviation of light penetration value was observed at POME concentration of 90% and 100%. Absence of chlorophyll functioning in light spectrum absorption caused the deviation. The approximate error calculated were 8.25% and 4.63% respectively. The deviation was less than 10% and was in the acceptable range.

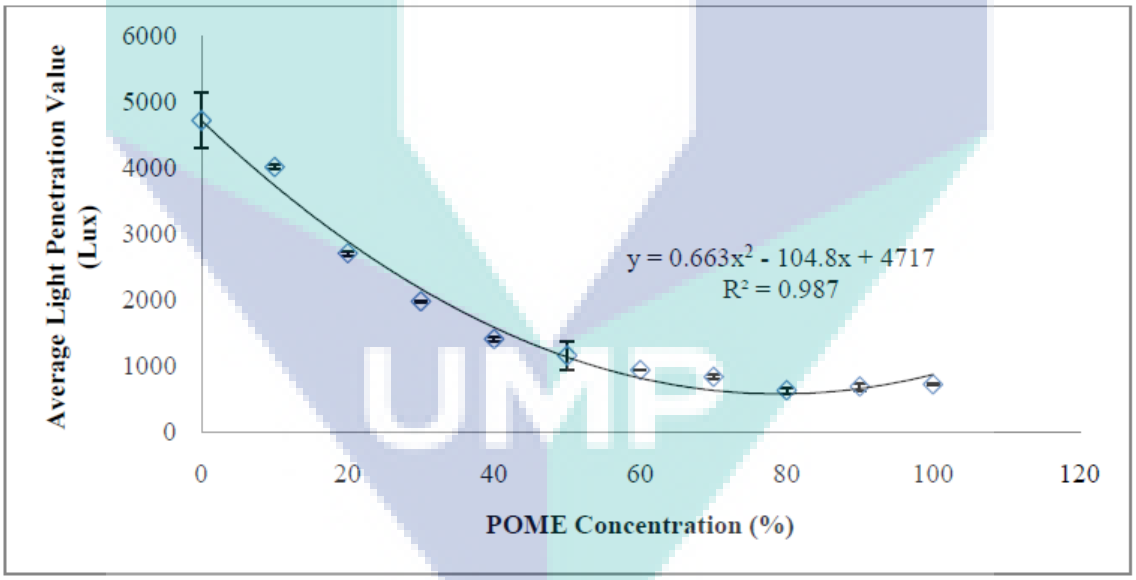

Figure 4.1: The graph of average light penetration value for each dilution of POME.

The result obtained was in agreement with the theory where penetrated light value would be decreasing as the concentration of POME was increasing. As stated by Neoh *et al.* (2012), lignin, degraded product, tannin and humic acids were the main elements contributed to the colourisation of POME. The colours in the POME were able to block the light radiance, hence affecting the light penetration. Nonetheless, when POME was

diluted, the colour intensity would also reduce which then allowing greater amount of light penetrated.

The trend of the cell amount differences were presented in Figure 4.2. As mentioned, the initial cell density was a constant variable throughout the experiment and the number should be approximately 1 x 10^5 cells/ml after the dilution step. The final microalgae cell density attained rise from 0 to 20% of POME concentration and achieved maximum density at 30 % v/v POME concentration. The cell density dropped thereafter. As the POME concentration was increased, there was a difficulty to differentiate the POME particles and microalgae cell. Consequently, the microalgae cell was hardly defined under high magnification resolution microscope. Thus, the cell number for 70 % v/v - 90 % v/v POME concentration was uncountable.

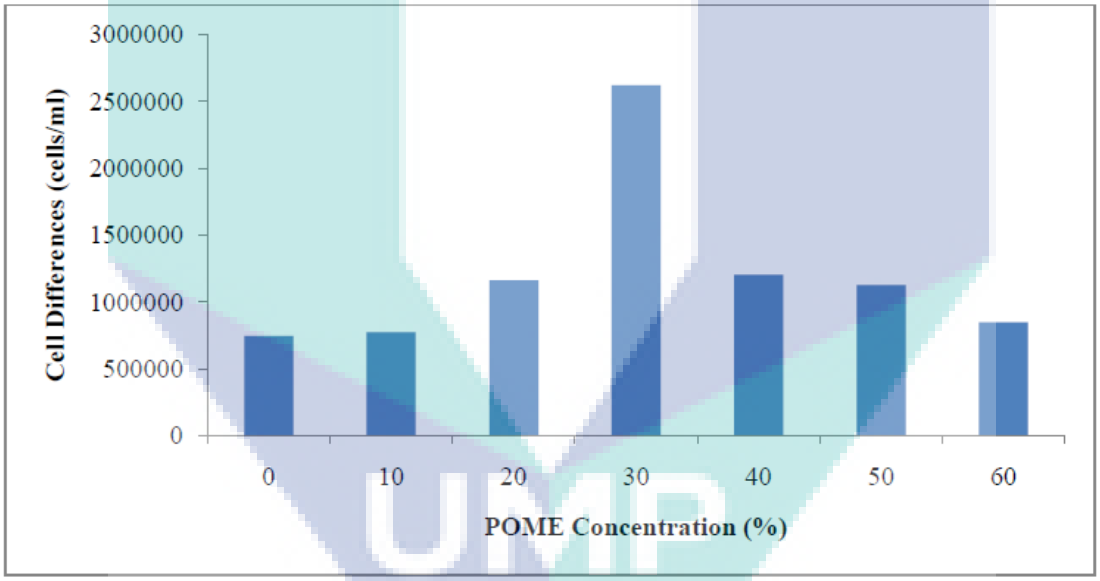

Figure 4.2: The amount of cell differences for different POME concentration.

The selection of initial cell density was based on the research of Daneshvar *et al.* (2007) where the *Cosmarium sp.* algal concentrations used in the batch decolourization experiments were in the range of 0 - 7.5 x 10^6 cells/ml. In their study, Daneshvar *et al.* (2007) reported that *Cosmarium sp.* of 4.5 x 10^6 cells/ml gave the highest efficiency on colour removal of Malachite Green, a synthetic colour dye, compare to other cell concentrations of 0, 1.5, 3, 6, and 7.5 x 10^6 cells/ ml. The sample of dye used was 100 ml. In current work, the minimum volume of POME used was 25 ml (10 %v/v). The

comparison of ratio can act as a reference for determination of seeding density in this experiment.

The final cell density counted also showed that the mix culture of microalgae was grown at a high growth rate and for instance, the final cell density for 30% v/v of POME concentration was approximately 25 times to its initial cell density. Besides, a considerable amount of microalgae biomass was produced only after five days of cultivation. It will become a significant problem to measure the final cell density if a high initial cell density was applied. Thus, an initial low cell density of 1 x 10^5 cells/ml was chosen. Nonetheless, the autoclaved and centrifuged POME (treated POME) was used instead of raw POME in order to facilitate the counting of microalgae cells. The treated POME allowed higher penetration of light which then encouraged the growth of the mix culture of microalgae.

Considering the light penetration alone was insufficient to determine its effect to microalgae's growth rate in different POME concentration, Hermanto (2009) proposed that the light intensity and dilution were the main input controller where other factors such as nutrient, temperature and pH were remained in the optimal condition. Fernandez *et al.* (1997) studied on the scenario of light attenuation by microalgae mass culture in a tubular photo-bioreactor by considering the Beer-Lambert's Law. The light attenuation determined how fast the light intensity decrease along a fix distance. The basic Beer-Lambert's equation without considering other factors was shown below:

$$A_t = \ln \frac{I_0}{I} \qquad \text{Eq. (4.1)}$$

where A_t is the light attenuation inside the experimental rig while I_o and I were the irradiation measured at the inlet and outlet of the experimental rig.

The incident irradiation (I_O) was 6,000 Lux and (I) is the measured irradiation for different POME concentration. Next, the calculated light attenuation values based on Equation 4.1 was illustrated in Figure 4.3. As observed, the light attenuation was increase gradually until it reached POME concentration of 60% and slowly level off at 100% of POME concentration. The R^2 value (0.988) shows that the data fitted well on a parabolic curve. However, the point evaluated for 80 % v/v of POME concentration

was slightly deviate from the curve.

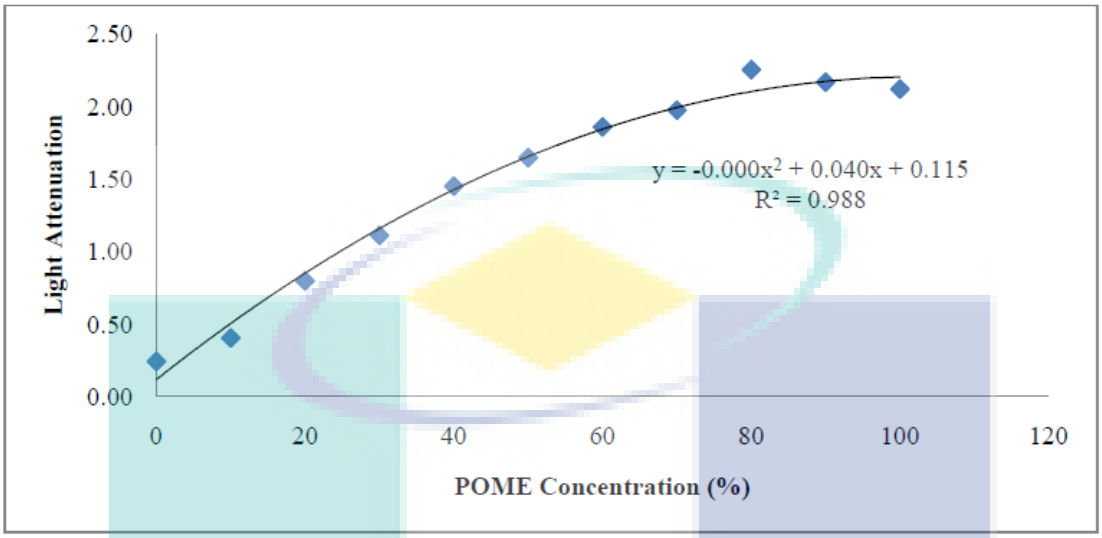

Figure 4.3: The light attenuation for each POME Concentration based on basic Beer-Lambert Law.

The results showed that Beer-Lambert's Law fit the curve linearly when the concentration of POME was low. At high POME concentration, the curve had a hyperbolic tendency. Fernandez *et al.* (1997) mentioned that there were limitations for the Beer-Lambert's Law application and the most remarkable assumption was the condition is based on homogeneous absorption where the scattering effect was not considered.

Most mathematical model developed was mainly aimed to estimate the light attenuation due to biomass concentration. Molina *et al.* (1994) emphasized the importance of determining the interaction between light radiation and biomass while applying the engineering approach. In this approach, the light attenuation has been considered as a function of geometry system as well as biochemical composition of the biomass. However, in the experiment, the concentration of substrate (POME) was the one that being manipulated and the cell density was maintained constant. As mentioned previously, POME particles and pigmentation highly affect the light penetration. The radiation attenuation phenomenon might be related to the light absorption by POME particles. Hence, the mathematical model was applied by substituting the concentration

of POME to estimate the light intensity profile.

There was another approach to estimate the light intensity inside the experimental apparatus (photo-bioreactor) as proposed by Hermanto (2009). In order to find the average light intensity, the extended Beer- Lambert's Law (Eq. 4.2) which considered the path length of the rig and POME concentration was applied.

$$I_{avg} = I_{in} \cdot \left(\frac{1}{b}\right)\left(1 - e^{-a_c \cdot C_{POME} \cdot b}\right) \cdot \frac{1}{C_{POME} \cdot a_c} \quad \text{Eq. (4.2)}$$

where I_{in} indicate the incident irradiation (6,000 Lux), a_c was the averaged spectral absorption coefficient (200 m^2/kg as cited by Barbosa *et al.*, 2003), C_{POME} was the concentration of POME whilst b was the experimental rig light path (0.26 m).

The profile of light intensity based on the extended Beer-Lambert's Law and the comparison with cell differences was plotted in Figure 4.4. The trend of light intensity decreased gradually and showing an asymptotic phenomenon as the POME concentration was increased. Besides, from Equation 4.2, it can be figured out the relationship of POME concentration and average light intensity where the average light intensity was inversely proportional to concentration of POME. In the experiment, however, the light scattering effect cannot be ignored. Again from the extended Beer-Lambert's Law, the concentration of POME affected the average light intensity in the experimental rig. The particles in POME might contribute to the light absorption, yet, it might cause light scattering phenomenon. Though the initial cell density was maintained constant, the microalgae tend to self-shade themselves. All the factors lead to the declination of the average light intensity generated through extended Beer-Lambert's Law.

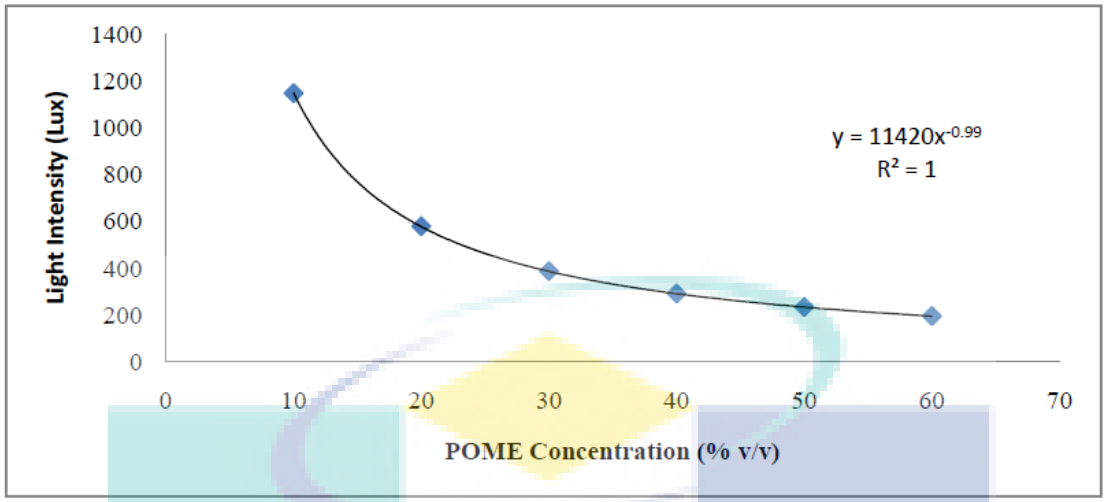

Figure 4.4: The profile of light intensity based on extended Beer Lambert Law.

For 0 to 30% v/v POME concentration, the microalgae growth pattern was not proportional to the amount of substrate (light intensity) provided. The plausible explanation was that the particles content for 0 to 30 %v/v POME concentration was low and the effect of increment on the amount of particles can be ignored. Thus, the growth rate of microalgae was increased along with the increment of POME concentration. However, when the microalgae was cultivated in wastewater, shading effect due to high particulate matter might occurred and this will prevent microalgae which were not floating on the surface from getting the light that serve as energy source.

The volumetric productivity and the efficiency of light utilization was important in characterize the microalgae cultivation system. The volume productivity was the product of specific growth rate and biomass density. The increment of light utilization will increase the volumetric productivity. Hence, light energy was the limiting substrate for growth and light falling on the cultivation system should be distributed evenly.

4.2 Kinetic Study of Microalgae Growth Rate

From the previous section, it was found that microalgae mixture experience optimum growth at 30% v/v of POME concentration. Next, the growth rate of microalgae mix culture was monitored in the medium of 0%v/v (control) to 30%v/v of POME concentration. Figure 4.5 showed the growth pattern of mix culture of microalgae in the cultivation of 0% v/v (control) to 30% v/v of POME concentration. The lag phase

of microalgae was approximately one day and followed by the exponential (growth) phase. The microalgae cultivated with POME lower than 30% v/v experienced a longer exponential phase (three days). Eventually, the cells entered the stationary phase after four or five days.

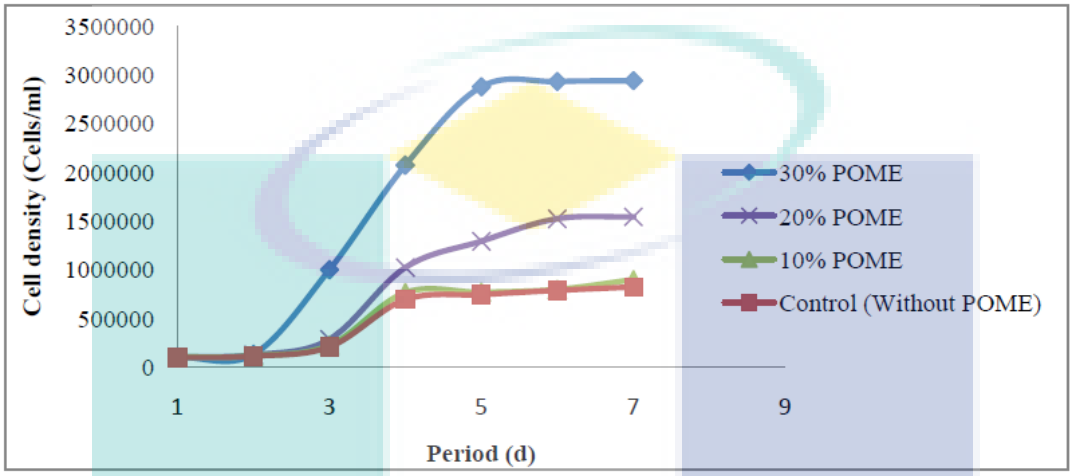

Figure 4.5: The cell density for control and 10 v/v% - 30 v/v% POME concentration within 7 days.

The growing trend of the microalgae was similar to the study of Hadiyanto and Nur (2012). Their study showed that the specific growth rate of *Chlorella sp*. was the highest by utilizing 70% POME concentration compared to 20% and 50%. The study of Hadiyanto and Nur (2012) presented the growth pattern of *Chlorella sp.* The cells entered the stationary phase after day 12, and then reach the death phase at day 15. Besides, Toyub *et al.* (2007) revealed the growth profile of green algae, *C. ellipsoidea*, in different concentrations of fertilizer factory effluent media (FFEM). The initial cell density of *C. ellipsoidea* using was 2.5×10^5 cells/ml which then attained a maximum density of 198.49 $x10^5$ cells/ml in Bold's Basal Medium (BBM) followed by 182.07, 157.41, 142.34, 137.57 and 121.35 ($x10^5$cells/ml) in 50, 55, 45, 60 and 40 % of FFEM respectively on the 10th day of culture. The microalgae cells in this study took only four to five days to reach stationary phase. Under the preliminary observation, it was predicted that the growth rate of the microalgae cells in this study was in a rapid speed. The abnormal speed of the microalgae growth might be due to the mix culture of the microalgae in current cultivation system created a competitive environment. The cells compete in order to absorb the substrate which was the POME as an energy source. This in turns cause a rapid speed of microalgae's growth.

In this experiment, the light intensity provided in the rig was 6,000 Lux. The utilization of artificial light might be provided in excess amounts which pushed the cells to the death phase. Dauta *et al.* (1990) determined the growth rate of four freshwater microalgae (*Chlorella vulgaris, Fragilaria crotonensis, Staurastrum pingue* and *Synechocystis minima)* over a wide range of light intensities (370 Lux to 59,200 Lux) and temperatures (10°C - 35°C). Under room temperature (21°C), the optimum growth rate of freshwater microalgae can be achieved when the light intensity was approximately 4,440 Lux. It revealed that high light intensity (800 $\mu E/m^2s$ or 59,200 Lux) not necessary served as the best condition for algae to grow and in contrary, it might lead to photoinhibition. If the light illumination was maintained relatively high, the condition stressed the cells and pushed the cells into death phase.

Their study also showed that the higher the cultivation temperature, the lesser the amount light intensity was needed to achieve optimum growth rate. When the temperature was in the range of 25°C - 35°C, the optimum growth rate of microalgae can be achieved with the light intensity of 740-3,700 Lux. The heat generated by the light intensity cannot be ignored and it will contribute to the rise of surrounding temperature. Rise of temperature supported the growth of mix culture of microalgae. Hence, the cells reached death phase in a short period of cultivation.

Table 4.1 tabulated the specific growth rate, biomass yield and productivity of the cells in different POME concentration. As shown in the table, the microalgae in the POME concentration of 30% v/v had the highest specific growth rate (1.39 d^{-1}), biomass yield (0.50 g cells/g substrate and 0.68 g/mol photons) and biomass productivity (0.61 g/L.d) compared to the others. The specific growth rate, biomass yield and biomass productivity increase followed by the increment of substrate (POME) concentration. It should be noted that the biomass productivity of the microalgae in the control is slightly higher than the 10% v/v cultivation. This might due to the weight of biomass in the control at the seventh day was slightly higher than the biomass' weight in 10% v/v POME.

Table 4.1: The Specific Growth Rate and Biomass Yield of Microalgae in each POME concentration.

POME Concentration (% v/v)	Specific Growth Rate (d^{-1})	Biomass Yield (g cells/g POME)	Biomass Yield (g/mol photons)	Biomass Productivity (g/L.d)
0 (control)	1.21	-*	0.59	0.10
10	1.24	0.22	0.61	0.08
20	1.29	0.46	0.63	0.34
30	1.39	0.50	0.68	0.61

***** The biomass yield is based on POME substrate, thus there is no value for control.

The specific growth rate again reflected that the mix culture of microalgae experienced high growth rate. A comparison was done by referring to the study of Hadiyanto and Nur (2012) and Toyub *et al.* (2007). It showed that the specific growth rate of *Chlorella sp.* and *C. ellipsoidea* for their studies was in the range of 0.40-0.60 d^{-1} which was lower than the specific growth rate of mix culture of microalgae in this study.

In terms of biomass yield based on light energy, the values are increasing as the light intensity was decreasing from 0 to 30% v/v POME as calculated by using extended-Beer Lambert Law. According to Zijffers *et al.* (2010), by supplying high photon flux or light rate, a relatively constant yield between 0.6 and 0.8 g/mol photons was obtained. Typically, they applied the range of 5- 48 µE/m^2s or 370-3,552 Lux for their study of maximum photosynthetic yield of green microalgae in photobioreactors. Thus, even a higher flux, i.e. 6,000 Lux was applied in this experiment, the biomass yield based on light energy (0.59-0.68 g/mol photons) is corresponding with the study of Zijffers *et al.* (2010). Besides, Zijffers *et al.* (2010) also stated that different biomass concentration will lead to different light gradient in photobioreactor, thus creating different light exposure patterns to microalgae but it did not affect the biomass yield. This explained even the light intensity decreased along the increment of POME concentration, the biomass yield still able to increase.

A comparison of biomass productivity was done using different microalgae strains as shown in Table 4.2. Recent work by Luisa and Oliveira (2008), Pruvost *et al.* (2009)

and Zhang *et al.* (2001) proved that microalgae that cultured in an open pond gave lower biomass productivity (an average of 0.13 g/L.d) compared to those cultured in photobioreactor (PBR). As observed, the biomass productivity of microalgae in this study for samples of 20% v/v and 30% v/v are similar with the biomass productivity of *Neochloris oleabundans* and *Nannochloropsis sp.* cultured in PBR. This proved that light condition is a limiting factor for the growth of microalgae. Differences in biomass productivity of *Neochloris oleabundans* and *Nannochloropsis sp.* present when aeration (CO_2 injection) supplied to the cultivation system. Similar condition was provided to the microalgae in this experiment. Thus the biomass productivity obtained slightly higher compare to *Nannochloropsis sp*. Even though the light intensity in 10% v/v was higher than 20% v/v and 30% v/v, the contribution of POME to sustain the microalgae growth cannot be neglected. Hence, the biomass productivity showing an increment trend from 10 to 30% v/v.

Table 4.2: The Productivity of Different Microalgae Strains.

Microalgae Strain	**Productivity (g/L.d)**	**Cultivation System**	**Growth Condition**
Spirulina maxima	0.21	Open pond	Grown initially in airlift bioreactors and then in polyethylene bags with bubbling air under lighting conditions (150 µE/m^2s or 11,100 Lux) at optimal temperature indoor, and finally in outdoor raceway agitated by paddle wheel ponds.
Chlorella vulgaris	0.18	Open pond	
Scenedesmus obliquus	0.09	Open pond	
Dunaliella tertiolecta	0.12	Open pond	
Nannochloropsis sp.	0.09	Open pond	
Neochloris oleabundans	0.09	Open pond	
Neochloris oleabundans (Continuous production without mineral limitation)	0.55	PBR	Culture at pH 7.5 and 25°C under 270 µE/m^2s or 19,980 Lux bubbled with air 0.5 L/min. The pH was maintained by injecting CO_2.
Nannochloropsis sp.	0.21	PBR	Culture at pH 7-8 and 27°C provided with1.5% CO_2.

In order to find the kinetic coefficients of Monod model, linear curve was plotted as

shown in Figure 4.6 and 4.7. The linear curve was plotted based on the specific growth rate and substrate concentration, in this case, light intensity based on extended Beer-Lambert Law and POME concentration. The plot based on light intensity (Figure 4.6) demonstrated a good relationship (R^2 = 0.929) for the specific growth rate and light intensity whereas the plotting based on the POME concentration illustrated an acceptable relationship (R^2 = 0.804) for the specific growth rate and POME concentration. By referring to Figure 4.6, the growth of the microalgae mix culture was satisfied though the amount of substrate (light intensity) was decreasing. As long as sufficient amount of light illumination is provided, the microalgae can utilised this light energy and enhance the growth. The minimum light intensity in a closed system must exceed a minimum value of 385 Lux as calculated for 30% v/v of POME concentration.

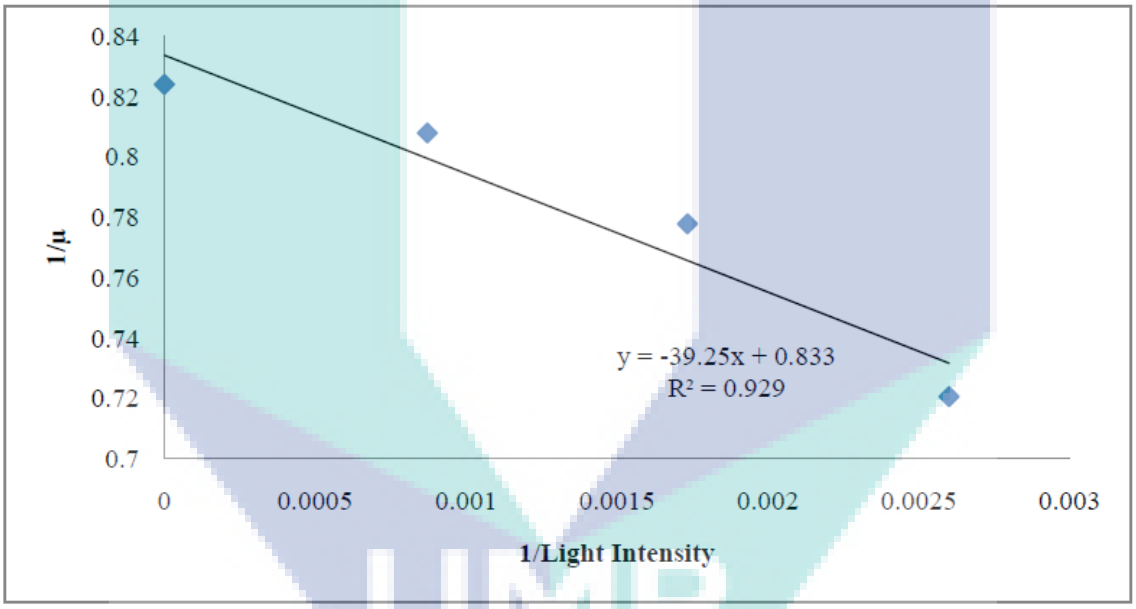

Figure 4.6: The Monod Model based on light intensity.

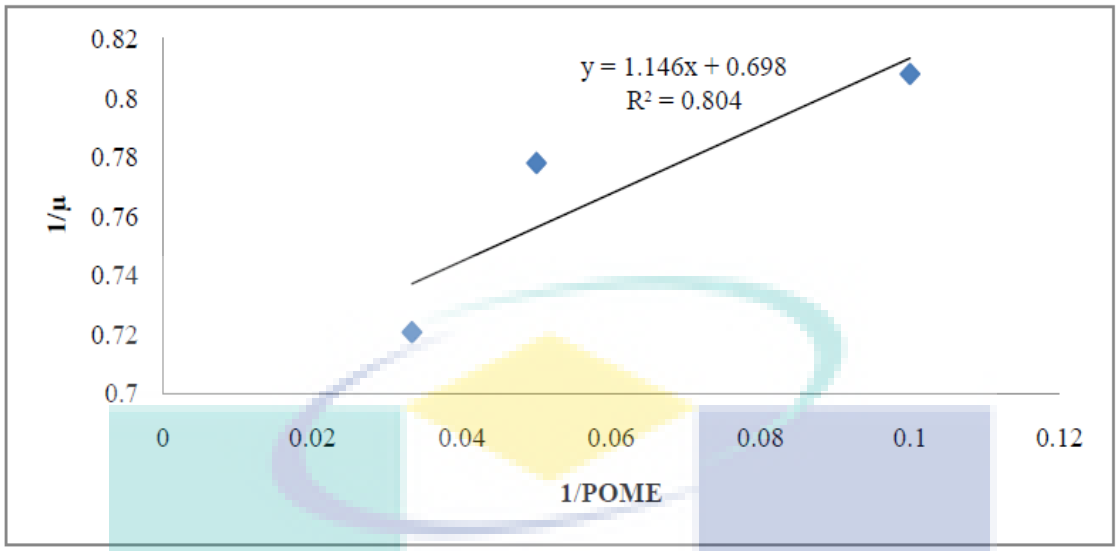

Figure 4.7: The Monod Model based on POME concentration.

Figure 4.7 which presented the relationship of specific growth rate and POME concentration showed a positive trend. As the amount of POME increased, the specific growth rate of microalgae mix culture was also increased. All of the kinetic coefficients that were calculated from Figure 4.6 and 4.7 were tabulated in Table 4.3.

Table 4.3: Result of the Application of Light Intensity and POME as Substrate Utilisation Models.

Substrate	**Equation**	**μ_{max}**	**K_S**	**R^2**
Light Intensity from Extended Beer-Lambert	y = 39.25x + 0.833	1.20	47.1	0.929
POME	y = 1.146x + 0.698	1.43	1.64	0.804

POME was proven to be an alternative source of nutrients to promote the microalgae's growth. While carried out the experiment, only the two main variables were considered. However, microalgae growth can be reduced from maximum by suboptimal temperature, pH and other nutrient conditions. As justified by Dauta *et al.* (1990), the success of a model related to the accuracy of the basic conditions and at such, the determinant factors in plankton physiology and ecology needed to be refined to produce better cell yield.

Generally, the Monod model built able to determine the feasibility of the model and the number of process that can be performed in a year. The growth rate cannot be increased beyond the maximum growth rate (μ_{max}) due to inherent metabolic reaction rate limitations. While the significance of Monod constant (K_s) is to justify the half of the maximum growth rate when the substrate concentration is equal to Monod constant.

In the real environment, although sufficient illumination and ideal photoperiod can be provided in order to promote the microalgae growth, there were several limitations. Cultivation of microalgae in the aerobic zone of the facultative pond is possible and microalgae tends to utilise the content of POME such as nitrogen and phosphate as energy sources. The main limitation to the growing of microalgae culture is the depth of aerobic zones. The depth of the pond plays an important role in light penetration. As discussed in previous section, the optimum growth occurred at 30% v/v of POME concentration with the light illumination supplied in all directions. In a smaller scale, the growth of microalgae in an undiluted sample is proven to be slow and the colourisation of POME blocked the light irradiance which then caused the shading effect. Obstacles are created when the light penetrated through the POME promotes the growth of surface microalgae only. The addition of surface microalgae cells further block the light penetration and cause the POME in the aerobic zones cannot be treated fully. This will later cause an elevation of BOD level. In addition, the POME in industry can neither be heated to eliminate the growth of heterotrophic organisms such as bacteria nor diluted to provide optimum condition for microalgae growth.

4.3 BOD and Colour Removal Efficiency

The BOD_5 and colour intensity of the diluted POME after seven days were given in Table 4.4. The values are compared with the value of BOD_5 and colour intensity of the autoclaved, centrifuged and undiluted POME. For the removal efficiency, the data is tabulated as shown in Figure 4.8.

Table 4.4: The BOD_5 and Colour Intensity Values for Undiluted POME and Samples at t = 7 days.

POME Concentration	BOD_5 (mg/L)	Colour Intensity (ADMI)
Treated POME without dilution	39.5	395
10	17.2	392
20	19.4	350
30	18.6	322

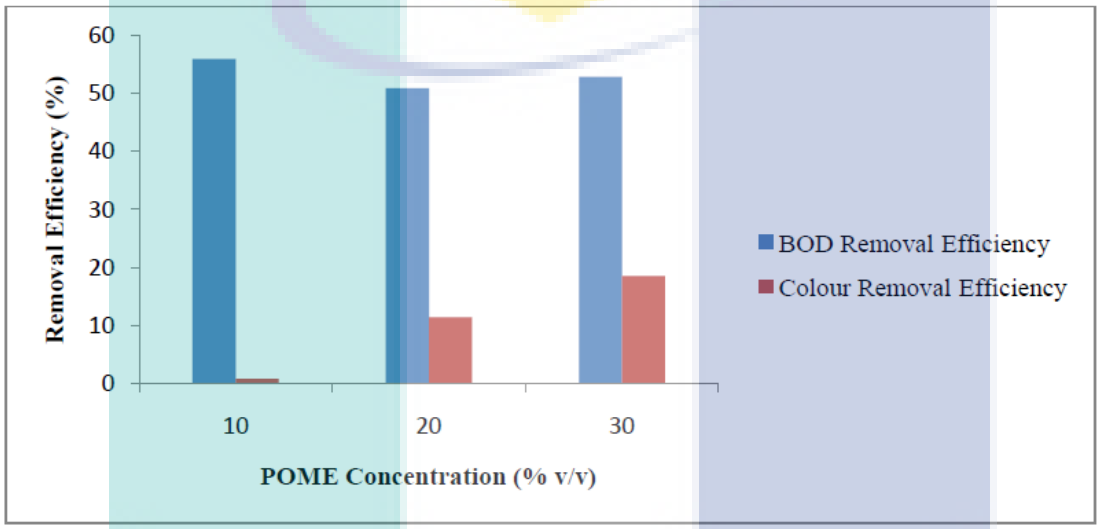

Figure 4.8: The removal efficiency of the BOD and colour.

By definition, biochemical oxygen demand or BOD was the amount of dissolved oxygen needed by aerobic biological organisms in a water system to break down organic material present at certain temperature over a specific time period. In the experiment, the BOD_5 of the POME and the removal efficiency of the BOD by utilizing microalgae mix culture were determined. As for 30% v/v of POME sample, the BOD_5 of the sample after seven days of cultivation was 18.6 mg/L and the removal efficiency was 53%.

Although the value fulfil the requirement of the Department of Environment which set the minimum discharge BOD value of 20 ppm or 20 mg/L, the removal efficiency was considered as low if compared to the study of Choi and Lee (2012) which applied the *Chlorella Vulgaris* on the removal of BOD in wastewater. An average of 81% of removal efficiency of BOD can be achieved in the study of Choi and Lee (2012).

Surprisingly, the BOD removal efficiency in the 30% v/v of POME sample was slightly lower than those in 10% v/v of POME sample which gave a BOD removal efficiency of 56%. The result showed that even though 30% v/v of POME sample equipped with higher microalgae growth rate, the removal efficiency still remained the same. The ratio of POME concentration and the respective growth profile of microalgae led to the similar removal efficiency of BOD. Choi and Lee (2012) also mentioned that there was no significant change in the BOD removal efficiency with the increment of microalgae dose. High BOD removal efficiency can be achieved by using low dose of microalgae.

As for initial reading of BOD_5 value of POME (39.5 mg/L), the value was tremendously lower than the influent reading proposed by several studies with an approximate value of 25,000 mg/L. The POME used in the experiment was collected in a facultative pond and treated by autoclaved and centrifugation before applied. A series of pre-treatment of POME was done before the POME channelled to the facultative pond. In the pre-treatment stage, the suspended solid and some portion of organic content were removed and successfully lowered the BOD value. Vijayaraghavan *et al.* (2007) clarified that the removal efficiency of BOD at pre-treatment stage can reached 71%. Assuming that in the influent of facultative pond, 71% of BOD successfully removed, in this case, the initial reading of our samples should be approximately 7,250 mg/L.

After the POME was treated by autoclaved, the dissolved oxygen level was brought down drastically. In general, the bacteria presented in the sample were killed and the demand for oxygen was depleted. It then leads to a low dissolved oxygen profile and further depreciates the BOD level of the sample.

Nitrates and phosphates in a body of water can contribute to high BOD levels. Nitrates and phosphates were essential nutrients and can cause microalgae to grow quickly. Under this circumstance, the BOD level can be decreased with the successive growth of microalgae. When microalgae growth rate was high, they also entered death phase rapidly. The phenomenon was proven by the kinetic studies as shown in the previous section. This contributed to the organic waste in the water, which is then decomposed by bacteria. Probably, a high BOD level will be resulted.

In term of decolourization of the POME, as an estimation of the decolourization potential of microalgae, a comparison of colour intensity was done. Figure 4.9 shows the initial colour intensity of treated POME whereas Figure 4.10 depicts the colour intensity of the each samples after seven days. As observed, the colour intensity was slightly changed. It should be emphasized that the dilution of POME was done and the colour intensity would be lower than the initial colour intensity as shown.

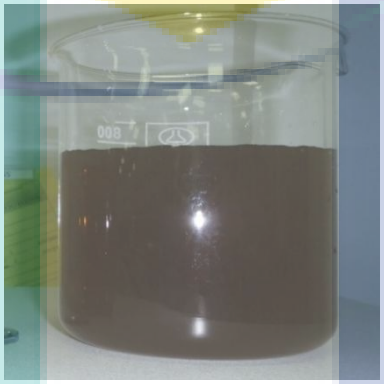

Figure 4.9: Treated POME.

Figure 4.10: The centrifuged sample after seven days for various %v/v POME.

As shown in Figure 4.8, the POME sample with 30% v/v gave the highest colour removal efficiency (18.5%) compared to others at the seventh day. The low decolourisation yield indicates that growing microalgae in POME does not give a significant result in uptake of coloured compound in POME.

To the best of the knowledge, there is no study related to the colour removal in treated

POME by using microalgae. The studies reported in the literature was the optimization of decolourisation of POME by growing fungus cultures, *Aspergillus fumigatus* (Neoh *et al.*, 2012) and the removal of phenolic compound in facultative POME sample using lactic acid bacteria (Limkhuansuwan and Chaiprasert, 2010). Neoh *et al.* (2012) mentioned that the total phenolic compounds and lignin were directly proportional to colour. Limkhuansuwan and Chaiprasert (2010) found that by using lactic acid bacteria (*L. plantarum* SF5.6), the phenol removal of POME was 34% while the decolourisation yield was 15.88%. The colour removal efficiency by lactic acid bacteria was similar with the microalgae in this study.

Several factors will depreciate the colour removal efficiency of microalgae. Firstly, in term of surface area, smaller cells are generally better adapted to coloured compound as their higher surface/volume ratio provides more surface area per volume for coloured compound uptake at the cell surface. Under comparison of size, diameter of fungus cell was in the range of 2 – 10 µm (Neoh *et al.*, 2012) whereas the diameter of microalgae cell can be few micrometers until hundreds micrometers (Lam and Lee, 2011). In other words, the size of microalgae might bigger compare to fungus cell. The lower surface/volume ratio lead to low decolourisation yield.

In term of morphology, the fungus pellet had a highly porous mycelium matrix and the appearance of hyphae provided a high surface area for coloured compound uptake (Neoh *et al.*, 2012). Figure 4.11 shows the microalgae structure under microscope with the magnification factor of 20 times. In general, microalgae cell is a simple structure. The size and the structure of the microalgae will directly affect the availability of surface area for coloured compound absorption hence induced low colour removal efficiency. Besides, the growth rate of the microalgae also determines the colour removal efficiency. The available surface area for decolourisation will increase with the rapid growth speed of microalgae.

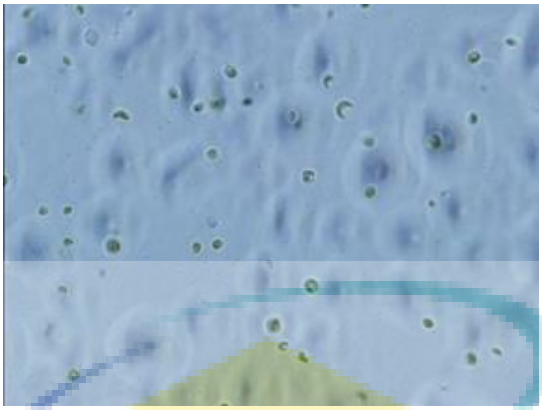

Figure 4.11: The microscopic image of mix culture of microalgae.

Next, the other factor that will influence the colour removal efficiency is the properties of the coloured compound. Daneshvar *et al.* (2007) showed that the dye (Malachite Green) removal efficiency was approximately 70% with the initial cell density of 3 x 10^6 cells/ml. The dye was a synthetic origin with complex aromatic molecular structures. The uptake of the dye molecules by the microalgae, *Cosmarium sp*. was eased by the present of weak Van der Waals forces between the dye molecules. For the phenolic compound which contributed to the colourisation of POME, the uptake of the compound curbed by the complex bonding appeared in phenolic compound. Phenolic compounds binds to POME through a variety of mechanisms in aqueous media, including hydrogen bonding, covalent bonding, hydrophobic interactions and ionic bonding (Xu and Diosady, 2001). The mechanisms create the barrier of phenolic compound uptake by microalgae as microalgae unable to break the bonding between POME and phenolic compound. As a result, the decolourisation yield was not satisfied.

In the literature, the pH factor cannot be excluded. Neoh *et al.* (2012) stated that higher decolourisation will be yielded at low pH as the high concentration of H^+ ions will lead to occurrence of adsorption as the repulsive forces between the cells and lignin were reduced. Daneshvar *et al.* (2007) studied the effect of pH of microalgae cultivation on the dye removal. The decolourisation rate reached a peak of 92.4% at pH 9.0. This was contradicted with the statement of Neoh *at al.* (2012). This could be explained by the isoelectric point of microalgae which is at pH 3.0 according to Daneshvar *et al.* (2007). Under the cultivation pH lesser than 3.0, the repulsive forces will be created between H^+ ions and phenolic cations. At a higher pH value which was greater than pH 3, the surface of biomass was negatively charged and the electrostatic force of attraction

between biomass and phenolic cations will be improved. This was supported by the study of Xu and Diosady (2001). They suggested that the removal of phenolic compounds by diafiltering the alkaline extract prior to isoelectric precipitation was effective and about half of the extractable phenolic acids and condensed tannins were actually unbound at extraction pH (pH 12). In the experiment, the neutral pH (7 ± 0.5) was applied but the pH was expected to be dropped to acidic range (less than pH 4.0) over the end of the experiment due to the secretion of glycolic acid into the culture medium under aerobic condition (Larsdotter, 2006). The initial pH will cause lesser yield on decolourisation. The decrement of the pH day by day added the difficulty of the decolourisation.

4.4 Effect of Operating Parameters on the Activated Sludge System

Since the BOD reduction and colour removal efficiency by using microalgae treatment system as the tertiary treatment of POME could not meet the standard set by the DOE, investigation has moved on to using activated sludge system in a sequential batch reactor as the other alternatives. As the operating parameters of the activated sludge system would greatly influence the results of treatment, a few important parameters had been examined and reported in this section.

4.4.1 Initial pH of POME

First operating parameter investigated in this study is initial pH of POME. The pH of POME seems to always increase gradually to alkaline region, around 8.0 to 9.0. This was reported as due to the high absorption or precipitation of available phosphate in the soil during biodegradation process of POME. However, this pH range is not the optimal environmental pH for the healthy grow of microorganism. Thus, a success treatment of POME required it to have a suitable pH environment for microorganism growth and a final pH that comply with the discharge standard limit set by the Malaysian Department of the Environment at pH 5.5 – 9.0. In this study, HRT was set at 48 hours, SRT was 7 days, MLVSS in the system was 1,500 mg/L with an initial BOD value of 650 mg/L and the pH was varied from 4.0 to 10.0.

Figure 4.12(a) shows that when the initial pH of POME is in the acidic region (pH 4.0 – 6.0), the pH attained after treatment is within pH 6.0 to 7.0. Above these initial pH

range, there was a drastic increment to the final pH achieved. When the initial pH of POME is above pH 6.5, pH after treatment would be above pH 8 and increased with the increment of initial pH. Hence, the ideal initial pH of POME seems to be pH 4 to 6.5. Conventionally, it is common to have the initial pH of POME at the acidic region (pH 4.0 – 6.0) in anaerobic system, and the available phosphorus content will be removed in anoxic condition (Yamashita & Ikemoto, 2014). Thus, there is no significant increment of POME pH at the end of the treatment. However, due to the incomplete cycle of phosphorus removal, the excess phosphorus has been carried over to facultative or aerobic system, where these phosphorus will be absorbed and precipitated in the soil during biodegradation process of POME; thus, gradually increase the POME pH along the facultative and aerobic system. (Huan, 1987; Okwute & Isu, 2007; Eze *et al.*, 2013).

Figure 4.12(b) also depicts the specific oxygen uptake rate (SOUR) of the microorganism in the POME. Generally, the SOUR achieved by the system was lower than the recommended value provided in the guidance document of the Malaysian Department of Environment. The recommended value of SOUR is between 8 - 20 mg O_2/h/g MLVSS (Technical Guidance Document DOE-IETS-1, 2010), but at all initial pH of POME, the SOUR value attained was less than 5 mg O_2/h/g MLVSS. The highest SOUR value obtained is at the initial pH of 6.5 (4.2 mg O_2/h/g MLVSS) where Ohimain et al. (2013) also reported a similar result. The microbial population in their study had shown the highest grow rate and SOUR value at pH 6.59 ± 0.05. This indicated that the bacteria able to grow better in the POME at the pH of 6.5.

As optimum organic removal of waste water, a good treatment system should attain a MLVSS:MLSS ratio above 80 (Harun & Annuar, 2014). Figure 4.12(c) illustrates that the only system barely achieved this ratio was the one with initial pH of 7.0. This result disclosed that the conditions of POME are not ideal for the sustaining growth of the microorganism in which the reason behind required further investigation. Figure 4.12(d) and (e) show the percentage reduction of BOD and COD respectively. Maximum BOD reduction was around 62% at initial pH of 7.5. Thus, the final BOD reading after treatment was at a value of ~ 247 mg/L, which is not even met the old regulation set (< 200 mg/L). Maximum COD reduction was at a value of 68% which occurred at initial pH of 7.5. Therefore, the ideal initial pH of POME is around pH 6.5 to 7.5. pH 6.5 was

chosen as the optimal for the following study because above this value, the pH of POME after treatment will exceed the maximum value allowed by the standard.

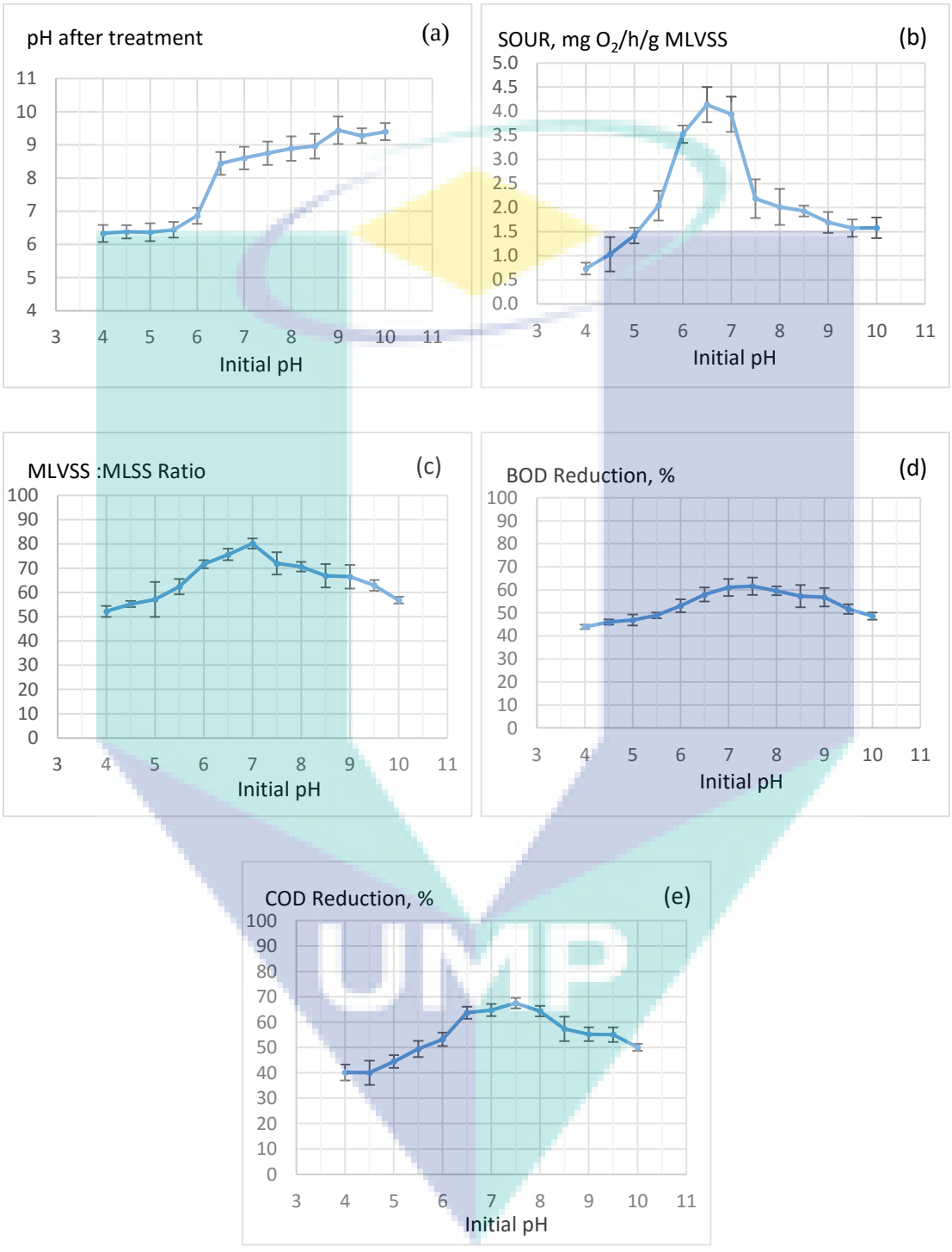

Figure 4.12: Effect of initial pH of POME on the (a) pH after treatment, (b) specific oxygen uptake rate, (c) MLVSS:MLSS ratio, (d) BOD reduction, (e) COD reduction. Operating conditions: HRT = 24 hours, SRT = 7 days, MLVSS = 2,000 mg/L, BOD initial = 650 mg/L.

4.4.2 Hydraulic retention time (HRT)

Hydraulic retention time is a key parameter to determine the period for the treatment of POME up to the required standard. Too short a period may not enough to enable a complete treatment of the waste, while too long a period is not practical for a high capacity incoming waste like POME. This is mainly due to a big land area would be required for the construction of the plant, which is a significant extra cost to the newly set up plant and a limitation to the existing plant. In this study, the initial pH of the POME was adjusted to 6.5, SRT was 7 days, MLVSS of the system was at a value of 2,000 mg/L with the initial BOD of 650 mg/L and the HRT was varied from 12 h to 144 h.

Figure 4.13(a) shows that when the HRT was increased from 12 h to 24 h, the pH of POME after treatment increase from 7.3 to 8.3. Above HRT of 24 h, the pH value after treatment increased insignificantly and the value is always less than pH 9.

Figure 4.13(b) illustrates the SOUR of the system at various HRT. It can be seen that the SOUR value was still below the healthy level of 8 mg O_2/h/g MLVSS irrespective of the HRT period. The maximum SOUR occurred between 48 to 60 h HRT at a value of ~ 4.6 mg O_2/h/g MLVSS. Above this HRT, SOUR was constant at a value of 4 mg O_2/h/g MLVSS.

Similarly, Figure 4.13(c) depicts the ratio of MLVSS:MLSS increased when the HRT was increased and attained a maximum value around 80 at HRT between 48 to 60 h. Further increased in the HRT causes a decreased in the MLVSS:MLSS ratio. This may be due to the depletion of nutrients when times prolong that causing the death of some of the microorganism.

As shown in the Figure 4.13(d) and (e), the maximum reduction of BOD (64%) and COD (62%) occurred at HRT of 48 h. Thus, the optimal HRT chosen for the following study is 48 h.

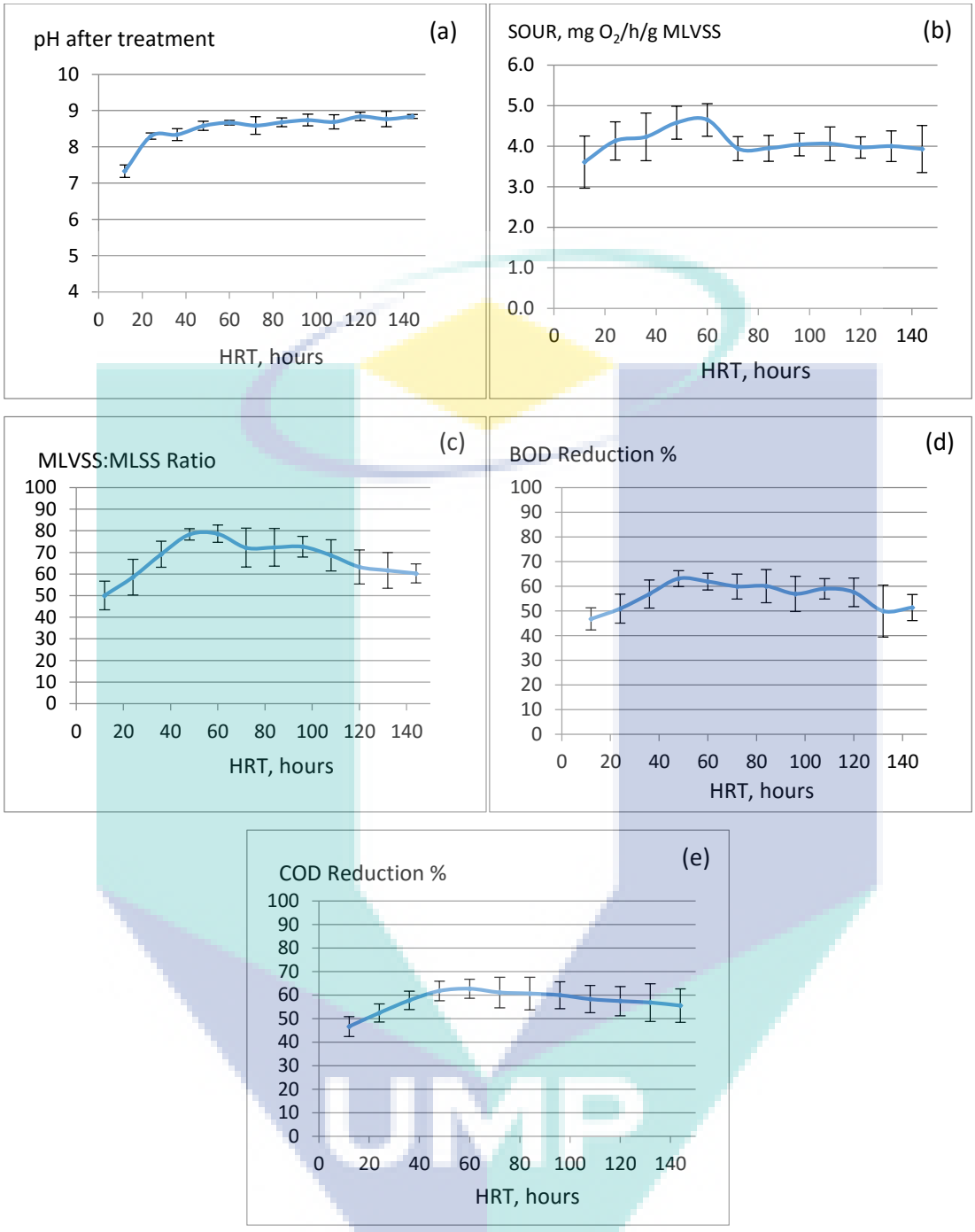

Figure 4.13: Effect of hydraulic retention time on the (a) pH after treatment, (b) specific oxygen uptake rate, (c) MLVSS:MLSS ratio, (d) BOD reduction, (e) COD reduction. Operating conditions: pH = 6.5, SRT = 7 days, MLVSS = 2,000 mg/L, BOD initial = 650 mg/L.

4.4.3 Organic loading rate (OLR)

Organic loading rate gives the indication of the susceptibility of the treatment system. It is intended to measure the amount of organic load that is able to be treated by the system. OLR is important to determine if this system is suitable to be used as a polishing plant and capable to treat the incoming waste up to the desired standard. In this experiment, the OLR was varied between 200 to 1000 mg/L, while the initial pH was adjusted to 6.5, HRT was fixed at 48 h, SRT was 7 days and the MLVSS of the system was at a value of 2,000 mg/L.

Figure 4.14(a) depicts that when the OLR increased from 200 mg/L to 400 mg/L, the pH after treatment increased from 7.2 to 8.5. The pH value was fairly constant between OLR of 400 mg/L to 800 mg/L. Above OLR of 800 mg/L, the pH after treatment increased rapidly when the OLR increased. The pH value exceeded the maximum allowable limit of the standard; hence, this system is not suitable to treat the POME with the incoming organic loading of more than 800 mg/L.

SOUR of the system at varies OLR was shown in the Figure 4.14(b). The SOUR at all the OLR values was less than 6 mg O_2/h/g MLVSS. The maximum SOUR of 5.5 mg O_2/h/g MLVSS was achieved at OLR value of 650 mg/L. It is obvious that the SOUR of the system was improved as compared to the previous two sections. Nevertheless, it is still below the recommended value of 8 mg O_2/h/g MLVSS.

Figure 4.14(c) illustrates that when the OLR was increased, the MLVSS: MLSS ratio also increased. A maximum value of 80 was attained at OLR value of 600 mg/L. At this value of OLR, percentage reduction of BOD and COD were 60% and 67% respectively (Figure 4.14(d) and (e)). These were also the maximum reduction that could be achieved in this study. Based on the results obtained in this section, the OLR was fixed at a value of 650 mg/L in the following studies.

Figure 4.14: Effect of organic loading rate on the (a) pH after treatment, (b) specific oxygen uptake rate, (c) MLVSS:MLSS ratio, (d) BOD reduction, (e) COD reduction. Operating conditions: pH = 6.5, HRT = 48 h, SRT = 7 days, MLVSS = 2,000 mg/L.

4.4.4 Initial mixed liquor volatile suspended solids (MLVSS)

Initial mixed liquor volatile suspended solids represents the amount of microorganism available to digest and treat the POME. It is resemble the inoculum size in a fermentation. A sufficient amount of inoculum is required to start a fermentation and digest (or treated) the organic compound in the POME to meet the discharge standard within the HRT. Thus, the MLVSS was varied from 1,000 to 10,000 mg/L at a fixed HRT of 48 h in this study. The pH of the initial POME was adjusted to 6.5 with the OLR of 650 mg/L and SRT of 7 days.

Illustrated in Figure 4.15(a) is the pH after treatment, where there is only an insignificant slight increased could be observed when initial MLVSS was increased from 2,000 mg/L to 10,000 mg/L. pH after treatment was around 7.2 when initial MLVSS was at a value of 1,000 mg/L, but above this value the pH was above 8. Nonetheless, the pH after treatment was still within the standard limit in the range of study.

When the initial MLVSS was between 5,000 mg/L to 6,000 mg/L, the SOUR attained for the system was around 5.5 mg O_2/h/g MLVSS, which is the maximum SOUR could be achieved by the system within the range of MLVSS studied (Figure 4.15(b)). Unfortunately, it is still below the recommended limit.

The maximum ratio of MLVSS:MLSS achieved by the system in the range of MLVSS studied was only 80 (Figure 4.15(c)). This maximum ratio occurred at initial MLVSS value of 5,000 mg/L. Likewise, the maximum BOD reduction (61%) and COD reduction (68%) also happened at the same MLVSS value (Figure 4.15(d) and (e)). Therefore, initial MLVSS value of 5,000 mg/L seems to be an optimal value to initiate the treatment process.

Practically, it is hard to get initial MLVSS of more than 2,000 mg/L in the existing plant. A concentration step of activated sludge would be required if a higher amount of MLVSS is desired. This would indicate an extra process unit prior to the polishing plant, which also means extra cost and space. Since it is practically not feasible, the following study would still use MLVSS of 2,000 mg/L.

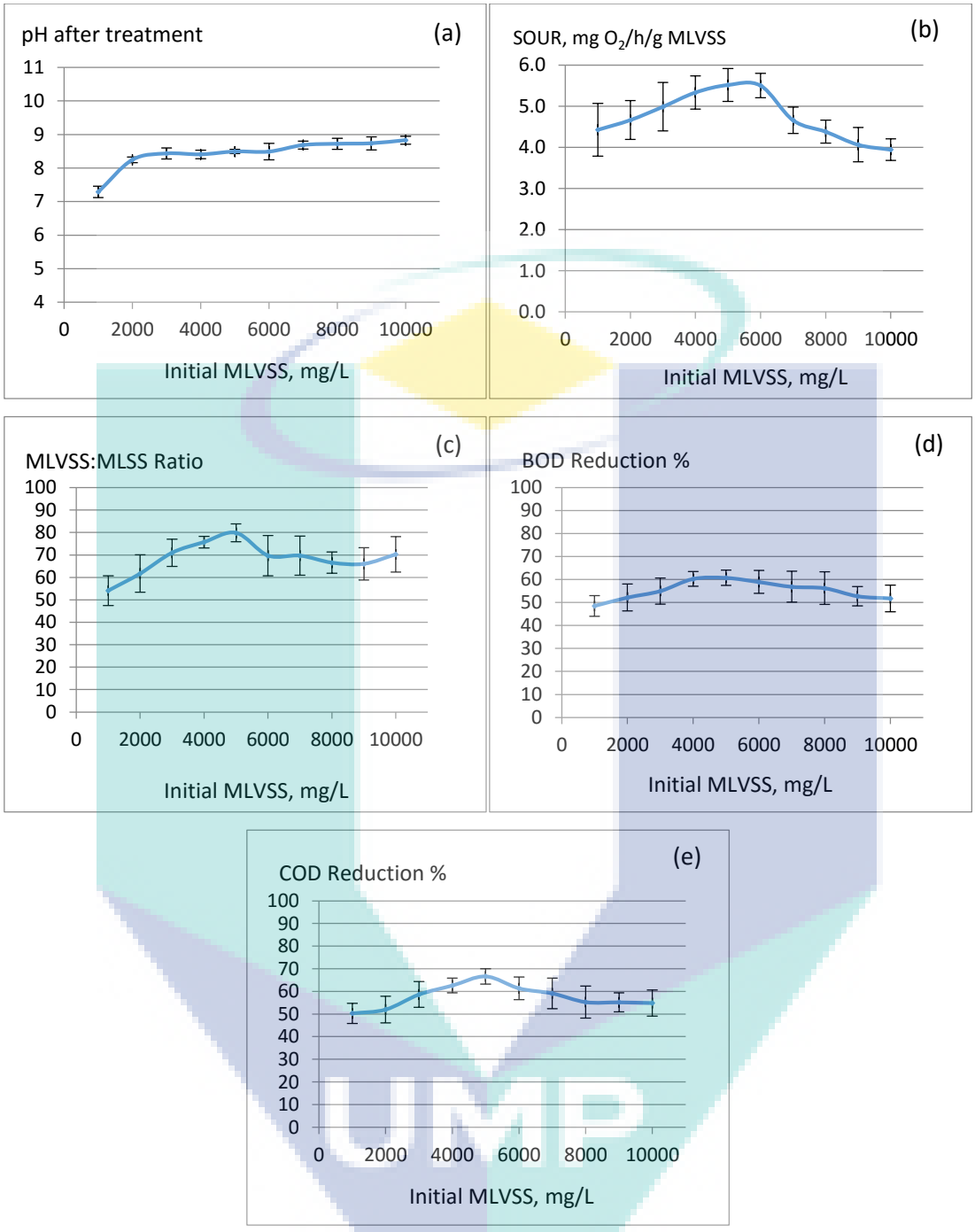

Figure 4.15: Effect of mixed liquor volatile suspended solid on the (a) pH after treatment, (b) specific oxygen uptake rate, (c) MLVSS:MLSS ratio, (d) BOD reduction, (e) COD reduction. Operating conditions: pH = 6.5, HRT = 48 h, SRT = 7 days, BOD initial = 650 mg/L.

4.4.5 Solid retention time (SRT)

Solid retention time is the sludge age, or in other words, the amount of time the sludge remain in the reactor before it is being used as the inoculum. It determines the maturity of the sludge and the amount of active microorganism that has adapted to the environment. The SRT was varied from 2 days to 20 days in this study, where the initial pH of the POME was adjusted to 6.5 with a MLVSS of the system of 2000 mg/L and initial BOD value of 650 mg/L. The HRT was fixed at 48 h.

Figure 4.16(a) depicts that when SRT was increased from 2 days to 6 days, the pH after treatment increased from 7.3 to 8.6 and remained quite constant up to SRT value of 12 days. The pH after treatment increased steadily if SRT was increased above 12 days. Too old a sludge seems to give some negative impact to the system.

Figure 4.16(b) shows that the maximum SOUR achieved was around 5.5 mg O_2/h/g MLVSS at SRT of about 10 days. Below or above this value, the SOUR values were lower, indicating a non-healthy growth of the microorganism.

The maximum ratio of MLVSS:MLSS also took place at SRT of 10 days. The ratio was only around 80 (Figure 4.16(c)). Figure 4.16(d) and Figure 4.16(e) show the percentage reduction of BOD and COD respectively. The maximum BOD reduction was around 60% at SRT of 10 days, while the maximum COD reduction was about 67% at the same SRT value. Even though the overall performance of the system was not entirely efficient, the best SRT within the range of study was still able to be identified, which was 10 days.

From all the results from Section 4.4.1 to Section 4.4.5, it seems like there was some limitation in the system that retarding the growth of microorganism. The percentage reduction of BOD and COD was not changed significantly even though the operating conditions were improved. This revealed that a portion of organic compound in the POME might be non-degradable or required long time to degrade. As a result, when the degradable food has depleted, the microorganism would be starved and died. Therefore, no further BOD or COD reduction could be achieved. In addition, there might be oxygen limitation or nutrient limitation in the system as the SOUR was always at the low value which indicating unhealthy grow of the microorganism.

Figure 4.16: Effect of solid retention time on the (a) pH after treatment, (b) specific oxygen uptake rate, (c) MLVSS:MLSS ratio, (d) BOD reduction, (e) COD reduction. Operating conditions: pH = 6.5, HRT = 48 h, MLVSS = 2000 mg/L, BOD initial = 650 mg/L.

4.4.6 Molasses concentration as external carbon source

In view of the possible nutrient limitation, this section examined the effect of adding molasses as an external carbon source to the activated sludge system. Molasses was known to be a type of carbon source that is typically supplemented to the fermentation system. As the biological treatment of POME resembling fermentation, cheap carbon source like molasses was chosen. Molasses concentration up to 100 mg/L was supplemented to the POME at the beginning of the reaction in this experiment. Other operating conditions were fixed at the optimal value obtained from the previous sections, except MLVSS. That is initial pH of 6.5, 48 h HRT with the initial BOD of 650 mg/L at SRT of 10 days.

Figure 4.17(a) illustrates that addition of molasses did not affect the pH after treatment irrespective of the molasses concentration. The pH after treatment is still within the range of standard set by the Malaysian Department of Environment.

When the concentration of molasses added was increased, there was an increment in the SOUR of the system (Figure 4.17(b)). However, at molasses concentration greater than 60 mg/L, the SOUR remained constant at the value of 8.3 mg O_2/h/g MLVSS. Since the higher SOUR, the healthier is the microorganism. This system is only barely exceed the minimum requirement of 8 mg O_2/h/g MLVSS, which indicating the vulnerability of the system. In other words, any disturbance would easily upset the system.

Figure 4.17(c) shows that once the molasses concentration fed exceeded 50 mg/L, the MLVSS:MLSS ratio would be more than 80. Nevertheless, differences in the ratio was not significant as indicated by the error bar. Correspondingly, the percentage reduction of BOD (Figure 4.17(d)) and COD (Figure 4.17(e)) was not showing substantial changed around the value of 65%. This mean that addition of molasses did not aid in the treatment of POME even though it facilitates the healthy grow of the microorganism. Molasses, as a degradable organic component, was only functioned as the food to the microorganism, thus improved the SOUR and support the growth of the microorganism. Other organic components in the POME, however, were non-degradable or slow degradable. As a consequence, there was no further reduction of BOD or COD could be observed in the time frame and operating conditions employed in this study. In order

to confirm the postulation of the existing of slow or non-degradable organic compound, further investigation was required.

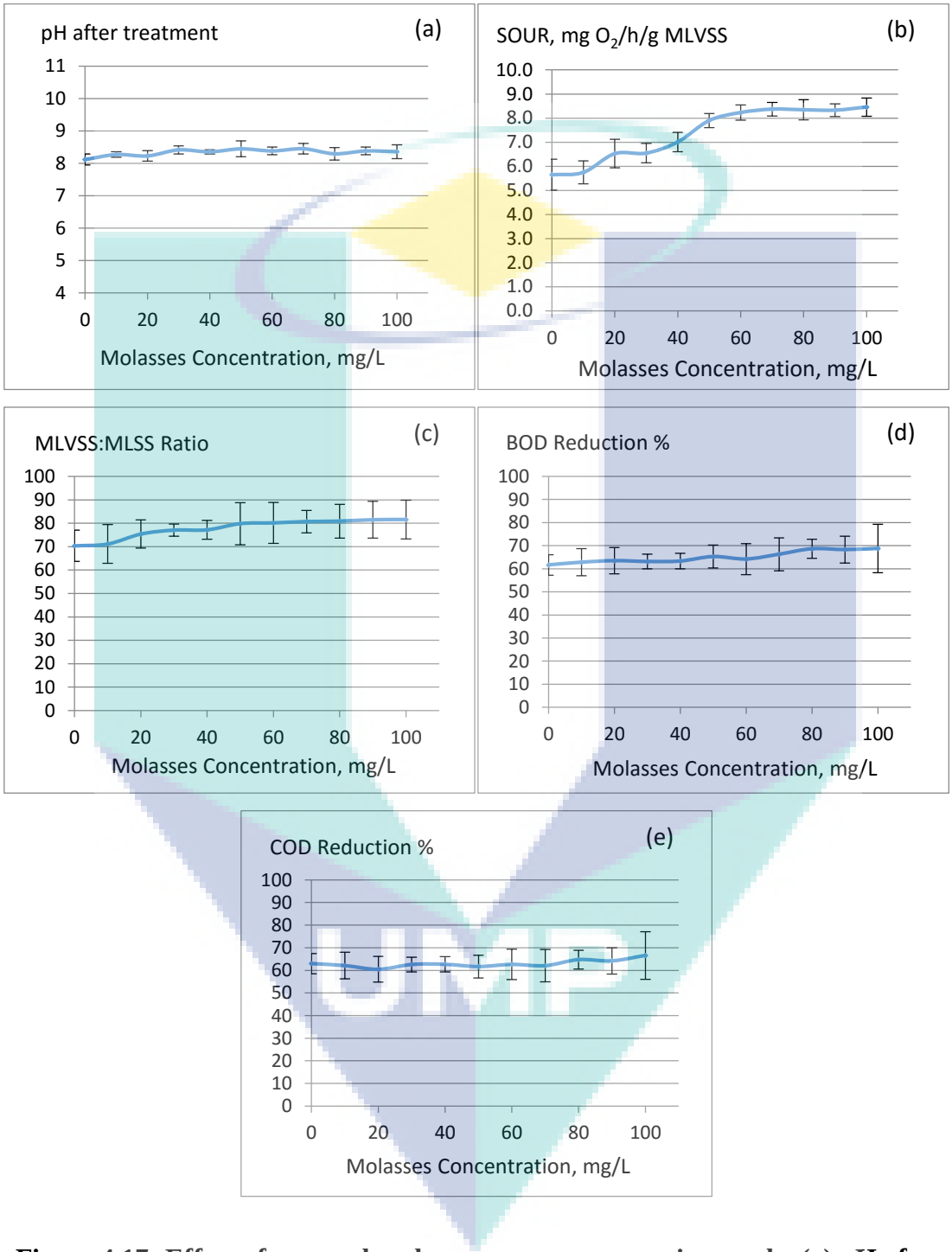

Figure 4.17: Effect of external carbon source concentration on the (a) pH after treatment, (b) specific oxygen uptake rate, (c) MLVSS:MLSS ratio, (d) BOD reduction, (e) COD reduction. Operating conditions: pH = 6.5, HRT = 48 h, SRT = 10 days, MLVSS = 2000 mg/L, BOD initial = 650 mg/L.

CHAPTER 5
CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this study, the minimum light penetration that must be provided in order to optimize the microalgae growth was determined to be approximately 2,000 Lux. The highest cell number (2.73×10^6 cells/ml) was obtained in 30% v/v of POME sample. It can be concluded that microalgae can grow in low light intensity condition and performed optimum growth once the light penetrated exceeded 2,000 Lux. Another factor which contributed the growth of microalgae was the POME as it contained several nutrients that were essential for the growth. However, as the POME concentration increased, the deep color of the POME blocked the light penetrated and thus reduce the growth of microalgae.

The respective kinetic study was carried out to observe the growth profile of microalgae. The kinetic study proven that mix culture of microalgae in 30% v/v POME experienced highest growth rate compare to the mix culture in control as well as 10 and 20% v/v POME samples. The specific growth rate of microalgae in 30% v/v POME was 1.39 d^{-1} and the biomass yield based on POME and light energy were 0.50 g cells/g POME and 0.68 g/mol photons. Finally, the biomass productivity calculated was 0.61 g/L.d. Besides, the maximum growth rate and saturated constant were determined through the Monod model based on light intensity (obtained from extended Beer-Lambert Law) and the values were 1.20 d^{-1} and 47.1 respectively. Whereas the maximum growth rate and saturated constant based on POME concentration were 1.43 d^{-1} and 1.64 respectively. In the kinetic study, it can be inferred that the growth of mix culture of microalgae was in a rapid rate.

In terms of BOD and color removal, the removal efficiency in 30% v/v sample was 52.9% and 18.5% respectively. The ratio of POME concentration and the respective growth profile of microalgae led to the similar removal efficiency of BOD. The decolourisation yield was unsatisfied and this might be regarded to several factors such as the morphology of microalgae which lessen the availability of the capacity for

colored compound uptake, the physical properties of the lignin and phenolic particles and the pH of the environment.

In the study of operating parameters for activated sludge system, initial pH at 6.5, hydraulic retention time of 48 h, solid retention time of 10 days, initial MLVSS value at 2000 mg/L and organic loading rate of 650 mg/L, together with the feeding of 60 mg/L of molasses at the beginning of treatment, enable a BOD reduction of up to 65%. Even though these operating conditions are practically feasible, the final BOD value still do not meet the new requirement for discharge. Hence, purely feeding carbon source to the activated sludge system do not aids in digesting all the organic compound in the POME as some of those maybe existed as non-degradable or slow-degradable organic compounds. Consequently, further investigation is required in this case.

5.2 Recommendation and Future Work

As for recommendation, to perform an effective decolourisation of POME, instead of using the mix culture of microalgae, utilizing single strain of microalgae is preferable due to the performance of the microalgae can be easily observed. To justify the ideal strain in tertiary treatment of POME, screening on the several microalgae strains can be done before applying in the POME treatment. The screening of the microalgae strains mainly focused on the growth profile of the microalgae and the decolourisation yield on POME. There are several advantages by using single microalgae strains. The environmental condition can be optimized by referring to the physical properties of microalgae and thus obtain the optimum growth of microalgae. Besides, a less competitive environment can be created to prolong the life of the microalgae. An effective decolourisation yield can be foreseen if the ideal microalgae strain is experiencing an ideal growth rate. Even though the mix culture of microalgae undergo a rapid growth rate, it does not represent the amount of microalgae that work in decolourisation is in majority.

In order to increase the BOD and color removal efficiency, the experiment can adapt a facultative environment to culture the microalgae as well as bacteria. As mentioned, the biomass of the microalgae tends to contribute to the increment of BOD value. The biomass is a potential food source for the aerobic bacteria and the oxygen created by

microalgae can be utilized by bacteria to oxidize the organic contents of POME. The compounds in turn able to promote the growth of microalgae. It is expected that the bacteria tend to oxidize the phenolic compound to a stable form and aid in the decolourisation.

Nevertheless, the pH of the culture need to be inspected from time to time. The influence of the pH values to the microalgae growth rate and decolourisation yield can be determined. The adjustment on the pH also can be done in order to obtain the optimum yield.

Determination of slow- and non-degradable BOD amount in POME are required to have a more detail insight of the system in order to re-design and improve the polishing stage of POME waste treatment.

REFERENCES

Abdel-Raouf, N., Al-Homaidan, A.A, Ibraheem, I.B.M. (2012). Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences,* 19: 257-275.

Abdurahman, N.H., Rosli, Y.M., Azhari, N.H. (2012). The Performance Evaluation of Anaerobic Methods for Palm Oil Mill Effluent (POME) Treatment: A Review. *International Perspectives on Water Quality Management and Pollutant Control*, 88-100.

APHA. (1992). *Standard methods for the examination of water and wastewater* (18th ed.). Washington D.C, USA: American Public Health Association.

Augustin, M.B., Sengpracha, W.P., Phutdhawong,W. (2008). Electrocoagulation of palm oil mill effluent. *International Journal of Environmental Research and Public Health,* 12: 177-180.

Badroldin, N. A. (2010). *Treatment of palm oil mill effluent (POME) using hybrid up flow anaerobic sludge blanket (HUASB).* PhD Thesis. Johor, Malaysia: Universiti Tun Hussein Onn, Malaysia.

Barbosa, M.J., Hoogakker, J., Wijffels, R.H. (2003). Optimisation of cultivation parameters in photobioreactors for microalgae cultivation using the A-stat technique. *Biomolecular Engineering, 20* : 115-123.

Benjamas, C., Salwa, T. (2012). Enhanced growth and lipid production of microalgae under mixotrophic culture condition: Effect of light intensity, glucose concentration and fed-batch cultivation. *Bioresource Technology,* 110: 510-516.

Chan, Y.J., Chong, M.F., Law, C.L. (2010).Biological treatment of anaerobically digested palm oil mill effluent (POME) using a lab scale sequencing batch reactor (SBR). *Journal of Environmental Management,* 91: 1738-1746.

Choi, H. J., Lee, S.M. (2012). Effects of microalgae on the removal of nutrients from

wastewater: various concentrations of *Chlorella vulgaris*. *Environmental Engineering Research*, 17: 3-8.

Daneshvar, N., Ayazloo, M., Khatee, A.R., Pourhassan, M. (2007). Biological decolorization of dye solution containing Malachite Green by microalgae Cosmarium sp. *Bioresource Technology*, 98: 1176-1182.

Dauta, A., Devaux, J., Piquemal, F., Boumnich, L. (1990). Growth rate of four freshwater algae in relation to light and temperature. *Journal of Hydrobiologia*, 207: 221-226.

Dotto, G.L., Pinto, L.A.A. (2012). Analysis of mass transfer kinetics in the biosorption of synthetic dyes onto Spirulina platensis nanoparticles. *Biochemical Engineering Journal*, 68: 85-90.

Eduardo, J., Carlos, H.G.S, Lucy, M., Telma, T.F. (2009). Effect of light cycles (night/day) on CO2 fixation and biomass production by microalgae in photobioreactors. *Chemical Engineering and Processing*, 48: 306-310.

Emma, S., Rosalam, S. (2012). Conversion of microalgae to biofuel. *Renewable and Sustainable Energy Reviews*, 16: 4316-4342.

Eze, V.C., Owunna, N. D. and Avoaja, D.A. (2013). Microbiological and physicochemical characteristics of soil receiving palm oil mill effluent in Umuahia, Abia State, Nigeria. *Journal of Natural Sciences Research*, Volume 3, No. 7.

Fernandez, F.G., Camacho, F.G., Perez, J.A., Sevilla J.M., Molina, E. (1997). *A model for light distribution and average solar irradiance inside outdoor tubular photobioreactors for the microalgal mass culture.* PhD Thesis. University of Almeria, Spain.

Hadiyanto, H., Nur, M.M.A. (2012). Potential of Palm Oil Mill Effluent (POME) as medium growth of chlorella sp for bioenergy production. *International Journal of*

Environment and Bioenergy, 3: 67-74.

Haruna H. and Nor-Anuar. A. (2014). Development and utilization of aerobic granules for soy sauce wastewater treatment: Optimization by response surface methodology. *Jurnal Teknologi*, 69:5, 31-37.

Hermanto, M. (2009). *Identification of Algae Growth Kinetics.* PhD Thesis. Wageningen University, Netherlands.

Hirooka, T., Akiyama, Y., Tsuji, N., Nakamura, T., Nagase, H., Hirata, K., Miyamoto, K. (2003). Removal of hazardous phenols by microalgae under photoautotrophic conditions. *Journal of Bioscience and Bioengineering*, 95: 200-203.

Huan, K.L. (1987). Trials on long-term effects of application of POME on soil properties, oil palm nutrition and yields. In: Proceedings of the International Oil Palm, Palm Oil Conferences (eds.) Abdul Halim, B., Chew, P.S., Woond, B.J. and PushParajah, E., 2: 575-598.

John, R.B., Robert, W.S. (2004). Aerobic Treatment of Wastewater and Aerobic Treatment Unit. Text in (M.A. Gross and N.E. Deal, eds.) University Curriculum Development for Decentralized Wastewater Management. National Decentralized Water Resources Capacity Development Project. University of Arkansas, Fayetteville, AR.

Kitaya, Y., Azuma,H., Kiyota, M. (2005). Effects of temperature, CO_2/O_2 concentrations and light intensity on cellular multiplication of microalgae, *Euglena gracilis*. *Advances in Space Research*, 35: 1584-1588.

Kumar, A. Ergas, S., Yuan, X., Sahu, A., Zhang, Q., Dewulf, J., Malcata, F.X., Langenhove, H.V. (2010). Enhanced CO2 fixation and biofuel production via microalgae: recent developments and future directions. *Trends in Biotechnology*, 28: 371–380.

Lam, M.K., Lee, K.T. (2011). Renewable and sustainable bioenergies production from palm oil mill effluent (POME): win-win strategies towards better environmental protection. *Biotechnology Advances*, 29: 121-141.

Larsdotter, K. (2006). Wastewater treatment with microalgae- a literature review. *Vatten*, 62: 31-38.

Limkhuansuwan, V., Chaiprasert, P. (2010). Decolorization of molasses melanoidins and palm oil mill effluent phenolic compounds by fermentative lactic acid bacteria. *Journal of Environmental Sciences*, 22: 1209-1217.

Luisa, G., Oliveira, C.A. (2008). Microalgae as raw material for biofuels production. *Journal of Industrial Microbiology and Biotechnology* 36: 269-274.

Mata, T.M., Melo, A.C., Simoes, M., Caetano, N.S. (2012). Parametric study of a brewery effluent treatment by microalgae *Scenedesmus obliquus*. *Bioresource Technology*, 107: 151-158.

Molina, G.E., Garcia, C.F., Sanchez, P.J., Sevilla, J.M., Fernandez, F.G., Contreras, G.A. (1994). A mathematical model of microalgal growth in light limited chemostat culture. *Journal of Chemical Technology and Biotechnology*, 61: 167-173.

Neoh, C.H. Yahya, A., Adnan, R., Majid, Z.A. and Ibrahim,Z. (2012). Optimization of decolorization of palm oil mill effluent (POME) by growing cultures of *Aspergillus fumigatus* using response surface methodology. *Environment Science Pollution Research*. Doi: 10.1007/s11356-012-1193-5.

Ohimain, E.I., Izah S.C. and Jenakumo N. (2013). Physicochemical and microbial screening of palm oil mill effluents for amylase production. *Greener Journal of Biological Sciences*. 3: 307–318.

Okwute, L. O. and Isu, N. R. (2007). The environmental impact of palm oil mill effluent (pome) on some physicochemical parameters and total aerobic bioload of soil at a dump

site in Anyigba, Kogi state, Nigeria. *African Journal of Agricultural Research*, 2: 656 - 662.

Pruvost, J., Vooren, G.V., Cogne, G., Legrand, J. (2009). Investigation of biomass and lipids production with *Neochloris oleoabundans* in photobioreactor. *Bioresources Technology*, 100: 5988-5995.

Richard, C. (2007). *Culturing of chlorella vulgaris-standard operating procedure*. Retrieved from http://www.biosci.rdg.ac.uk/Research/ed/daphnia.htm

Roleda, M.Y., Stephen, P.S., Raymond J.G. L., John, G. D., Elanor, M. B., Michele, S.S. (2013). Effects of temperature and nutrient regimes on biomass and lipid production by six oleaginous microalgae in batch culture employing a two-phase cultivation strategy. *Bioresource Technology,* 129: 439-449.

Samori, G., Samori,C., Franca, G., Rossella, P. (2013). Growth and nitrogen removal capacity of *Desmodesmus communis* and of a natural microalgae consortium in a batch culture system in view of urban wastewater treatment: Part I. *Journal of Water Research*, 47: 791-801.

Suzana, W., Ani, I., Sitti, R.M.S. (2013). The influence of light intensity and photoperiod on the growth and lipid content of microalgae *Nannochloropsis sp. Bioresource Technology*, 129: 7-11.

Technical Guidance Document Series Number: DOE-IETS-1. (2010). Guidance document on performance monitoring of industrial effluent treatment system specified in regulation 9(a) environmental quality (industrial effluent) regulation 2009. Third Edition. Page 19.

Toyub, M.A, Ahmet, S.R., Miah, M.I., Habib, M.A.B. (2007). Growth performance and nutritional value of *chlorella ellipsoidea* in fertilizer factory effluent media. *Asian Fisheries Science,* 20: 65-79.

Vijayaraghavan, K., Ahmad, D., Ezani,M. (2007). Aerobic treatment of palm oil mill effluent. *Journal of Environmental Management,* 82: 24-31.

Wong, K. (1980). Application of ponding system in the treatment of palm oil mill and rubber mill effluent. *Journal of Pertanika,* 3: 133-141.

Wu, T.Y., Mohammad, A.W., Jamaliah, M.J., Nurina, A. (2010). Pollution control technologies for the treatment of palm oil mill effluent (POME) through end-of-pipe process. *Journal of Environmental Management,* 91: 1467-1490.

Xu, L., Diosady, L.L. (2001). *The removal of phenolic compounds for the prodcution of high quality canola protein isolates.* Toronto, Canada: Department of Chemical Engineering and Applied Chemistry, University of Toronto.

Yacob, S., Hassan, M.A.,Yoshihito, S., Minato, W., Sunderaj, S. (2005). Baseline study of methane emission from open digesting tanks of palm oil mill effluent treatment. *Journal of Chemosphere,* 59: 1575–1581.

Yamashita, T. and Ikemoto, R.Y. (2014). Nitrogen and phosphorus removal from wastewater treatment plant effluent *via* bacterial sulfate reduction in an anoxic bioreactor packed with wood and iron. *International Journal of Environmental Research and Public Health*, 11: 9835-9853.

Zaini Ujang, Salmiati Salmiati and Mohd Razman Salim (2010). Microbial Biopolymerization Production from Palm Oil Mill Effluent (POME), Biopolymers, Magdy Elnashar (Ed.), ISBN: 978-953-307-109-1, InTech, Available from: http://www.intechopen.com/books/biopolymers/microbial-biopolimerization-production-frompalm-oil-mill-effluent-pome-

Zhang, C.W., Zmora, O., Kopel, R., Richmond, A. (2001). An industrial-size flat plate glass reactor for mass production of *Nannochloropsis sp. (Eustigmatophyceae)*. *Aquaculture,* 195: 35-49.

Zielinska M., Bernat K., Cydzik-Kwiatkowska A., Wojnowska-Baryla. I. (2012). Respirometric activity of activated sludge in sequencing batch reactor depending on substrate and dissolved oxygen concentration. *Environmental Protection Engineering.* Vol 38. No.2.

Zijffers, J.F., Schippers, K.J., Zheng, K., Janssen, M., Tramper, J., Wijffels, R.H. (2010). Maximum photosynthetic yield of green microalgae in photobioreactors. *Marine Biotechnology*, 12: 708-718.

Zulkarnain, M.I., Jamal, P. and Alam, M.Z. (2012). Evaluation of palm oil mill effluent treatment with concomitant phenolics production by *Aspergillus niger* IBS-103ZA. *Australian Journal of Basic and Applied Sciences*, 6: 55-61.