AEROBIC TREATMENT AND BIODEGRADATION OF PALM OIL MILL EFFLUENT BY INDIGENOUS MICROORGANISMS

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AEROBIC TREATMENT AND BIODEGRADATION OF PALM OIL MILL EFFLUENT BY INDIGENOUS MICROORGANISMS

by

BALA JEREMIAH DAVID

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DEDICATION

This thesis is dedicated to:

My beloved late Father: (WO) Polycarp A. David (JP) (RTD),
Whose words of encouragement and push for tenacity ring in my ears.

Time may pass and fade away, but memories of you will always stay. In God's care you rest above, in our hearts you rest with love.

Deep in our hearts a memory is kept of you and we shall never forget. Simple words but very true,
we'll always love and remember you.

To my beloved mother: Mrs. Abigail David Polycarp,
For her endless love, support and encouragement.

To Treasure, my lovely wife: Wazotah Jeremiah

Who have always loved me
unconditionally and whose good examples have taught me to work hard for
the things that I aspire to achieve. I am truly thankful for having you in my life.

To my beloved sons

Maxwell Polycarp Jeremiah and Magnus Polycarp Jeremiah
Both of you have been my best cheerleaders.

To my beloved younger brothers and sisters

Captain Nimrod David Polycarp, Engineer Wilfred David Polycarp, Rahab David Polycarp, Esther David Polycarp, Barr. Tsumbuji Phinehas David Polycarp (ASP), Ato Reuben David Polycarp, and Queen David Polycarp,
For their unrelenting encouragement, love and ceaseless prayers.

Thank you Lord (God), for always being there for me.

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Finally, I would like to leave the remaining space in memory of my beloved late Father.

Baba, you fell asleep (Thursday 3rd October 2013) without goodbye, but memories of you will never die. A precious one from us has gone. A voice we love is still. A place is vacant in our hearts which no one else can fill. After our lonely heartaches and our silent tears, we will always have beautiful memories of our father that we loved so dear. Baba, enter thy Master's joy. God bless you Baba and keep you in His care, until we meet again.

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LIST OF ABBREVIATIONS

ABFC	All bacteria-fungi combination
BFSW	Bacteria-fungi stepwise
BOD_5	Biochemical oxygen demand
CMC	Carboxymethyl cellulose
COD	Chemical oxygen demand
DO	Dissolved oxygen
HPLC	High Performance Liquid Chromatography
MSM	Mineral salt medium
NA	Nutrient agar
O & G	Oil and grease
ODB	Oil degrading bacteria
ODF	Oil degrading fungi
OMWW	Olive mill wastewater
PB	Palm oil mill effluent bacteria
PDA	Potato Dextrose agar
PF	Palm oil mill effluent fungi
POA	Palm oil agar
POME	Palm oil mill effluent
rRNA	Ribosomal ribonucleic acid
THB	Total heterotrophic bacteria
THC	Total heterotrophic counts
THF	Total heterotrophic fungi
TSS	Total suspended solid

RAWATAN AEROBIK DAN BIODEGRADASI EFFLUEN KILANG KELAPA SAWIT OLEH MIKROORGANISMA ASLI

ABSTRAK

Biodegradasi mikrob air buangan yang melibatkan penggunaan pelbagai mikroorganisma telah menunjukkan keupayaan degradasi sisa organik dalam air sisa yang berkesan yang telah menarik perhatian sejak kebelakangan ini. Kajian ini bertujuan untuk menentukan keupayaan biodegradasi oleh mikroorganisma-mikroorganisma asli yang dipencilkan daripada efluen kilang minyak kelapa sawit (POME) sebagai substrat untuk mengurangkan keperluan oksigen biokimia (BOD_5), permintaan oksigen kimia (COD), jumlah pepejal terampai (TSS) dan minyak dan gris (O & G) daripada POME dan untuk mengenalpasti strain yang paling sesuai untuk teknologi rawatan biologi POME dalam keadaan aerobik. Pencirian sampel POME telah dijalankan mengikut kaedah-kaedah piawai untuk pemeriksaan air dan air sisa. Pemencilan dan pengenalpastian mikroorganisma asli adalah tertakluk kepada kaedah piawai mikrobiologi dan aturan 16S rRNA dan gen 18S rRNA masing-masing. Aktiviti enzim mikrob yang dipencil dikaji untuk mengesan lipase dan selulase dalam media pepejal oleh plat asai. Pengurangan peratusan BOD_5, COD, TSS dan O & G oleh pencilan terpilih ditentukan. Analisis HPLC telah dijalankan untuk menunjukkan degradasi selulosa. Penjujukan 16S rRNA dan 18S rRNA daripada pencilan-pencilan telah dikenalpasti sebagai *Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, *Providencia vermicola* 104PB, *Klebsiella pneumonia* 105PB, *Bacillus subtilis* 106PB, *Aspergillus fumigatus* 107PF, *Aspergillus nomius* 108PF, *Aspergillus niger* 109PF dan *Meyerozyma guilliermondii* 110PF. Semua pencilan yang dikenalpasti telah disaring untuk hidrolisis lipid dan selulosis kaedah plat. Strain-strain menunjukkan tahap

aktiviti lipase dan selulase yang berbezapada media pepejal. Profil pertumbuhan pencilan bakteria dan kulat telah dilakukan menggunakan medium cecair garam mineral (MSM).*Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, *Bacillus subtilis* 106PB, *Aspergillus fumigatus* 107PF dan *Aspergillus niger* 109PF dapat memaparkan pertumbuhan baik yang setanding dan degradasi minyak dan selulosa dalam MSM dan telah dipilih untuk inokulasi POME dan pengurangan kandungan organik. Hasil kajian menunjukkan bahawa parameter fizikokimia (COD, BOD_5, TSS dan O & G) telah dikurangkan dengan ketara melalui rawatan secara aerobik. Kecekapan peratus pengurangan parameter oleh pencilan mikrob (individu dan gabungan) adalah antara 35.93– 95.20% untuk bakteria dan 43.09– 92.37% untuk kulat dan 84.45– 92.23% untuk kombinasi campuran. Analisis varians (ANOVA) menunjukkan bahawa terdapat perbezaan yang signifikan ($P<0.05$) dalam peratus pengurangan parameter untuk *Bacillus cereus* 103PB dan *Aspergillus niger* 109PF berbanding dengan isolat lain. Kultur campuran bakteria dan kulat dalam kajian ini menunjukkan perbezaan tinggi peratus pengurangan parameter yang signifikan ($P<0.05$) berbanding dengan kultur tulen tunggal dan merupakan yang paling berkesan.Analisis varians (ANOVA) juga menunjukkan bahawa terdapat perbezaan yang signifikan ($P<0.05$) dalam pengurangan parameter untuk suhu pada 37°C dan 30°C bagi strain bakteria manakala tiada perbezaan yang signifikan ($P>0.05$) dalam kadar pengurangan parameter untuk suhu 28°C dan 30°C bagi strain kulat. Sampel POME tidak steril menunjukkan pengurangan parameter ketara yang lebih tinggi daripada sampel POME steril. Walau bagaimanapun, penemuan utama kajian ini adalah bahawa prestasi kultur campuran boleh disebabkan oleh aktiviti sinergi organisma. Pecahan selulosa yang mewakili bahan-bahan selulosa dalam POME oleh mikroorganisma kepada glukosa membuktikan

biodegradasi selulosa. Kajian ini akan membantu dalam memahami peranan bakteria tulen dan kulat yang diasingkan daripada POME dalam teknologi rawatan biologi air kumbahan seperti pemprosesan minyak seperti POME.

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ABSTRACT

Microbial biodegradation of wastewaters involving the application of variety of microorganisms has demonstrated effective degradability of organic wastes in wastewaters which has attracted attention in recent time. This study was designed to determine the biodegradation ability of indigenous microorganisms isolated from palm oil mill effluent (POME) as substrate for the reduction of biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solids (TSS) and oil and grease (O & G) from POME and to identify the most suitable strain(s) for a biological treatment technology of POME under aerobic condition. The characterization of POME sample was carried out according to standard methods for the examination of water and wastewater. Isolation and identification of indigenous microorganisms was subjected to standard microbiological methods and sequencing of the 16S rRNA and 18S rRNA genes respectively. Enzymatic activities of the isolated microbes were examined for the detection of lipase and cellulase on solid media by plate assay. Percent reduction of BOD_5, COD, TSS and O & G by selected isolates was determined. HPLC analysis was carried out to indicate cellulose degradation. Sequencing of the 16S rRNA and 18S rRNA genes of the isolates suggests that they were identified as *Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, *Providencia vermicola* 104PB, *Klebsiella pneumonia* 105PB, *Bacillus subtilis* 106PB, *Aspergillus fumigatus* 107PF, *Aspergillus nomius* 108PF, *Aspergillus niger* 109PF and *Meyerozyma guilliermondii* 110PF. All identified isolates were screened for lipid hydrolysis and cellulolysis of cellulose by plate assay. Strains showed varying degree of lipase and cellulase

activity on solid media. Growth profile of the bacteria and fungi isolates was conducted in mineral salt medium (MSM) liquid medium. *Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, *Bacillus subtilis* 106PB, *Aspergillus fumigatus* 107PF and *Aspergillus niger* 109PF were able to display comparable good growth and degradation of oil and cellulose in MSM and were selected for POME inoculation and reduction of organic load. Results revealed that the physicochemical parameters (COD, BOD_5,TSS and O&G) were reduced remarkably with aerobic treatments. Percent reduction of the parameters by the microbial isolates (individual and combination) ranged from 35.93% – 95.20% for bacteria and 43.09 – 92.37% for fungi and 84.45 – 92.23% for mixed combination. Analysis of variance (ANOVA) showed that there were significant differences ($P<0.05$) in the percentage reduction of the parameters for *Bacillus cereus* 103PB and *Aspergillus niger* 109PF as compared to other isolates. Mixed cultures of bacteria and fungi in the present study showed high significant differences ($P<0.05$) in the percentage reduction of the parameters as compared to pure single cultures and were the most effective. Analysis of variance (ANOVA) also revealed that there were significant differences ($P<0.05$) in reduction of the parameters for temperatures at 37°C and 30°C for bacterial strains while no significant differences ($P>0.05$) in the rate of reduction of the parameters for temperatures at 28°C and 30°C for fungal strains. Non-sterile POME sample showed significantly higher reduction of the parameters than sterile POME sample. The breakdown of cellulose which represents the cellulosic materials in POME by microorganisms into glucose suggests biodegradation of cellulose. The study would help in understanding the role of indigenous bacteria and fungi isolated from POME in biological treatment of wastewaters such as those of oil processing like POME.

CHAPTER ONE

1.1 Introduction

Biodegradation of a compound is often a result of the actions of multiple organisms. When microorganisms are imported to a contaminated site to enhance degradation we have a process known as bioaugmentation (Takeno et al., 2005). Biodegradation is a natural process by which microbes alter and break down oil and organic pollutants in wastewater into other substances. The resulting products can be carbon dioxide, water, and simpler compounds that do not affect the environment. This acceleration can be accomplished by two forms of technology (Boopathy, 2000). These are addition of nutrients and/or addition of microbes. These additions are necessary to overcome certain environmental factors that may limit or prevent biodegradation. At present, employing the biochemical abilities of microorganisms is the most popular strategy for the biological treatment of contaminated soil, waters and wastewaters (Okonko and Shittu, 2007).

Only a few investigations have been conducted on degradation process for the reduction of organic wastes present in oily wastewaters such as POME (Wu et al., 2010). The total suspended solids (TSS) which is the cellulolytic material in wastewaters such as POME is still present after treatment using various physical and chemical treatment processes remains a problem to be resolved further (AbdulKarim et al., 2011). The use of microorganisms in biological treatment of wastewaters offers an alternative solution to reduce the COD, BOD, TSS content of effluents (Alam et al., 2009).

El-Masry et al. (2004) have found the use of microorganisms to be very efficient method for reducing BOD, COD, TSS, oil and grease. Thus, the exploitation of microorganisms for biological treatment offers a very efficient tool for purifying contaminated effluents and water (Glazer and Nikaido, 1995). In addition, Maygaonkar et al. (2012) reported that the physical and chemical treatment of industrial effluents is found to be insufficient whereas the biological treatment is most often found to be effective. Biofilm systems have shown attractive success in the removal of oil and grease from oily contaminated industrial wastewaters (Fogarty and Kelly, 1990; Gehara, 1999).

The biological processes for wastewater treatment consist of mixed communities with a wide spectrum of microorganisms, including bacteria, protozoa, fungi, rotifers and possibly algae (Sethupathi, 2004). In addition, the mixed microbial combination consortium showed the maximum percentage reduction of organic load. Combination of those mixed cultures display metabolic versatility and superiority to pure cultures (Sugiura et al., 1997; Sathishkumar et al., 2008; Hamme et al., 2000). Sathishkumar et al. (2008) reported that a microbial consortium containing a number of microorganisms is considered to be well suited for the degradation of industrial wastewaters. De Felice et al. (1997) used a combination of bacteria and yeast to degrade olive oil mill wastewater (OMW). The yeast, *Yarrowia lipolytica*, reduced 80% of COD from OMW. Oswal et al. (2002) has used the combination of *Yarrowia* with a consortium of bacteria and algae developed from garden soil, achieving 95% of COD reduction for the treatment of POME. Other investigators have reported reduction of organic load (COD, BOD, TSS and O & G) from POME and bakery wastewater with cultures of microorganisms. *Acinetobacter* sp. (KUL8), *Bacillus* sp. (KUL39), and *Pseudomonas*

sp. (KLB1 (Bhumibhamon et al., 2002) and *Trichoderma harzianum* and *Penicillium* (Abdul karim et al., 2011).

Microbial degradation of organic wastes in wastewaters using microorganisms such as bacteria, molds and yeasts had shown to be capable of completely degrading organic matter in oily wastewaters (Dhouib et al., 2006). Microbial degradation of oily wastewaters involves the application of variety of microorganisms which has demonstrated effective degradation of oil in wastewater (Erguder et al., 2000; Kissi et al., 2001; Dhouib et al., 2006; Wu et al., 2006c; El-Bestawy et al., 2005). Similarly, treatment of wastewater containing high concentrations of oil and grease matter with photosynthetic bacteria from food and agricultural wastewater has been reported to achieve remarkable reduction of COD and oil and grease (Sasaki et al., 1991; Hassan et al., 1997; Takeno et al., 2005).

So far to the best of our knowledge no work has been done on the cellulosic degradation in oil-contaminated wastewater. The indigenous microorganisms (bacteria and fungi) were isolated from POME. The POME sample was used as a substrate in the present study. The total suspended solids (TSS) exhibited as cellulose in POME is considered as organic matter (Iwara et al., 2011) which constitutes about 50% of the POME (Chin et al., 1996). The exploration and degradation analyses are important in the control of biological and physical wastewater treatment processes.

1.2 Problem statement

It has been reported that one of the major sources of water pollution is suspended solids. Suspended solids in POME are considered as organic matter (Iwara et al., 2011). The total suspended solids (TSS) which represent the cellulosic materials in POME sample still remain to be explored. Various physical and chemical treatment processes have been designed to treat agricultural industrial effluents such as POME, however, the problem of chemical residues and total suspended solids (TSS) which is still present after the treatment process remain to be resolved further (Abdul karim et al., 2011).

In consequence, TSS reduces the light penetration of water body and this reduces the ability of algae to produce food and oxygen (Alade et al., 2011). Suspended solids interfere with effective drinking water treatment and contribute to turbidity, or cloudiness of the water (Jameel and Olanrewaju, 2011).The amount of suspended solids in a water sample may be used as a general indicator of the overall quality of the sample. Suspended solids analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with discharge regulations (APHA, 2005).

The application of microorganisms such as *Trichoderma viride* spores, *T. viride* mycelium, *Yarrowia lipolytica* and *Saccharomyces cerevisiae* for the treatment of industrial wastewaters such as POME was not that effective in organic load reduction (Jameel and Olanrewaju, 2011). This may be due to the fact that these microorganisms are not indigenous to POME (Jameel and Olanrewaju, 2011; Abass et al., 2012; Soleimaninanadegani and Manshad, 2014). This gap offered researchers a greater

opportunity to explore the organic load reduction of oil contaminated wastewater in this study by using microbes that were isolated from POME as the substrate.

Very few investigations have been conducted on aerobic digestion process for the treatment of organic pollutants present in POME and POME related wastewater (Wu et al. 2010) as such there seem to be dearth of information on the microbiology of POME in literatures (Ohimain et al. 2012a). The interaction and integration of microbes in the particular wastewater (POME and POME related wastewater) to reduce organic pollutant loads in form of cellulose (TSS) and lipid (oil & grease) was not clearly explain anywhere.

Hence this study explored the degradation and enzymatic breakdown of organic matter in palm oil mill effluent (POME) particularly total suspended solids (TSS) exhibited as cellulose in POME by their indigenous (autochthonous) isolates.

1.3 Aim and objectives of the study

This study has the general aim to understanding the interaction and integration of microbes via aerobic treatment of POME sample with specific objectives as follows:

1. To isolate, identify, screen, and detect lipolytic and cellulolytic indigenous microbial isolates from palm oil mill effluent (POME).
2. To determine the percent reduction of BOD_5, COD, TSS, oil and grease in POME by the selected indigenous microorganisms.
3. To determine the biodegradation potential of cellulose in POME sample.

1.4 Significance of the study

The present study demonstrated the degradation ability and the interaction of the indigenous microorganisms of POME that exhibit the reduction of organic load in POME. The study revealed the identification of the most suitable microbial strain(s) for organic load reduction of wastewater such as palm oil mill effluent. It also helps in understanding the role of bacteria and fungi isolated from POME in biological treatment of wastewaters such as those of oil processing like palm oil mill effluent.

The use of indigenous bacteria and fungi isolated from POME somehow help to explain the conversion of organic matter/compound present in any oil contaminated wastewater of agricultural industry. The use of bacteria and fungi in biological treatment of wastewater such as palm oil mill effluent offers an alternative solution to reduce the organic load of the effluent. This study also has revealed the presence of potential bacterial and fungal strains responsible for the degradation, utilization and reduction of organic pollutants.

1.5 Scope of study

The present study covers the determination of microbiological analysis of palm oil mill effluent (POME). This involves Isolation and enumeration of total heterotrophic indigenous, palm oil-utilizing and cellulose utilizing bacteria and fungi from POME. Screening for lipid and cellulose hydrolysis on solid media by plate assay in order to detect lipase and celllulase producing bacteria and fungi on solid media. Growth profile of bacterial and fungal isolates that utilize palm oil and cellulose in mineral salts medium (MSM) was carried out. Physicochemical parameters (BOD_5, COD, TSS, oil and grease) was analysed to evaluate POME characteristics before and after treatment.

Percent reduction of the organic load by individual bacterial and fungal strains (pure culture) and combination of bacterial and fungal strains (mixed cultures) in sterile and non- sterile POME sample was investigated. Aerobic process was conducted to measure the degradation of cellulose by using high performance liquid chromatography (HPLC).

CHAPTER TWO

LITERATURE REVIEW

2.1 Composition of wastewaters

Watercourses receive pollution from many different sources, which vary both in strength and volume. The composition of wastewater is a reflection of the life styles and technologies practiced in the producing society (Horan, 1990). It is a complex mixture of natural organic and inorganic materials as well as man-made compounds. Organic and inorganic substances which are released into the environment as a result of domestic, agricultural and industrial water activities lead to organic and inorganic pollution. Three quarters of organic carbon in wastewaters are present as carbohydrates, fats, proteins, amino acids, and volatile acids (Abdel-Raouf et al., 2012). The inorganic constituents include large concentrations of sodium, calcium, potassium, magnesium, chlorine, sulphur, phosphate, bicarbonate, ammonium salts and heavy metals (Lim et al., 2010).

Different sources of pollutants include ‘‘Discharge of either raw or treated wastewaters from towns and villages; discharge from manufacturing or industrial plants; run-off from agricultural land; and leachates from solid waste disposal sites’’ these sites of pollution have problems so that a solution is sought (Horan, 1990). Scarcity of water, the need for energy and food are forcing us to explore the feasibility of wastewater recycling and resource recovery.

Wastewater environment is an ideal media for a wide range of microorganisms specially bacteria, fungi, viruses and protozoa. The majority is harmless and can be used in biological wastewater treatment, but some wastewaters also contain pathogenic microorganisms (Abdel-Raouf et al., 2012). Bacteria which cause cholera, typhoid and tuberculosis; fungi which cause dermatophytosis; viruses which cause infectious hepatitis; protozoa which cause dysentery and the eggs of parasitic worms are all found in various wastewaters depending on their sources (Shaaban et al., 2004).

2.2 Biodegradation and bioremediation of wastewaters

Biodegradation and bioremediation has been successfully used in the clean-up of petroleum hydrocarbon pollutants (Okoh, 2006), refinery effluents (Ojumu et al., 2005), textile effluents (Asamudo et al., 2005; Bako et al., 2008), wastewaters (Okonko and Shittu, 2007) and palm oil mill effluent (Oswal et al., 2002). The benefits of bioremediation and biodegradation are enormous. It saves cost; it is ecofriendly, ecologically simple, destroys contaminants (not moving them from one place to another) as well as treating wastes on site (Nadeau et al., 1993). The application of bioremediation will be an important aspect of waste management now and into the future as more is learned about this technology. El- Masry et al. (2004) and Bhumibhamon et al. (2002) have reported bioremediation of oily polluted industrial wastewater with combination of bacterial isolates for the reduction of COD and O & G. While El- Bestawy et al. (2005) also reported the combination of *Pseudomonas* sp. and *Pseudomonas. diminuta* as mixed cultures for reducing COD in contaminated industrial effluents.

Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities (Mueller et al., 1996). By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms (Okonko and Shittu, 2007). It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment (Takeno et al., 2005). The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site (Vidali, 2001).Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes (Okonko and Shittu, 2007). Bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate (Vidali, 2001).

The use of microorganisms to destroy, or reduce the concentration of hazardous waste on a contaminated site is called bioremediation. Such a biological treatment system has various applications, including, clean-up of contaminated sites such as water, soils, sludges, and wastewater streams (Boopathy, 2000). Bioremediation methods use microorganisms that occur naturally in the environment and degrade (mineralize) contaminants to less toxic or harmless products like carbon dioxide and water. Biological processes or microbial processes have been used successfully to remediate soils contaminated with petroleum hydrocarbons and their derivatives (Mulligan et al., 2001).

Bioremediation technology using microorganisms was reportedly invented by George M. Robinson. Research on the use of bioremediation to clean-up oil spills dates back to 1942, when the American Petroleum Institute began subsidizing this research (U.S. Congress, 1991). In the simplest terms, bioremediation is the use of microorganisms (bacteria or fungi) to decompose toxic pollutants into less harmful compounds. It is a means of promoting the degradation of an organic compound. To fully understand bioremediation, the term biodegradation must be discussed. Biodegradation is a process by which microbes break down organic compounds into other substances. The resulting products can be carbon dioxide, water, and partially oxidized biologically inert by-products (Bragg et al., 1992).

Bioremediation technologies can be broadly classified as *ex situ* and *in situ*. *Ex situ* technologies are those treatments which involve the physical removal of the contaminated material for treatment process. In contrast, *in situ* techniques involve treatment of the contaminated material in place. Some of the examples of *in situ* and *ex situ* bioremediation are as follows:

Land farming: Solid-phase treatment system for contaminated soils: may be done *in situ* or *ex situ*.Composting: Aerobic, thermophilic treatment process in which contaminated material is mixed with a bulking agent; can be done using static piles or aerated piles. Bioreactors: Biodegradation in a container or reactor; may be used to treat liquids or slurries. Bioventing: Method of treating contaminated soils by drawing oxygen through the soil to stimulate microbial activity. Biofilters: Use of microbial stripping columns to treat air emissions. Bioaugmentation: Addition of bacterial cultures to a contaminated medium; frequently used in both *in situ* and *ex situ* systems. Biostimulation: Stimulation of indigenous microbial populations in soils or ground

water by providing necessary nutrients. Intrinsic bioremediation: Unassisted bioremediation of contaminant; only regular monitoring is done. Pump and treat: Pumping ground water to the surface, treating, and reinjecting (Boopathy, 2000).

Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminant increase in numbers when the contaminant is present; when the contaminant is degraded, the biodegradative population declines. The residues for the treatment are usually harmless products and include carbon dioxide, water, and cell biomass. Generally, bioremediation is useful for the mitigation of pollution load in the environment. Many compounds that are legally considered to be hazardous can be transformed to harmless products. This eliminates the chance of future liability associated with treatment and disposal of contaminated material. Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible. Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation. Bioremediation can prove less expensive than other technologies that are used for clean-up of hazardous waste.

Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation. There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound. Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial

populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants. It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations. Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly dispersed in the environment. Contaminants may be present as solids, liquids, and gases. Bioremediation often takes longer than other treatment options, such as excavation and removal of soil or incineration. Regulatory uncertainty remains regarding acceptable performance criteria for bioremediation. There is no accepted definition of "clean", evaluating performance of bioremediation is difficult, and there are no acceptable endpoints for bioremediation treatments (Vidali, 2001).

2.3 Sources of oily wastewaters

The largest source of wastewater is produced during oil extraction processes in most oil mills and the mill effluents such as palm oil mill effluent (POME) may be categorized as an oily wastewater (Ahmad et al., 2005b).

Oil contaminated wastewaters comes from variety of sources such as palm oil mills, olive oil mills, crude oil production, oil refinery, petrochemical industry, metal processing, compressor condensates, lubricant and cooling agents, car washing, restaurants (Lan et al., 2009). Oily wastewaters contain toxic substances such as phenols, petroleum hydrocarbons, polyaromatic hydrocarbons, which are inhibitory to plant and animal growth, equally, mutagenic and carcinogenic to human being. Similarly, oily wastewaters contain high oil content, chemical oxygen demand (COD) and colour. The increase in global demand for edible oils has in the last few decades

resulted in a tremendous increase in the cultivation of oil seeds, particularly of soybean and oil palm (Yacob et al., 2006). The oil seeds are usually processed to obtain the oil contents which are subsequently processed for human consumption and industrial applications. Thus the vegetable oil industries are, equally, associated with oil extraction, refining, transportation, uses and reuses. However, these industries have been linked with environmental pollutions resulting from oil spill, oily effluent discharge into water bodies and oily sludge discharge into the environment indiscriminately, untreated or in conditions below the standard discharge limits (Alade et al., 2011).

2.3.1 Palm oil mill effluent (POME)

Characteristics of palm oil mill effluent depend on the quality of the raw material and palm oil production processes in palm oil mills. The extraction of crude palm oil from fresh fruit bunches (FFB) requires huge amounts of water (Rupani et al., 2010; Mohammed et al., 2014; Ma, 1999a and b; Ahmad et al., 2003). Sethupathi (2004) has categorized three major processing operations responsible for producing the POME. Sterilization of FFB, clarification of the extracted crude palm oil (CPO), hydrocyclone separation of cracked mixture of kernel and shell hydrocyclone contributes about 36, 60 and 4% of POME respectively in the mills. Yacob et al. (2006) estimated that about 0.5- 0.75 tonnes of POME will be discharged from mill for every tonne of fresh fruit bunch.

Palm oil mill effluent (POME) is an important source of inland water pollution when released into local rivers or lakes without treatment. POME contains lignocellulosic wastes with a mixture of carbohydrates and oil. Chemical oxygen

demand (COD) and biochemical oxygen demand (BOD) of POME are very high and COD values greater than 80,000 mg/L are frequently reported. Incomplete extraction of palm oil from the palm nut can increase COD values substantially (Oswal et al., 2002). The high COD value is responsible for distraction of aquatic life (Maygaonkar et al., 2012).

Wastewater composition depends mainly on the season, raw matter quality and the particular operations being conducted at any given time. Typically, palm oil mill wastewater is low in pH because of the organic acids produced in the fermentation process, ranging about 4-5. It also contains large amounts of total solids (40,500 mg/L), oil and grease (4000 mg/L) (Ma, 2000). Wastewater includes dissolved constituents such as high concentration of protein, carbohydrate, nitrogenous compounds, lipids and minerals, which may be converted into useful materials using microbial processes. The effluents from palm oil mill can cause considerable environmental problems, if discharged untreated (Singh et al., 2010). Therefore, the challenge of converting POME into an environmental friendly waste requires an efficient treatment and effective disposal technique.

This wastewater is a viscous, brownish liquid containing about 95–96% water, 0.6–0.7% oil and 4–5% total solids (including 2–4% SS, mainly debris from the fruit). It is acidic (pH 4–5), hot (80–90°C), nontoxic (no chemicals are added during oil extraction), has high organic content (COD 50,000 mg/L, BOD 25,000 mg/L) and contains appreciable amounts of plant nutrients (Singh et al., 1999).

Palm oil is one of the two most important vegetable oils in the world's oil and fats market following soya beans. Oil palm (*Elaeis guineensis*) is the most productive oil producing plant in the world, with one hectare of oil palm producing between 10 and

35 tonnes of fresh fruit bunch (FFB) per year (Ma et al., 1996). The palm has a life of over 200 years, but the economic life is 20-25 years (nursery 11-15 months, first harvest is 32-38 months from planting and peak yield is 5-10 years from planting) (Igwe and Onyegbado, 2007). Usually, the harvested part is the fruit "fruit bunch "whereby oil is obtained from the fleshy mesocarp of the fruit. Oil extraction from flesh amounts to at least 45-46% while kernel accounts for at least 40-50%. The palm has a highly varied nutrient demand which depends mainly on the yield potential determined by the genetic make-up of the planting material and on yield limit set by climatic factors such as water, effective sunshine and temperature (Igwe and Onyegbado, 2007).

Crude palm oil contains fatty acid ester of glycerol commonly referred to as triglycerides, therefore, contributing to the worlds need of edible oil and fats. It is composed of approximately 50% saturated fats (primarily palmitic acid) and 40% unsaturated fats (principally linolenic and oleic acid); a unique composition if compared with other major fats. The distinctive colour of the oil is due to the fat soluble carotenoids (pigment) which are also responsible for its vitamins E (tocopherols and tocotrienols) content (Igwe and Onyegbado, 2007).

Fresh fruit bunches (FFB) harvested from the oil palms are processed in palm oil mills and turned into crude palm oil and the palm kernel. Generally, palm oil mills are located in the plantations to facilitate the transportation and processing of the FFB. The palm oil milling process basically involves the physical extraction of the palm products (Hii et al., 2012).

There exists several processing stages in the extraction of crude palm oil from fresh fruit bunches. The first stage is sterilization. This involves subjecting freshly harvested fruit bunches brought to the mill to a high pressure steam (120 to 140°C at 40

psi (275790 Newton / square meter [N/m^2]) with a minimal delay so as to inactivate the lipolytic enzymes that causes oil hydrolysis and fruit deterioration. This stage also prevent further formation of free fatty acids due to enzyme action and preparing the fruit bunches for the subsequent sub processes (Igwe and Onyegbado, 2007; Hii et al., 2012). The next stage is called bunch stripping. This offers a means of separating the fruits from the bunch stalks by mechanical stripping. The separated and sterilized fruits thereafter undergo a process of digestion. This is achieved by reheating the fruits using steam to a temperature of 80-90°C. This prepares the fruits for oil extraction by rupturing the oil bearing cells in the mesocarp and loosening the mesocarp from the nuts. Oil extraction followed by clarification and purification are the last processes of oil extraction. The crude oil is extracted from the digested fruit mash by the use of the screw press without kernel breakage (Hui, 1992).

The extracted liquid and nuts are discharged from the screw press. However, the extracted oil contains varying amounts of water, solids and dissolved impurities that must be removed. The fiber particles from the pressed crude oil are first removed by passing the oil over a vibrating screen; sand and dirt are allowed to settle. Water is removed by settling or centrifuging and finally by vacuum drying. It is worthy to note that the moisture content of the clarified crude oil is still about 0.1-0.25% of moisture (Hui, 1992). This helps in maintaining oxidative stability and also prevents the deposition of small amounts of soluble solids known as gums. The final product is consumed locally as crude palm oil or can further be refined (Igwe and Onyegbado, 2007).For every 100 kg of fruit bunches, typically 22 kg of palm oil and 1.6 kg of palm kernel oil can be extracted (Gunawan et al., 2009). However, enormous and large

amounts of palm oil residues or pollutants are produced at the same time, which might result in serious environmental pollution (Hii et al., 2012).

2.3.2 Characteristics of palm oil mill effluent (POME)

Large amount of waste are produced in the palm oil mill industry. The process of oil extraction results in generation of liquid waste commonly named as palm oil mill effluent (POME) (Rupani et al., 2010; Mohammed, 2014). Palm oil mill effluent is generated mainly from oil extraction, washing and cleaning processes in the mill and these contains cellulosic material, fat, oil and grease etc (Agamuthu, 1995). Palm oil mill effluent also contains substantial quantities of solids, both suspended solids and total dissolved solids in the range of 18,000 mg/L and 40,500 mg/L respectively (Table 2.1). These solids are commonly named palm oil mill sludges (POMS). The solid waste that are produced in the process of extraction are the leaves, trunk, decanter cake, empty fruit bunches, seed shells and fiber from the mesocarp (Rupani et al., 2010).

Fresh POME is a hot, acidic (pH between 4 and 5), brownish colloidal suspension containing high concentrations of organic matter, high amounts of total solids (40,500 mg/L), oil and grease (4,000 mg/L) COD (50,000 mg/L) and BOD (25,000 mg/L) (Ma, 2000). The characteristics of typical POME are given in Table 2.1. According to Vairappan and Yen (2008), 66.8 million tonnes of POME was generated in year 2005. The raw or partially treated POME has an extremely high content of degradable organic matter. As no chemicals were added during the oil extraction process, POME is considered as non toxic, but it is identified as a major source of aquatic pollution by depleting dissolved oxygen when discharged untreated into the water bodies (Khalid and Wan Mustafa, 1992). However it also contains appreciable

amounts of N, P, K, Mg and Ca (Habib et al., 1997; Muhrizal et al., 2006), which are the vital nutrient elements for plant growth. Due to the non toxic nature and fertilizing properties, POME can be used as fertilizer or animal feed substitute, in terms of providing sufficient mineral requirements. Agamuthu et al. (1995) has also reported the increase of organic nitrogen leading to the production of a better fertilizer in POME.

Muhrizal et al. (2006) reported that POME contains high content of Al as compared to chicken manure and composted sawdust. According to Habib et al. (1997) toxic metals, such as Pb, can also be found in POME, but their concentrations are usually below sub lethal levels (greater - than 17.5 μg /g) (James et al., 1996). According to James et al. (1996), Pb is found in POME as a result of contamination from plastic and metal pipes, tanks and containers where Pb is widely used in paints and glazing materials. The effluent discharge standards limits for crude palm oil mills are presented in Table 2.2.

Table 2.1: Characteristics of raw palm oil mill effluent (POME)

Parameters	Value
Temperature (°C)	80[a] - 90[a]
pH	4.5[b] - 4.7[a]
Biochemical Oxygen Demand ($BOD_{3)}$, 3days at 30 °C	25,000[a] - 52,100[b]
Chemical Oxygen Demand (COD)	50,000[a] - 95,000[b]
Total Solids (TS)	40,500[a] - 43,000[b]
Total Suspended Solids (TSS)	12,500[b] - 18,000[a]
Total Volatile Solids (TVS)	34,000[a]
Oil and Grease (O&G)	4,000[a] - 11,000[b]
Ammonia-Nitrate (NH_3-N)	35[a]
Total Kjeldahl nitrogen (TKN)	750[a]

*All values, except pH and temperature, are expressed in mg/L

Source: Ma (2000) [a]; Soleimaninanadegani and Manshad (2014) [b].

Table 2.2: Effluent discharge standards limits for crude palm oil mills.

Parameters	Discharge effluent standard	
	Malaysia	Thailand
Chemical oxygen demand (COD)	-	< 1000
Biochemical oxygen demand (BOD)	100	<100
Total suspended solids (TSS)	400	<150
Volatile suspended solids (VSS)	-	-
Oil & grease (O&G)	50	<25
pH	5-9	5-9

All parameters are in mg/L except pH.

Source: Malaysia: Environmental Quality Act 1974 (2014); Thailand: Environmental Management Guideline (1997).

2.4 Treatment of POME

The treatment technologies for POME are usually combination of physical, chemical and biological processes of wastewater treatment. There are four types of treatment processes commonly employed to treat POME in most of the local palm oil mills and this vary from one mill to the other. This may be attributed to the plant design or inclusion of new production and treatment technologies.

The pre-treatment of POME is physical wastewater treatment process, which includes stages such as screening, sedimentation and oil removal in oil traps prior to the secondary treatment in biological treatment systems (Jameel and Olanrewaju, 2011). Residual oil and fat are usually recovered by oil trappers, titled-plate separators, dissolved air flotation units, centrifuges and electro-flotation systems (Phalakornkule et al., 2010). Prior to the separation by physical treatment, as found in some mills, coagulants and flocculants such as ferric chloride, aluminum sulphate, lime and polyelectrolyte are used to reduce the total fatty matter and other suspended solids (Industrial Processes and The Environment, 1999).

POME contains large amount of organic matter, which is generally biodegradable and this facilitates the application of biological treatment based on anaerobic, aerobic and facultative processes (Jameel et al., 2011).The biological treatment depends greatly on a consortium of active microorganisms, which utilizes the organic substances present in the POME as nutrients and eventually degrades these organic matters into simple by-product gases such as methane, carbon dioxide, hydrogen sulphide and water (Jemeel et al., 2011). The biological treatment process involves a large reservoir to hold the POME in place for the effective biodegradation which often take some days depending on the type and population of the

microorganisms. This development led to the use of ponds and tanks that characterized most of the mills (Khalid and Wan Mustafa, 1992).

Land application of POME has become a standard practice in mills where they have plantations nearby. The anaerobically digested POME contains high concentrations of plant nutrients. The application of the effluent to the cropland, not only provides nutrient and water to the vegetation, but also a means of alternative disposal of the effluent. This has resulted in substantial saving in fertilizer bills and increased income due to higher crop yield. However such practices are only feasible to the plantation groups with enough land in the vicinity of their palm oil mills (Ahmad et al., 2003; Foo and Hameed, 2010).

Evaporation is one of the most widely used unit operation in water regeneration in a wide range of processing applications. POME is made up of about 95 - 96% water thus by this evaporation technology the water could be recovered and the residual solid can be concentrated for advanced utilization (Ma, 2000). The energy requirement is the major consideration in the evaporation technology. However the heat required is largely inherent in the fresh POME itself which is discharged at a temperature of above 80°C. The additional energy can be economically generated from the surplus electricity normally generated by palm oil mills or combustions of empty fruit bunch (EFB) (Ma, 2000).

Various attempts have been made to treat POME by biological treatment either anaerobic or aerobic and physicochemical treatment methods. Many studies have been conducted with various methods and technologies to treat POME before discharge into receiving water bodies (Rupani et al., 2010).

The most commonly used treatment method is the anaerobic treatment which is carried out by open pond system (Chin et al., 1996) or open digesting tank system (Yacob et al., 2006). Other anaerobic treatment processes includes anaerobic suspended growth process (Norli et al., 2006), attached growth on a rotating biological contactor (RBC), expanded granular sludge bed (EGSB) reactor, upflow anaerobic sludge - fixed film bioreactor (UASFF) and closed digesting tank system (Najafpour et al., 2005). Other improved anaerobic treatment methods includes membrane anaerobic system (MAS) (Abdulrahman et al., 2011), semi - commercial closed digester tank with sludge recycling and anaerobic sequencing batch reactor with ozonation pretreatment (Chaiprapat and Laklam, 2011). POME treatment has been successfully conducted with up-flow anaerobic sludge blanket (UASB) reactor, achieving COD removal efficiency up to 98.4% (Borja and Banks 1994). The anaerobic contact digestion process has been implemented in POME (Ibrahim et al. 1984). Borja and Banks (1995) have also utilized anaerobic filter for POME treatment. The advantages and disadvantages between anaerobic and aerobic treatment of POME are presented in Table 2.3.

The physicochemical method of POME treatment includes methods such as sedimentation by coagulation and flocculation (Ahmad et al., 2005a; Bhatia et al., 2007), membrane filtration process (Wu et al., 2007), adsorption (Ahmad et al., 2005c) and electrocoagulation (Phalakornkule et al., 2010; Agustin et al., 2008).

Table 2.3: Advantages and disadvantages between anaerobic and aerobic treatment of POME

Treatment Types	Advantages	Disadvantages	Reference
Anaerobic	Low energy requirements (no aeration), producing methane gas as a valuable end product, generated sludge from process could be used for land applications.	Long retention time, slow start-up (granulating reactors), large area required for conventional digesters.	Metcalf (2003), Borja et al. (1996).
Aerobic	Shorter retention time, more effective in handling toxic wastes.	High energy requirement (aeration), rate of pathogen inactivation is lower in aerobic sludge compared to anaerobic sludge, thus unsuitable for land applications.	Doble and Kumar (2005).

2.5 Treatment of wastewaters

Some of the common conventional methods of wastewater treatment include flotation, gravitational methods, chemical treatment, biological treatment, dissolved air flotation (DAF) and use of membranes (Chowdhury et al., 2006). Oil droplets of about 50 μm have been removed effectively by low cost equipment such as API separators, Corrugated Plate Interceptors (CPI), and Parallel Plate Interceptors (PPI), for primary treatment of oily water (Wang et al., 2004). Oil droplets less than 50 μm size have been removed by packed beds and dissolved air flotation (DAF) (Rubio et al., 2002). De-emulsification which is effective in breaking stable emulsions using chemicals such as sulphuric acid, iron and aluminum sulphate and reducing the amount of oil in the water,