Microbiota of Palm Oil Mill Wastewater in Malaysia

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Abstract: This study was aimed at identifying indigenous microorganisms from palm oil mill effluent (POME) and to ascertain the microbial load. Isolation and identification of indigenous microorganisms was subjected to standard microbiological methods and sequencing of the 16S rRNA and 18S rRNA genes. Sequencing of the 16S rRNA and 18S rRNA genes for the microbial strains signifies that they were known as Micrococcus luteus 101PB, Stenotrophomonas maltophilia 102PB, Bacillus cereus 103PB, Providencia vermicola 104PB, Klebsiella pneumonia 105PB, Bacillus subtilis 106PB, Aspergillus fumigates 107PF, Aspergillus nomius 108PF, Aspergillus niger 109PF and Meyerozyma guilliermondii 110PF. The results indicate that the population of heterotrophic bacteria (THB) ranges from 9.5 × 10^5 to 7.9 × 10^6 cfu/mL. The population of heterotrophic fungi (THF) ranges from 2.1 × 10^4 to 6.4 × 10^4 cfu/mL. The population of indigenous microorganisms in the medium agar CMC ranges from 8.2 × 10^5 to 9.1 × 10^6 cfu/mL (bacteria) and 1.4 × 10^3 to 3.4 × 10^3 cfu/mL (fungi). The study concludes that the indigenous microorganisms are capable of surviving and thriving in the palm oil mill effluent (POME). This indicates that the isolation of indigenous microorganisms is important for bioremediation, bio-treatment and biodegradation of industrial waste.

Kata kunci: Biodegradasi, Industri, Malaysia, MALPOM Sdn. Bhd., Microbiota, Efluen Kilang Minyak Sawit (POME), Sisa Air

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vermicola 104PB, Klebsiella pneumoniae 105PB, Bacillus subtilis 106PB, Aspergillus fumigatus 107PF, Aspergillus nomius 108PF, Aspergillus niger 109PF and Meyerozyma guilliermondii 110PF. Results revealed that the population of total heterotrophic bacteria (THB) ranged from $9.5 \times 10^5$ – $7.9 \times 10^6$ cfu/mL. The total heterotrophic fungi (THF) ranged from $2.1 \times 10^4$ – $6.4 \times 10^4$ cfu/mL. Total viable heterotrophic indigenous microbial population on CMC agar ranged from $8.2 \times 10^5$ – $9.1 \times 10^6$ cfu/mL and $1.4 \times 10^3$ – $3.4 \times 10^3$ cfu/mL for bacteria and fungi respectively. The microbial population of oil degrading bacteria (ODB) ranged from $6.4 \times 10^5$ – $4.8 \times 10^6$ cfu/mL and the oil degrading fungi (ODF) ranged from $2.8 \times 10^3$ – $4.7 \times 10^4$ cfu/mL. The findings revealed that microorganisms flourish well in POME. Therefore, this denotes that isolating native microorganisms from POME is imperative for effectual bioremediation, biotreatment and biodegradation of industrial wastewaters.

**Keywords:** Biodegradation, Industry, Malaysia, MALPOM Sdn. Bhd., Microbiota, Palm Oil Mill Effluent (POME), Wastewater

**INTRODUCTION**

Industrial wastewaters are essential habitat for diverse microbes. Generally, some of the microorganisms have been used for biotreatment of wastewaters (Abdel-Raouf et al. 2012; Bala et al. 2014a, 2014b, 2014c; Bala et al. 2015a, 2015b; Bala 2016). Microorganisms domiciled in diverse wastewaters can also cause diseases such as tuberculosis, cholera, typhoid, dermatomycosis, hepatitis and dysentery (Shaaban et al. 2004).

Palm oil industry has become one of the most important agricultural based industries in Malaysia that produce colossal amount of oily liquid wastewater universally named as palm oil mill effluent (POME) (Ahmad et al. 2005; Rupani et al. 2010; Mohammed et al. 2014). Palm oil mill wastewater is produced during oil extraction processes in palm oil mill industries. POME is an extremely polluting wastewater that contaminates the environment when released directly into rivers, streams or lakes devoid of treatment.

POME; in addition include large amounts of solids, both suspended solids and total dissolved solids in the range of 18,000 mg/L and 40,500 mg/L correspondingly. These solids are usually named palm oil mill sludges (POMS). The solid waste that are formed in the process of extraction are the leaves, trunk, decanter cake, empty fruit bunches, seed shells and fibre from the mesocarp (Rupani et al. 2010).

Raw POME is a warm, acidic (pH between 4 and 5), brownish colloidal suspension having lofty concentrations of organic matter, elevated amounts of total solids (40,500 mg/L), oil and grease (4,000 mg/L), chemical oxygen demand (COD) (50,000 mg/L) and biochemical oxygen demand (BOD) (25,000 mg/L) (Ma 2000). The wastewater from palm oil mill can cause significant ecological problems, if released untreated (Singh et al. 2010). The chemical oxygen demand (COD) and biochemical oxygen demand (BOD) values of palm oil mill wastewater are high enough to cause serious pollution and environmental problem to the rivers. Chemical oxygen demand and biochemical oxygen demand of palm oil mill
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wastewater are very high and COD values greater than 60,000 mg/L are often reported (Bala et al. 2015a; Bala 2016). Accordingly, the adverse environmental impact from the palm oil industry cannot be overlooked. Consequently, the challenge of converting POME into an environmental friendly waste necessitates a well-organised treatment and effectual removal method.

The physicochemical properties of POME are well documented. Conversely, the microbiological aspect is overlooked; as such there seem to be dearth of information on the microbiota been documented proving that a well-developed understanding of these is needed. Therefore, this study represents one of the few studies in Malaysia. The diverse microbiota communities are known to participate effectively in the biodegradation and bioremediation of POME. Consequently, the study on the microbiological characteristics of POME lays a basis to promote better understanding of the types and nature of microorganisms domicile in POME. This will provide evidence of the microbiota characteristics of POME. Their involvement in biodegradation and biotreatment of POME may possibly abet in achieving higher reduction of organic load present in POME. This study was designed to explore the microorganisms associated with palm oil mill wastewater and to establish the microbial load from MALPOM Sdn. Bhd. in Pulau Pinang, Malaysia.

MATERIALS AND METHODS

Sample Collection and Preservation

Raw POME was collected aseptically from MALPOM Sdn. Bhd., Pulau Pinang, palm oil mill industry in a sterile microbiological container (20 L) and brought back to the laboratory. In collecting raw POME sample from the POME holding tank, the mouth of the tap connected to the holding tank was swabbed with cotton wool soaked in ethanol. This was done in order to disinfect the mouth of the tap. The tap was allowed to run for few minutes and the container was used to collect the POME sample and quickly corked. Prior to sample collection, the POME sample inside the container was inverted a few times in ordered to rinse the inside wall of the container. The sample was later poured out into the surrounding. This step was done three times and the container was finally placed to collect the POME sample. The POME sample was kept in an ice box while transporting to School of Industrial Technology laboratory, Universiti Sains Malaysia and preserved at 4°C until further experiment in order to stop the wastewater from undergoing biodegradation due to microbial action (American Public Health Association [APHA] 2005). Sample was brought out from the refrigerator and left at room temperature before use.
Isolation and Enumeration of Total Heterotrophic Indigenous Palm Oil-Utilising and Cellulose Utilising Bacteria From POME

The populations of microorganisms in the raw POME sample was enumerated using standard spread plate method (APHA 2005; Bala et al. 2015a; Bala 2016). The POME was well shaken to homogenised suspension and thereafter, ten-fold (10-fold) serial dilution was made by aseptically transferring one milliliter (1 mL) of the homogenised suspension into a sterile test tubes containing nine milliliter (9 mL) of sterile, distilled water. Then, using a sterile pipette, 0.1 mL aliquots of the dilutions were aseptically removed with a sterile pipette and separately spread plated with flamed-sterilised glass spreader (bent glass rod) on well-dried Nutrient Agar (NA), oil agar (Palm Oil Agar [POA]) Mineral Salts Medium (MSM) for bacteria and Carboxymethyl cellulose (CMC) agar plates for bacteria in triplicates for the enumeration of viable heterotrophic bacteria, palm oil utilising and cellulose utilising bacteria respectively. The plates were inoculated using spread plate technique (APHA 2005; Bala et al. 2015a; Bala 2016). The culture plates were incubated at 37°C for 24–48 h. Three uninoculated plates were used as control. After incubation, plates that contained 30–300 colony forming units (cfu) were selected and counted with the aid of a colony counter. Viable numbers of colonies on each plate were enumerated and expressed or recorded as colony forming units per milliliter (cfu/mL) of the sample. Colonies were purified by repeatedly subcultured aseptically on to fresh NA, oil agar and CMC agar and incubated at 37°C for 48 h to obtain discrete pure colonies. Pure colonies were then stored on NA, oil agar and CMC agar slants at 8°C to maintain viability for subsequent analysis and identification. Gram staining was performed for all the isolates. The medium was incorporated with Ketoconazole antifungal (known as funginox) to inhibit fungal growth.

Preparation and Composition of Mineral Salt Medium (MSM) for Palm Oil Utilising Bacteria

The MSM (oil agar medium) for palm oil utilising bacteria was prepared according to the MSM composition of Zajic and Supplisson (1972). The composition of the medium was NH₄Cl (4.0 g), K₂HPO₄ (1.8 g), KH₂PO₄ (1.2 g), MgSO₄·7H₂O (0.2 g), NaCl (0.1 g), FeSO₄ (0.01 g), 15 g agar and distilled water, 1 L). The medium was used for isolation, enumeration and identification of palm oil-utilising bacteria (oil degraders). The medium was prepared by the addition of 1% (v/v) palm oil as sole source of carbon and energy, sterilized with 0.45 µm pore size Millipore filter paper to sterile MSM, which has been cooled to 45°C under aseptic condition. 200 mg ketoconazole antifungal (known as funginox) was added to prevent fungal growth. The MSM and palm oil were then mixed thoroughly and dispensed into sterile Petri dishes to solidify.
Isolation and Enumeration of Total Heterotrophic Indigenous Palm Oil-Utilising and Cellulose Utilising Fungi from POME

The standard procedures for serial dilution aforementioned for bacterial isolation were followed for fungal isolation. Thereafter, using a sterile pipette, 0.1mL aliquots of the dilutions were aseptically removed with a sterile pipette and separately spread plated with flamed-sterilised glass spreader (bent glass rod) on well-dried Potato Dextrose Agar (PDA), oil agar (POA) MSM for fungi and Carboxymethyl cellulose (CMC) agar plates for fungi in triplicates for the enumeration of viable heterotrophic fungi, palm oil utilising and cellulose utilising fungi respectively. The plates were inoculated on the surface using the standard spread plate technique (APHA 2005). The plates were allowed to remain undisturbed for 25 min in the laminar flow before been inverted and incubated.

The culture plates were incubated at 28°C for 5–7 days (APHA 2005). Three uninoculated plates were used as control. After incubation, viable numbers of colonies on each plate were enumerated and expressed or recorded as colony forming unit per milliliter (cfu/mL). Colonies were purified by repeatedly sub culturing aseptically on to fresh PDA, oil agar and CMC agar and incubated at 28°C for 5–7 days to obtain discrete pure colonies. Pure colonies were then stored on PDA, oil agar and CMC agar slants at 8°C to maintain viability for subsequent analysis and identification. Staining was also performed for all the isolates using lacto phenol cotton blue solution. The medium was incorporated with Altacef antibiotic to inhibit bacterial growth.

Preparation and Composition of Mineral Salt Medium (MSM) for Palm Oil Utilising Fungi

The MSM (oil agar medium) for palm oil utilising fungi was prepared according to the MSM composition of Mills et al. (1978) as modified by Okpokwasili and Okorie (1988). The composition of the medium was NaCl, 10.0g; MgSO₄·7H₂O, 0.42g; KCl, 0.29 g; KH₂PO₄, 0.83 g; Na₂HPO₄, 1.25 g; NaNO₃, 0.42 g; agar, 20 g; distilled water, 1 L and pH of 7.2. The medium was used for isolation, enumeration and identification of palm oil-utilising fungi (oil degraders). The medium was prepared by the addition of 1% (v/v) palm oil as sole source of carbon and energy, sterilised with 0.45 μm pore size Millipore filter paper to sterile MSM, which has been cooled to 45°C under aseptic condition. 250 mg Altacef antibiotic, was added to prevent bacterial growth. The MSM and palm oil were then mixed thoroughly and dispensed into sterile Petri dishes to solidify.

Identification of Bacteria Isolates by Sequencing of 16S rRNA Gene

Initial identification of individual bacterial isolates was achieved by standard tests (Bergey et al. 1994). Such identification included the shape of cells, Gram’s reaction and colony morphology on solid nutrient media. Genetic identification of bacterial
isolates was performed by determining nucleotide sequences of 16S rRNA genes using commonly used primers (Table 1) for amplifying the DNA between positions 27 and 1492 of bacterial 16S rRNA genes. Genetic identification of the pure cultures of bacterial isolated from POME were sent to Centre for Chemical Biology (CCB), Universiti Sains Malaysia for sequencing of the 16S rRNA gene. Inoculum preparation was carried out by inoculating bacteria strains in nutrient broth, fungi in potato dextrose broth, incubated for 24 h (bacteria), 2–3 days (fungi) at 37°C and 28°C respectively.

**Table 1:** Genetic Identification of bacterial isolates in POME

| Strains               | Nucleotide Sequences 16S rRNA gene | Sequences of Primers  |
|-----------------------|------------------------------------|-----------------------|
| Micrococcus luteus    | 101PB                              | 27F: 5’- AGAGTTTGATCMTGGCTCAG-3’ |
| Stenotrophomonas maltophilia | 102PB                       | 1492R: 5’-GGGTTACCTTGTTACGACTT-3’ |
| Bacillus cereus       | 103PB                              |                        |
| Providencia vermicular | 104PB                              |                        |
| Klebsiella pneumoniae | 105PB                              |                        |
| Bacillus subtilis     | 106PB                              |                        |

**Identification of Fungal Isolates by Sequencing of 18S rRNA Gene**

Initial identification of individual fungal isolates was based on microscopic staining of fungi using lactophenol blue solution (Lactophenol cotton blue solution) and macroscopic appearance which comprise pigmentation/colour, identified on the basis of cultural (colour and colonial appearance of fungal colony) and morphological characteristics in lacto-phenol blue solution wet mount by compound microscope. Genetic identification of fungal isolates was performed by determining nucleotide sequences of 18S rRNA genes using commonly used primers (Table 2) for amplifying the DNA. Genetic identification of the pure cultures of fungal isolated from POME were sent to CCB, Universiti Sains Malaysia for sequencing of the 18S rRNA gene.

**Table 2:** Genetic Identification of fungal isolates in POME

| Strains               | Nucleotide Sequences 18S rRNA genes | Sequences of Primers  |
|-----------------------|------------------------------------|-----------------------|
| Aspergillus fumigatus | 107PF                              | ITS1 F: 5’-TCCGTAGGTGAAACCTGCGG -3’ |
| Aspergillus nomius    | 108PF                              | ITS4 R: 5’-TCCTCCGCTTATTGATATGC-3’ |
| Aspergillus niger     | 109PF                              |                        |
| Meyerozyma guillermondii | 110PF                         |                        |
RESULTS AND DISCUSSION

Microbial Populations of POME Sample

The microbial population, total heterotrophic bacteria (THB) and total heterotrophic fungi (THF) of POME are presented in Table 3 and oil degrading bacteria (ODB) and oil degrading fungi (ODF) are presented in Table 4.

Table 3: Microbial populations of POME.

| Media                        | Isolates     | Total heterotrophic counts (THC) |
|------------------------------|--------------|----------------------------------|
| Nutrient agar (NA)           | Bacteria     | $9.5 \times 10^5 - 7.9 \times 10^6$ cfu/mL |
| Potato Dextrose agar (PDA)   | Fungi        | $2.1 \times 10^4 - 6.4 \times 10^4$ cfu/mL |
| Carboxymethyl cellulose (CMC) agar | Bacteria | $8.2 \times 10^5 - 9.1 \times 10^6$ cfu/mL |
| Carboxymethyl cellulose (CMC) agar | Fungi     | $1.4 \times 10^3 - 3.4 \times 10^3$ cfu/mL |

Table 4: Oil degrading microbes of POME.

| Media                        | Isolates     | Counts (cfu/mL) |
|------------------------------|--------------|-----------------|
| Oil agar (MSM) Palm oil agar (POA) | Bacteria | $6.4 \times 10^5 - 4.8 \times 10^6$ |
| Oil agar (MSM) Palm oil agar (POA) | Fungi     | $2.8 \times 10^3 - 4.7 \times 10^4$ |

The population of total heterotrophic bacteria (THB) ranged from $9.5 \times 10^5 - 7.9 \times 10^6$ cfu/mL. The total heterotrophic fungi (THF) ranged from $2.1 \times 10^4 - 6.4 \times 10^4$ cfu/mL. Total viable heterotrophic indigenous (authochthonous) microbial population on CMC agar ranged from $8.2 \times 10^5 - 9.1 \times 10^6$ cfu/mL and $1.4 \times 10^3 - 3.4 \times 10^3$ cfu/mL for bacteria and fungi respectively. The microbial population of oil degrading bacteria (ODB) ranged from $6.4 \times 10^5 - 4.8 \times 10^6$ cfu/mL and the oil degrading fungi (ODF) ranged from $2.8 \times 10^3 - 4.7 \times 10^4$ cfu/mL (Tables 3 and 4). The findings revealed that ODB and ODF flourish well in oily waste water. Awotoye et al. (2011) reported THB, THF, ODF and ODB population of $1.8 \times 10^6$ cfu/g, $9.5 \times 10^2$ cfu/g, $1.2 \times 10^2$ cfu/g and $4.0 \times 10^2$ cfu/g in that order at the point of POME release from oil palm milling machine. Ugoji (1997) specified that THB and THF are $1.3 \times 10^6$ cfu/mL and $1.0 \times 10^3$ cfu/mL correspondingly in POME.

In a related study, Okwute and Isu (2007a; 2007b) have reported total aerobic bacterial populations of $9.6 \times 10^8$ cfu/mL, $1.64 \times 10^9$ cfu/mL and $1.07 \times 10^9$ cfu/mL in POME samples. In addition, Okwute (2013) has also confirmed the population of THB, THF and ODB as $4.0 \times 10^9$ cfu/mL, $2.6 \times 10^3$ cfu/mL and $2.6 \times 10^3$ cfu/mL in that order. The counts were also comparable to those described by Serikovna et al. (2013) with the index of $10^8$ cfu/mL, $10^7$ cfu/mL and $2 \times 10^8$ cfu/mL as well as Wu et al. (2009) who revealed in their study the count of $6.65 \times 10^6$ cfu/mL from oily wastewaters. Ohimain et al. (2012a) has also stated that the population of total heterotrophic bacteria (THB) ranged from
7.4 × 10^5 – 2.0 × 10^6 cfu/mL and total heterotrophic fungi (THF) ranged from 3.1 – 5.7 × 10^4 cfu/mL while the oil degrading bacteria (ODB) ranged from 6.5 × 10^5 – 2.0 × 10^6 cfu/mL and the oil degrading fungi (ODF) ranged from 3.1 – 5.6 × 10^4 cfu/mL in POME sample. Bala et al. (2012) has also reported similar counts from pharmaceutical wastewater. These corroborate the presence of diverse microorganisms in wastewaters (Bala 2016).

Results from the present study aforementioned confirmed some disparity in the microbial counts. The variations in the range of microbial populations are an indication of several reasons such as nutrient, minerals, temperature, oxygen level, acidity, volume of wastewater (Okereke et al. 2007), concentration of oil and grease and sugars in the POME. High population of bacteria in the POME may possibly be linked with contaminations from poor sanitation in the mills (Okechalu et al. 2011), and intermittent disinfection of the environment. Besides, it may also be due to the handling process and the existing environmental conditions in the mills. The presence and growth of viable bacteria and fungi in POME may possibly be associated with the fact that POME is rich in carbohydrates, proteins, nitrogenous compounds, lipids, minerals, cellulose, hemicelluloses and lignin (Hii et al. 2012). The microbes isolated in the present study conceivably derive their nutrients from the aforementioned compounds in raw POME.

The microbial species found in POME has the prospective to degrade carbon source present in the POME. Bala et al. (2014b) and Bala (2016) has reported that Micrococcus luteus 101PB, Stenotrophomonas maltophilia 102PB, Bacillus cereus 103PB and Bacillus subtilis 106PB showed high lipase activity on solid media indicating their ability for degrading lipid (oil) as carbon source and producing lipase enzyme. The types of organisms isolated in the present study were also identified as oil degrading microorganisms by Bharathi and Vasudevan (2001) and Rahman et al. (2002) because of their ability to hydrolyse lipid (oil). Biodegradation is connected with the capability of bacteria and fungi to grow on and degrade carbon sources in industrial wastewaters (Haimann 1995). The high organic matter in palm oil mill wastewater possibly will have played an essential role in the abundance of aerobic and facultative anaerobic microbial strains in the present study.

Genetic Identification of Bacteria and Fungi Isolates in POME Sample

Tables 1 and 2 present the microorganisms isolated from POME based on 16S rRNA gene and 18S rRNA genes for bacteria and fungi respectively. Identification of isolates was performed by determining nucleotide sequences of 16S rRNA and 18S rRNA genes for bacteria and fungi in that order. The isolates were identified by sequences analysis of 16S rRNA and 18S rRNA genes. Sequencing of the 16S rRNA and 18S rRNA of the microbial strains suggest that they were known as Micrococcus luteus 101PB, Stenotrophomonas maltophilia 102PB, Bacillus cereus 103PB, Providencia vermicola 104PB, Klebsiella pneumoniae 105PB, Bacillus subtilis 106PB, Aspergillus fumigatus 107PF, Aspergillus nomius 108PF,
Aspergillus niger 109PF and Meyerozyma guilliermondii 110PF. Plates and Figures showing identified bacteria and fungi in POME sample is presented in Appendix A to H.

The results from the present study revealed that the microbes isolated are comparable to those found in areas polluted with wastewaters (Abass et al. 2012; Soleimaninanadegani & Manshad 2014; Bala et al. 2015a) and crude oil or petroleum hydrocarbons (Okereke et al. 2007). Bala et al. (2012) had also reported the isolation of Bacillus subtilis from industrial wastewater. Conversely, Micrococcus luteus 101PB, Stenotrophomonas maltophilia 102PB, Bacillus cereus 103PB, Bacillus subtilis 106PB, Aspergillus fumigatus 107PF and Aspergillus niger 109PF are lipase and cellulase producing organisms isolated from the present study.

The development of spores makes POME microorganisms to be quiescent and highly resistant to lethal consequence of boiling, dry heating and ultra violet radiation from the sunlight (Okechalu et al. 2011). Palm oil mill wastewater is a possible habitat for lipolytic and cellulolytic bacteria and fungi since it is rich in nutrients such as lipids (oil) and cellulosic materials (Ohimain et al. 2012a; 2012b; Bala 2016).

Ohimain et al. (2012a) isolated lipase and cellulase producing Bacillus sp from POME collected from palm oil processing environment. Asikong (1994) identified Aspergillus sp. as fungal species linked with lipase and cellulase production. Aspergillus sp. is particularly reported to be good producers of cellulase and lipase. These enzymes are responsible for the breakdown of cellulose and oil in POME (Wong et al. 2008). Aspergillus niger and Aspergillus fumigatus have been well-known for their capability to survive in oily wastewater such as Palm oil mill wastewater due to the presence of nutrients such as lipids (oil). Fungi are particularly aerobic and can also grow under environmental strained conditions such as low pH and poor nutrient status. Lipase facilitates the hydrolysis of lipid causing succeeding breakdown into fatty acid and alcohol (Guehi et al. 2007; Ghosh et al. 1996). Other researchers have also isolated comparable microbes aforementioned above at 28°C-37°C from POME sample (Bhumibhamon et al. 2002; Ohimain et al. 2012a; 2012b; Okwute 2013; Soleimaninanadegani & Manshad 2014; Bala 2016).

The prevalence of these microbes (bacteria and fungi) in palm oil mill wastewater may perhaps be due to their capability to make use of oil and cellulose as their sole carbon source which has been formerly reported by Ojumu et al. (2005), Bala et al. (2014b), Bala et al. (2015b), and Bala (2016). The use of POME as a carbon source by these microorganisms has been reported by Wu et al. (2007), Sira et al. (2010) and Bala (2016). The presence of Micrococcus luteus 101PB, Stenotrophomonas maltophilia 102PB, Bacillus cereus 103PB, Bacillus subtilis 106PB, Aspergillus fumigatus 107PF and Aspergillus niger 109PF isolated from POME sample in the current study revealed that these microorganisms are capable of biodegradation of oily wastewaters as reported by other researchers (Ohimain et al. 2012a; 2012b; 2012c; Nwuche & Ogbonna 2011).
Microorganisms present in POME have been used for the treatment of wastewaters such as palm oil mill wastewater and olive oil mill wastewater for the reduction of COD (Oswal et al. 2002; Ohimain et al. 2012a; Kamal et al. 2011; Neoh et al. 2013; Nawawi et al. 2010; Ahmad et al. 2011; Bala et al. 2014c; Bala et al. 2015a; Bala 2016). During degradation process, oil and cellulose in POME are broken down by effective microbes which make use of the organic waste present in palm oil mill wastewater and degrades these organic matters into water and carbon dioxide (Singh et al. 2010; Jameel & Olanrewaju 2011). Aspergillus fumigatus 107PF, Aspergillus niger 109PF, Micrococcus luteus 101PB, Stenotrophomonas maltophilia 102PB, Bacillus cereus 103PB, and Bacillus subtilis 106PB have been isolated for POME with potential to degraded oil and cellulose (Bala et al. 2014b; Bala et al. 2015b; Bala 2016). The aforesaid microbes thus exhibited comparable biodegradation potential with published literatures. The oily habitat in palm oil mill wastewater possibly will make available a good environment for lipolytic microorganisms to grow due to the oil present in the wastewater which serves as carbon source. However, the present of these microbes in POME are useful in degrading contaminated pollutants in wastewaters such as crude oil (hydrocarbon) (Ohimain et al. 2012a; Soleimaninanadegani & Manshad 2014). Palm oil mill wastewater is inhabited by dissimilar types of microbes which plays a fundamental task in the biotreatment, bioremediation and biodegradation of oil-containing wastewaters (Hassen-Aboushiba et al. 2013; Tan et al. 2015).

Table 5: Cultural characteristics of bacteria isolated from POME.

| Organisms                        | Characteristics                                                                 | Gram's reaction |
|----------------------------------|-------------------------------------------------------------------------------|-----------------|
| *Micrococcus luteus 101PB*       | Circular, pinhead colonies which are convex with entire margins. Colonies produces a bright yellow, nondiffusible pigment | Positive cocci  |
| *Stenotrophomonas maltophilia 102PB* | Circular, smooth, convex, moist and pigmented colonies                        | Negative rod    |
| *Bacillus cereus 103PB*          | Large, irregular, opaque colonies. Smooth and moist colonies, whitish to cream | Positive rod    |
| *Providencia vermicola 104PB*    | Colonies are circular, shining, slimy, convex, and opaque with a brownish centre. Brown pigment is produced, colouring the medium around the colonies. Colonies are smooth with entire edges. | Negative rod    |
| *Klebsiella pneumoniae 105PB*    | Distinctive yeasty odor and bacterial colonies have a viscous/mucoid appearance | Negative rod    |
| *Bacillus subtilis 106PB*        | Dry, flat, and irregular, with lobate margins; colonies round or irregular; surface dull; become thick and opaque; whitish | Positive rod    |

Conversely, in view of the fact that most of the microbes domiciled in POME form spores, it facilitate their survival and continued existence in harsh or stressed normal conditions of palm oil mill wastewater such as absence of air or free oxygen (anaerobiosis), soaring concentration of oil and grease (Okechalu et al. 2011).
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2011; Ugoji 1997), and acidity (Leslie-Grady et al. 1999; Breccari et al. 1996; Poh & Chong 2009; Ugoji 1997). This corroborates with the study of Bala et al. (2015a) who reported in their investigation a low pH of 4.74 from raw palm oil mill wastewater in Malaysia. Under anaerobic conditions, methane and carbon dioxide are produced (Ugoji 1997). The anaerobic microflora inhabitant of palm oil mill wastewater sludge may well be valuable for the manufacture of biohydrogen and biogas production by fermentation during treatment (Vijayaraghavan & Ahmad 2006; Atif et al. 2005; Ismail et al. 2010). Table 5 revealed cultural characteristics of bacteria isolated from palm oil mill wastewater while Table 6 revealed microscopic, macroscopic morphology and cultural characteristics of fungi isolated from palm oil mill wastewater.

Table 6: Microscopic, macroscopic morphology and cultural characteristics of fungi isolated from POME.

| Organisms                  | Type of organisms | Microscopic morphology                                                                 | Macroscopic morphology                                      |
|----------------------------|-------------------|----------------------------------------------------------------------------------------|-------------------------------------------------------------|
| Aspergillus fumigatus 107PF| Filamentous mold  | Presence of rough conidiophore, with uni/biseriate phialides whose vesicle is round with radiate head. Brownish sclerotia were also observed | Presence of blue-green to yellow coloration from surface      |
| Aspergillus nomius 108PF   | Filamentous mold  | Presence of septate hyphae and colourless and rough conidiophores with swollen vesicles | A brownish colour with a creamy edge that appears golden in the reverse of the septate |
| Aspergillus niger 109PF    | Filamentous mold  | Presence of septate hyphae, long and smooth conidiophores, and long unbranded sporangiospores with large, round head | Brownish-black mycelium with dark spores and often appears golden on the reverse side |
| Meyerozyma guilliermondii 110PF| Yeast | Clusters of small blastospores along the pseudoconidia and particularly at septal points. Pseudoconidia are short and few in number | Colonies are flat, moist, smooth, and cream to whitish in colour |

CONCLUSION

Results from the current study revealed the presence of diverse types of microorganisms domiciled in palm oil mill wastewater. This conclusion suggests that microorganisms thrive well in palm oil mill wastewater. The investigation provides insight on the exploitation of microbial strains in biotreatment of industrial agricultural based wastewaters such as palm oil mill wastewater. The diversity of microbial strains isolated from palm oil mill wastewater provides a basis to promote better understanding of the types and nature of microorganisms domicile in palm oil mill wastewater. This will provide evidence on the microbiota
characteristics of palm oil mill wastewater. Conversely, this signifies the optimism for identification of native microbes from palm oil mill wastewater for biodegradation and bioremediation of industrial wastewaters. Study on metagenomic and transcriptomics characterisation is required for further identification of microbial strains diversity using Next-Generation Sequencing (NGS).

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APPENDIX A

Identified bacteria in POME sample.

| Strains                        | Image | Gram’s reaction       |
|--------------------------------|-------|-----------------------|
| Micrococcus luteus 101PB       |       | Gram positive cocci   |
| (Pure culture)                 |       |                       |
| Stenotrophomonas maltophilia 102PB |       | Gram negative rod     |
| (Pure culture)                 |       |                       |
| Bacillus cereus 103PB          |       | Gram positive rod     |
| (Pure culture)                 |       |                       |

Plate 1

Plate 2

Plate 3
| Strains                  | Image | Gram’s reaction          |
|-------------------------|-------|--------------------------|
| *Providencia vermicola* | ![Plate 4](image4.png) | Gram negative rod        |
| 104PB (Pure culture)    |       |                          |
| *Klebsiella pneumoniae* | ![Plate 5](image5.png) | Gram negative rod        |
| 105PB (Pure culture)    |       |                          |
| *Bacillus subtilis*     | ![Plate 6](image6.png) | Gram positive rod        |
| 106PB                   |       |                          |
APPENDIX B

Identified fungi in POME sample

Aspergillus fumigatus 107PF
(Microscopic staining)

Plate 7

Aspergillus nomius 108PF
(Microscopic staining)

Plate 8

Aspergillus niger 109PF
(Microscopic staining)

Plate 9
Meyerozyma guilliermondii 110PF
(Microscopic staining)

Plate 10

Aspergillus fumigatus 107PF
(Pure culture)

Plate 11

Aspergillus nomius 108PF
(Pure culture)

Plate 12
Aspergillus niger 109PF
(Pure culture)

Plate 13

Meyerozyma guilliermondii 110PF
(Pure culture)

Plate 14
APPENDIX C

Plates showing genomic DNA and purified PCR product of bacteria isolated from POME

Plate 15: Gel picture of genomic DNA: Lane 1: 101PB; 2: 102PB; 3: 103PB; 4: 104PB; 5: 105PB; 6: 106PB; M: Lambda/HindIII marker

Plate 16: Gel picture of purified PCR product: Lane 1: 101PB; 2: 102PB; 3: 103PB; 4: 104PB; 5: 105PB; 6: 106PB; M: 1 kb marker (Fermentas).
APPENDIX D

Plates showing genomic DNA and purified PCR product of fungi isolated from POME

Plate 17: Gel picture of genomic DNA: Lane 1: 107 PF; 2: 108 PF; 3: 109 PF; 4: 110 PF; M: Lambda/HindIII marker.

Plate 18: Gel picture of purified PCR product: Lane 1: 107 PF; 2: 108 PF; 3: 109 PF; 4: 110 PF; M: 1 kb marker (Fermentas).
APPENDIX E

DNA sequence of bacterial strains isolated from POME

TCGAACGATGAGCCACGCTCTGCGTGGTGATATGGTGCAACGGCTTGCAGAACGCTTGGGACATCAGGACATTAACAAGCCAGCTTACGCTCTCTGGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACTCTGTCGTAGGGGATAACGTAGGGAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGGACCTTCCGGGCCTTGCGCGATTGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCAAAGGGCAGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCCTACGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGCTGGCTAAATACCCGGTTGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTCGTATTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGATACTGGGCGACTAGAATGTTAGAGGGTAGCGGAATTCCTGGTGTAGCAGTGAAATGCGTAGAGATCAGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACATTGACACTGAGGCACGAAAGCGTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGCTAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAAACCTTACCTGGCCTTGACATGTCGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTACATAATGGTAGGGACAGAGGGCTGCAAGCCGGCGACGGTAAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATATTGAGTTTTCGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTTGTTGACCAGAAGCAGGTAGCTTAACCTTCGGGAGGGC

Figure 1: *Micrococcus luteus* 101 PB (Accession NO. AB539843.1)

GCTTGCTCTCTGGGTGCGAGTACGCTACGCTCTCTGGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACTCTGTCGTAGGGGATAACGTAGGGAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGGACCTTCCGGGCCTTGCGCGATTGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCAAAGGGCAGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCCTACGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGCTGGCTAAATACCCGGTTGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTCGTATTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGATACTGGGCGACTAGAATGTTAGAGGGTAGCGGAATTCCTGGTGTAGCAGTGAAATGCGTAGAGATCAGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACATTGACACTGAGGCACGAAAGCGTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGCTAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAAACCTTACCTGGCCTTGACATGTCGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTACATAATGGTAGGGACAGAGGGCTGCAAGCCGGCGACGGTAAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATATTGAGTTTTCGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTTGTTGACCAGAAGCAGGTAGCTTAACCTTCGGGAGGGC

Figure 2: *Stenotrophomonas maltophilia* 102PB (Accession No. JQ 619623.1)
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Figure 3: Bacillus cereus 103PB (Accession No. JF 432000.1)

Figure 4: Providencia vermicola 104PB (Accession No. KC775772.1)
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Figure 5: *Klebsiella pneumoniae* 105PB (Accession No. GU128173.1)

Figure 6: *Bacillus subtilis* 106PB (Accession No. KF62494.1)
APPENDIX F

DNA sequence of fungal strains isolated from POME

CCTTCCGTAGGTAACCTGGCAAGGAGATCATTACCAAGTGAGGCCCTCTGCGGTCACACCTCCC
ACCCGTTGTACACCGACATTGTGCTTTGCTTGCGGCGCGCCCGCGCGCGCGCGCGCGGAGGCCCTGG
ACCCGTGTCTATTGTACCTTGCTTCGGCGGGCCCGCCGGCCCGCCGGCCCGCCGGCCCGCCGGCCCG
CCGCCAAGACCCCACATGAACGCTGTTCTGAAAGTATGCACTCTGAGTTGATTATCGTAATCAGTTA
AAAACTTTCTAACAACAGGATCTCTTGGGTTCCGGCATCGATGAAAGAACGCGAGCAATGCGATAAA
TATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAAACGCACATTGCGCCCCCTGGTATTCC
GGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCGTCGCC
CAGCCGACACCCAACTTTATTTTTCTAAGGTGTGACCTCGGATCAGGTAGGGATACCCGCTGAAC
CTTAAGCATATCAATAAAGGGCGGAGGAA

Figure 7: Aspergillus fumigatus 107PF (Accession No. EU664647.1)

TTCCGTAGGTAACCTGGCAAGGAGATCATTACCAAGTGAGGCCCTCTGCGGTCACACCTCCC
ACCCGTTGTACACCGACATTGTGCTTTGCTTGCGGCGCGCCCGCGCGCGCGCGCGCGGAGGCCCTGG
ACCCGTGTCTATTGTACCTTGCTTCGGCGGGCCCGCCGGCCCGCCGGCCCGCCGGCCCGCCGGCCCG
CCGCCAAGACCCCACATGAACGCTGTTCTGAAAGTATGCACTCTGAGTTGATTATCGTAATCAGTTA
AAAACTTTCTAACAACAGGATCTCTTGGGTTCCGGCATCGATGAAAGAACGCGAGCAATGCGATAAA
TATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAAACGCACATTGCGCCCCCTGGTATTCC
GGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCGTCGCC
CAGCCGACACCCAACTTTATTTTTCTAAGGTGTGACCTCGGATCAGGTAGGGATACCCGCTGAAC
CTTAAGCATATCAATAAAGGGCGGAGGAA

Figure 8: Aspergillus nomius 108PF (Accession No. DQ467991.1)

TTCCGTAGGTAACCTGGCAAGGAGATCATTACCAAGTGAGGCCCTCTGCGGTCACACCTCCC
ACCCGTTGTACACCGACATTGTGCTTTGCTTGCGGCGCGCCCGCGCGCGCGCGCGCGGAGGCCCTGG
ACCCGTGTCTATTGTACCTTGCTTCGGCGGGCCCGCCGGCCCGCCGGCCCGCCGGCCCGCCGGCCCG
CCGCCAAGACCCCACATGAACGCTGTTCTGAAAGTATGCACTCTGAGTTGATTATCGTAATCAGTTA
AAAACTTTCTAACAACAGGATCTCTTGGGTTCCGGCATCGATGAAAGAACGCGAGCAATGCGATAAA
TATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAAACGCACATTGCGCCCCCTGGTATTCC
GGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCGTCGCC
CAGCCGACACCCAACTTTATTTTTCTAAGGTGTGACCTCGGATCAGGTAGGGATACCCGCTGAAC
CTTAAGCATATCAATAAAGGGCGGAGGAA

Figure 9: Aspergillus niger 109PF (Accession No. KC119204.1)

AACCTGGCAAGGAGATCATTACCAAGTGAGGCCCTCTGCGGTCACACCTCCC
ACCCGTTGTACACCGACATTGTGCTTTGCTTGCGGCGCGCCCGCGCGCGCGCGCGCGGAGGCCCTGG
ACCCGTGTCTATTGTACCTTGCTTCGGCGGGCCCGCCGGCCCGCCGGCCCGCCGGCCCGCCGGCCCG
CCGCCAAGACCCCACATGAACGCTGTTCTGAAAGTATGCACTCTGAGTTGATTATCGTAATCAGTTA
AAAACTTTCTAACAACAGGATCTCTTGGGTTCCGGCATCGATGAAAGAACGCGAGCAATGCGATAAA
TATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAAACGCACATTGCGCCCCCTGGTATTCC
GGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCGTCGCC
CAGCCGACACCCAACTTTATTTTTCTAAGGTGTGACCTCGGATCAGGTAGGGATACCCGCTGAAC
CTTAAGCATATCAATAAAGGGCGGAGGAA

Figure 10: Meyerozyma guilliermondii 110PF (Accession No. JN183444.1)
APPENDIX G

Phylogenetic trees of the identified bacterial isolates from POME

**Figure 11:** Phylogenetic tree of *Micrococcus luteus* 101PB based on 16S rRNA gene sequence comparisons

**Figure 12:** Phylogenetic tree of *Stenotrophomonas maltophilia* 102PB based on 16S rRNA gene sequence comparisons
**Figure 13:** Phylogenetic tree of *Bacillus cereus* 103PB based on 16S rRNA gene sequence comparisons

**Figure 14:** Phylogenetic tree of *Providencia vermicola* 104PB based on 16S rRNA gene sequence comparisons
Figure 15: Phylogenetic tree of *Klebsiella pneumoniae* 105PB, based on 16S rRNA gene sequence comparisons

Figure 16: Phylogenetic tree of *Bacillus subtilis* 106PB based on 16S rRNA gene sequence comparisons

APPENDIX H

Phylogenetic trees of the identified fungal isolates from POME
Figure 17: Phylogenetic tree of *Aspergillus fumigatus* 107PF based on 18S rRNA gene sequence comparisons

Figure 18: Phylogenetic tree of *Aspergillus nomius* 108PF based on 18S rRNA gene sequence comparisons
**Figure 19:** Phylogenetic tree of *Aspergillus niger* 109PF based on 18S rRNA gene sequence comparisons

**Figure 20:** Phylogenetic tree of *Meyerozyma guilliermondii* 110PF based on 18S rRNA gene sequence comparisons
REFERENCES

Abass A O, Jameel T A, Muyibi A S, Abdul Karim I M and Alam Z. (2012). Investigation of the viability of selected microorganisms on the biodegradation of palm oil mill effluents (POME). *International Journal of Chemical Environmental Engineering* 3(3): 182–186.

Abdel-Raouf N, Al-Homaidan A A and Ibraheem I B M. (2012). Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences* 19: 257–275. https://doi.org/10.1016/j.sjbs.2012.04.005

Ahmad A L, Ismail S and Bhatia S. (2005). Optimization of coagulation- flocculation process for palm oil mill effluent using response surface methodology. *Environmental Science and Technology* 39(8): 2828–2834. https://doi.org/10.1021/es0498080

Ahmad M N, Mokhtar M N and Baharuddin A S. (2011). Changes in physicochemical and microbial community during co-composting of oil palm frond with palm oil mill effluent anaerobic sludge. *BioResources* 6(4): 4762–4780.

American Public Health Association (APHA). (2005). *Standard methods for the examination of water and wastewater*. (21st edition). Washington, DC: American Public Health Association (APHA).

Awotoye O O, Dada A C and Arawomo G A O. (2011). Impact of palm oil processing effluent discharge on the quality of receiving soil and river in South Western Nigeria. *Journal of Applied Sciences Research* 7(2): 111–118.

Asikong B E. (1994). Studies on extracellular lipases of three fungi isolated from mouldy cocoa seed and palm fruit. MSc. Thesis. University of Calabar, Nigeria.

Atif A A Y, Razi A F, Ngan M A, Morimoto M, Iyuke S E and Veziroglu N T. (2005) Fed batch production of hydrogen from palm oil mill effluent using anaerobic microflora. *International Journal of Hydrogen Energy* 30: 1393–1397. https://doi.org/10.1016/j.ijhydene.2004.10.002

Bergey D H, Holt J G, Krieg N R and Sneath P H A. (1994). *Bergey’s manual of determinative bacteriology*, 9th edition. Philadelphia, PA: Lippincott Williams and Wilkins.

Bala J D, Yusuf I Z and Tahir F. (2012). Bacteriological assessment of pharmaceutical wastewater and its public health implications in Nigeria. The Icfai University Press (IUP) *Journal of Biotechnology* 6(1): 34–49.

Bala J D, Lalung J and Ismail N. (2014a). Biodegradation of palm oil mill effluent (POME) by bacteria. *International Journal of Scientific and Research Publications* 4(3): 502–511.

Bala J D, Lalung J and Ismail N. (2014b). Biodegradation potential and removal of oil and grease by bacteria isolated from Palm oil mill effluent (POME). *Proceedings of the International Conference on Beneficial Microbes ICOBM, 2014. Microbes for the benefits of Mankind*. 27–29 May 2014, Parkroyal Penang Resort, Penang, Malaysia, 138–144.

Bala J D, Lalung J and Ismail N. (2014c). Palm oil mill effluent (POME) treatment “Microbial communities in an anaerobic digester”: A review. *International Journal of Scientific and Research Publications* 4(6): 2250–3153

Bala J D, Lalung J and Ismail N. (2015a). Studies on the reduction of organic load from palm oil mill effluent (POME) by bacterial strains. *International Journal of Recycling of Organic Waste in Agriculture* 4(1): 1–10. https://doi.org/10.1007/s40093-014-0079-6
Bala J D, Lalung J, AL-Gheethi, A A S and Ismail N. (2015b). Reduction of oil and grease by fungi isolated from Palm oil mill effluent (POME). Proceedings of the 4th ICERT 2015: International Conference on Environmental Research and Technology: Exploring the Frontiers in Environmental Science and Technology Research, 27–29 May 2015, Parkroyal Hotel Penang, Malaysia, 79–91.

Bala J D. (2016). Aerobic treatment and biodegradation of palm oil mill effluent by indigenous microorganisms. PhD Dissertation. Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia.

Bharathi S and Vasudevan N. (2001). Utilization of petroleum hydrocarbons by Pseudomonas fluorescens isolated from petroleum contaminated soil. Environment International 26: 413–414. https://doi.org/10.1016/S0160-4120(01)00021-6

Bhumibhamon O, Koprasertsak A and Funthong S. (2002). Biotreatment of high fat and oil wastewater by lipase producing microorganisms. Kasetsart Journal 36: 261–267.

Breccari M, Bonemazzi F, Majone M and Riccardi C. (1996). Interaction between acidogenesis and methanogenesis in the anaerobic treatment of olive oil mill effluents. Water Research 30: 183–189. https://doi.org/10.1016/0043-1354(95)0086-Z

Ghosh P, Saxena R, Gupta R, Yadav R and Davidson S. (1996). Microbial lipases: production and applications. Sci Progress 79: 119–158.

Guehi T S, Dingkuhn M, Cros E, Fourny G, Ratomahenina R, Moulin G and Vidal A C. (2007). Identification and lipase-producing abilities of moulds isolated from ivorian raw cocoa beans. Research Journal of Agriculture and Biological Sciences 3: 838–843.

Haimann R A. (1995). Fungal technologies for the treatment of hazardous waste. Environmental Progress 14(3): 201–203. https://doi.org/10.1002/ep.670140320

Hassen-Aboushiba A, Ramli R and Sofian-Azirun M. (2013). Ecological characteristics of POME ponds with reference to study some of their invertebrate species in Peninsular Malaysia. Journal of Animal and Plant Sciences 23(5):1305–1315.

Hii K L, Yeap S P and Mashitah M D. (2012). Cellulase production from palm oil mill effluent in Malaysia: Economical and technical perspectives. Engineering in Life Sciences 12(1): 7–28. https://doi.org/10.1002/elsc.201000228

Ismail I, Hassan M A, Abdul Rahman N A and Soon C S. (2010). Thermophilic biohydrogen production from palm oil mill effluent (POME) using suspended mixed culture. Biomass and Bioenergy 34(1): 42–47. https://doi.org/10.1016/j.biombioe.2009.09.009

Jameel A T and Olanrewaju A A. (2011). Aerobic biodegradation of oil and grease in palm oil mill effluent using consortium of microorganisms In: Alam M D Z, Jameel A T and Amid A. (eds). Current research and development in biotechnology engineering at International Islamic University Malaysia (IIUM) Vol. III. Kuala Lumpur: International Islamic University Malaysia (IIUM) Press, 43–51.

Kamal S A, Jahim J M and Anuar N. (2011). Pre-treatment effect of palm oil mill effluent (POME) during hydrogen production by a local isolate clostridium butyricum, International Journal of Advanced Science, Engineering and Information Technology 2: 54–60.

Leslie-Grady C P, Daigger G T and Lim H C. (1999). Biological wastewater treatment. 2nd edition. Boca Raton, FL: CRC Press.

Ma A N. (2000). Environmental management for the oil palm industry. Palm Oil Developments 30: 1–10.

Mills A L, Breuil C and Colwell R R. (1978). Enumeration of petroleum-degrading marine and estuarine microorganisms by the most-probable number. Canadian Journal of Microbiology 24: 552–557. https://doi.org/10.1139/m78-089
Mohammed R R, Ketabachi M R and McKay G. (2014). Combined magnetic field and adsorption process for treatment of biologically treated palm oil mill effluent (POME). *Chemical Engineering Journal* 243: 31–42. https://doi.org/10.1016/j.cej.2013.12.084

Nawawi W M F W, Jamal P and Alam M Z. (2010). Utilization of sludge palm oil as a novel substrate for biosurfactant production. *Bioresource Technology* 101(23): 9241–9247. https://doi.org/10.1016/j.biortech.2010.07.024

Neoh C H, Yahya A, Adnan R, Majid Z A and Ibrahim Z. (2013). Optimization of decolorization of palm oil mill effluent (POME) by growing cultures of *Aspergillus fumigatus* using response surface methodology. *Environmental Science and Pollution Research* 20: 2912–2923. https://doi.org/10.1007/s11356-012-1193-5

Nwuche C O and Ogbonna J C. (2011). Isolation of lipase producing fungi from palm oil mill effluent (POME) dump sites at Nsukka. *Brazilian Archives of Biology and Technology* 54: 113–116. https://doi.org/10.1590/S1516-89132010001000015

Ohimain E I, Olukole C D, Izah S C, Eke R A and Onkonwo A C. (2012a). Microbiology of palm oil mill effluents. *Journal of Microbiology and Biotechnology Research* 2(6): 852–857.

Ohimain E I, Seiyaboh E I, Izah S C, Oghenegueke V and Perewarebo T. (2012b). Some selected physico-chemical and heavy metal properties of palm oil mill effluents. *Greener Journal of Physical Sciences* 2: 131–137.

Ohimain E I, Daokoru-Olukole C, Izah S C and Alaka E E. (2012c). Assessment of the quality of crude palm oil produced by smallholder processors in Rivers State, Nigeria. *Nigerian Journal of Agriculture, Food and Environment* 8(2): 28–34.

Ojumu T V, Bello O O, Sonibare J A and Solomon B O. (2005). Evaluation of microbial systems for bioremediation of petroleum refinery effluents in Nigeria. *African Journal of Biotechnology* 4(1): 31–35.

Okechalu J N, Dashen M M, Lar P M, Okechalu B and Gushop T. (2011). Microbiological quality and chemical characteristics of palm oil sold within Jos Metropolis, Plateau State, Nigeria. *Journal of Microbiology and Biotechnology Research* 1(2): 107–112.

Okereke J N, Obiekezie S O and Obasi K O. (2007). Microbial flora of oil-spilled sites in Egbeama, Imo State, Nigeria. *African Journal of Biotechnology* 6(8): 991–993.

Okpokwasili G C and Okorie B B. (1988). Biodeterioration potentials of microorganisms isolated from car engine lubricating oil. *Tribology International* 21(4): 215–220. https://doi.org/10.1016/0301-679X(88)90020-5

Okwute L O and Isu N R. (2007a). The environmental impacts 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(12): 656–662.

Okwute L O and Isu N R. (2007b) Impact analysis of palm oil mill effluent on the aerobic bacterial density and ammonium oxidizers in a dumpsite in Anyigba, Kogi State. *African Journal of Biotechnology* 6(2): 116–119.

Okwute L O. (2013). Bioremediation of soil polluted with palm oil mill effluent (POME) in Kogi State using microorganisms found in chicken dropping and cow dung. PhD dissertation. Department of Microbiology, Federal University of Technology Minna, Nigeria.

Oswal N, Sarma P M, Zinjarde S S and Pant A. (2002). Palm oil mill effluent treatment by a tropical marine yeast. *Bioresource Technology* 85(1): 35–37. https://doi.org/10.1016/S0960-8524(02)00063-9
Poh P E and Chong M F. (2009). Development of anaerobic digestion methods for palm oil mill effluent (POME) treatment. *Bioresource Technology* 100: 1–9. https://doi.org/10.1016/j.biortech.2008.06.022

Rahman K S M, Rahman J T, Lakshmanaperumalsamy P and Banat L M. (2002). Towards efficient crude oil degradation by a mixed bacterial consortium. *Bioresource Technology* 85: 257–261. https://doi.org/10.1016/S0960-8524(02)00119-0

Rupani P F, Singh R P, Ibrahim M H and Esa N. (2010). Review of current palm oil mill effluent (POME) treatment methods: Vermicomposting as a sustainable practice. *World Applied Sciences Journal* 11(1): 70–81.

Serikovna S Z, Serikovich K S, Sakenovna A S, Murzakhmetovich S S and Khamitovich A K. (2013). Screening of lipid degrading microorganisms for wastewater treatment. *Malaysian Journal of Microbiology* 9(3): 219–226.

Shaaban A M, Haroun B M and Ibraheem I B M. (2004). Assessment of impact of *Microcystis aeruginosa* and *Chlorella vulgaris* in the uptake of some heavy metals from culture media. In Proceedings of 3rd International Conference on Biology Science, Faculty of Science, Tanta University, 28–29 April. Volume 3, 433–450.

Singh R, Ibrahim M H, Esa N and Iliyana M. (2010). Composting of waste from palm oil mill: A sustainable waste management practice. *Reviews in Environmental Science and Biotechnology* 9(4): 331–344. https://doi.org/10.1007/s11157-010-9199-2

Sira P, Orathai P, Ratana R, Boonyarach K, Pastra S and Sumaeth C. (2010). Biosurfactant production by *Pseudomonas aeruginosa* SP4 using sequencing batch reactor: Effect of oil-to-glucose ratio. *Biochemical Engineering Journal* 49: 185–191. https://doi.org/10.1016/j.bej.2009.12.011

Soleimaninanadegani M and Manshad S. (2014). Enhancement of biodegradation of palm oil mill effluents by local isolated microorganisms. *International Scholarly Research Notices* 2014: 1–8. https://doi.org/10.1155/2014/727049

Tan K M, Liew W L, Muda K and Kassim M A. (2015). Microbiological characteristics of palm oil mill effluent. Paper presented at the *International Congress on Chemical, Biological and Environmental Sciences* (ICCBES). 7–9 May, Kyoto Research Park, Kyoto, Japan.

Ugoji E O. (1997). Anaerobic digestion of palm oil mill effluent and its utilization as fertilizer for environmental protection. *Renewable Energy* 10(2–3): 291–294. https://doi.org/10.1016/0960-1481(96)00080-8

Vijayaraghavan K and Ahmad D. (2006). Biohydrogen generation from palm oil mill effluent using anaerobic contact filter. *International Journal of Hydrogen Energy* 31: 1284–1291. https://doi.org/10.1016/j.ijhydene.2005.12.002

Wong K M, Nor A A, Suraini A, Vikineswary S and Hassan M A. (2008). Enzymatic hydrolysis of palm oil mill effluent solid using mixed cellulases from locally isolated fungi. *Research Journal of Microbiology* 3(6): 474–481. https://doi.org/10.3923/jm.2008.474.481

Wu T Y, Mohammad A W, Jahim J M and Anuar N. (2007). Palm oil mill effluent (POME) treatment and bioresources recovery using ultrafiltration membrane: effect of pressure on membrane fouling. *Biochemical Engineering Journal* 35: 309–317. https://doi.org/10.1016/j.bej.2007.01.029
Wu T Y, Mohammad A W, Jahim J M and Anuar N. (2009). A holistic approach to managing palm oil mill effluent (POME): Biotechnological advances in the sustainable reuse of POME. *Biotechnology Advances* 27: 40–52. https://doi.org/10.1016/j.biotechadv.2008.08.005

Zajic J E and Supplisson B. (1972). Emulsification and degradation of Bunker C fuel oil by microorganisms. *Biotechnology and Bioengineering* 14: 331–334. https://doi.org/10.1002/bit.260140306