Biodegradation of palm oil mill effluent (POME) by bacterial

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Abstract- Palm oil mill effluent (POME) is produced in large volumes by many of the palm oil mills in Malaysia and contributes a major source of pollution. The main aim of the present study was to evaluate the biodegradation potential of bacterial isolated from POME and to find the most suitable strain(s) for a biological treatment technology of POME. The isolates were identified by sequences analysis of 16S rRNA genes. Sequencing of the 16S rRNA of the isolates suggests that they were identified as *Micrococcus luteus*101PB, *Stenotrophomonas maltophilia*102PB, *Bacillus cereus*103PB, *Providencia vermicola*104PB, *Klebsiella pneumoniae*105PB and *Bacillus subtilis*106PB.Results revealed that total suspended solids (TSS), oil and grease were reduced dynamically with treatments after 5 days. *Bacillus cereus*103PB produced the highest activity in reducing TSS (71.63%), oil and grease (85.14%).Simulation study results showed comparable reduction of parameters measured. The results presented in this study of simultaneous removal of TSS, oil and grease, appears useful for practical wastewater treatment as a compact treatment system for POME. Thus the strain *Bacillus cereus*103PB is the most effective bacteria and the best candidate to use in biological treatment technology of POME having the highest TSS, oil and grease reduction rate. Hence *Bacillus cereus*103PB do possess the biodegradation ability and is able to reduce the pollutants of the effluent sample. These results suggested that *Bacillus cereus*103PB might be applicable to a wastewater treatment system for the removal of TSS and oil. The strain also produces extracellular lipase and cellulase which stimulates better waste treatment. This study demonstrated that POME degrading microorganisms can be isolated from POME polluted area and the degrading ability of these microorganisms is *a* clear indicator that these bacteria can be applied in the bioremediation techniques for biodegradation of POME to enhance treatment.

Index Terms- Biodegradation, oil & grease, physicochemical, POME, TSS, wastewater

I. INTRODUCTION

The palm oil industry is one of the major agro-industries in Malaysia. The production of palm oil, however, results in the generation of large quantities of polluted wastewater commonly referred to as palm oil mill effluent (POME). The most significant pollutant from palm oil mils is POME (Poh and Chong, 2009). Typically, 1 t of crude palm oil production requires 5–7.5 t of water; over 50% of which ends up as POME. 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 ^{0}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; Borja and Banks, 1996). POME contains about 4000–6000 mg/l of oil and grease (Ahmad et al., 2005).

The composition of POME are mainly water, oil, suspended solid, dissolved solid and sand (Ibrahim et al., 2012), total suspended solids (TSS), as well as cellulose wastes (Rashid et al., 2009), vegetative matter, colloidal slurry of water and solids including about 2% suspended solids originating mainly from cellulose fruit debris, that is, palm mesocarp (Bek-Nielsen *et al*., 1991).The suspended solids in POME which are the cellulolytic material derived from palm mesocarp are organic in nature and considered as organic matter(Chin et al., 1996) and constitute about 50% of the POME (Ho et al., 1983; Ho et al., 1984).

Treatment and disposal of oily wastewater, such as palm oil mill effluent is presently one of the serious environmental problems contributors. Palm oil mill wastes have existed for years but their effects on environment are at present more noticeable. The oily waste has to be removed to prevent interfaces in water treatment units, avoid problems in the biological treatment stages, and comply with water-discharge requirements (Ahmad et al., 2005). Palm oil mill effluent (POME) is an important source of inland water pollution when released into local rivers or lakes without treatment. POME contains lignocellulolic wastes with a mixture of carbohydrates and oil (Oswal et al., 2002).

Recently, various physical and chemical treatment processes have been designed to treat 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). The use of microorganisms in biological treatment of POME in this present study offers an alternative solution to reduce the TSS and organic load content of the effluent (Alam et al., 2009).

Palm oil industries are facing tremendous challenges to meet the increasingly stringent environmental regulations (Najafpour et al., 2006). Thus, it is obvious that the presence of high levels of fat, oil and grease in wastewater induces serious problems not only to the receiving water but also to treatment plants and waste collecting systems. Although oil is not generally thought of as a material which is discharged into Land Rivers, it can and does reach these waters; not only as tin colored films but also in sufficient volumes to necessitate the closing of abstraction points (El-Bestaway et al., 2005). It is therefore essential that the potential danger from oil pollution is fully appreciated.

The various effluent treatment schemes which are currently used by the Malaysian palm oil industry are listed in descending order: (a) anaerobic/facultative ponds (Rahim and Raj, 1982; Wong, 1980; Chan and Chooi, 1982), (b) tank digestion and mechanical aeration, (c) tank digestion and facultative ponds, (d) decanter and facultative ponds, and (e) physico-chemical and biological treatment (Andreasen, 1982).

The current methods adapted for the treatment of palm oil mill effluent (POME) in most of the mills in Malaysia is the ponding system in which about 85% of the mills practice (Poh and Chong, 2009). This is not very effective in treating the pollutants in the POME to the stringent standards required (Jameel and Olanrewaju, 2011); The status and concentration of the oily matter/oil residue (oil and grease) after the treatment process is given less attention and this suggest that these approach employed is not sustainable to minimize the environmental impact of oil and grease in POME. Moreover, the range of concentration of oil and grease in POME is relatively higher than those obtained in toxic wastewater (Jameel and Olanrewaju, 2011). Thus, the need for effective treatment process for POME.

The anaerobic digestion treatment of POME using various types of bioreactors by researchers and the ponding systems in the mills uses undefined microbial populations (McHugh et al., 2003) to reduce the polluting power of wastes and wastewaters. This involves a consortium of undefined microorganisms catalyzing a complex series of biochemical reactions that mineralize organic matter producing methane and carbon dioxide. These microorganisms are not established and hence the substrate they degrade and utilize is not ascertained. This lead to poor effluent discharge into the environment as the performance of the microorganisms with regards to the rate of reduction and removal of oily waste cannot be monitor since they are not known.

The present study will use defined/known microorganisms isolated from POME to inoculate the POME and monitor the percentage removal/reduction of the physicochemical parameters with a view to enhance treatment. This emphasizes the originality of the study and hence, this has therefore attracted the interest of this study.

Furthermore, since several researchers based their findings on the overall COD removal, methane production and not the individual microorganisms (using undefined microbial population) utilizing and degrading the components in POME making up the COD and BOD, tailored to the fact that, no work has been done on the isolation of different individual microorganisms breaking down and utilizing the different components in POME making up the COD and BOD in order to remove or reduce the organic load level. Therefore, this research to the best of our knowledge can be listed as a novel study.

Very few investigations have been conducted on aerobic digestion process for the treatment of oil and grease present in POME (Wu et al., 2010). The major problems lie in the establishment of the most suitable microbial population for POME waste to be treated (Yacob et al., 2006; Poh and Chong, 2009). Some aerobic treatment approaches include: degradation of POME using a tropical marine yeast (*Yarrowia lipolytica*) NCIM 3589 in a lagoon (Oswa et al., 2002), trickling filter (TF) (Norulaini et al., 2001) and rotating biological contactors (RBC) (Najafpour et al., 2005). Organisms used for these aerobic treatments by the investigators are isolated from different source while this present study will isolate indigenous organisms from POME for the treatment.

Microbial degradation of oil wastewater is a concern in recent years. A variety of microorganisms such as bacteria, molds, and yeasts, have been shown to be capable of completely degrading oil wastewater (Ammar *et al*., 2005; Dhouib *et al*., 2006; Erguder *et al*., 2000; Ettayebi *et al*., 2003; Kissi *et al*., 2001). Therefore, using of microorganisms for treatment and bioremediation purposes affords a very efficient tool for purifying contaminated effluents and natural water (Glazer & Nikaido 1995). Using bacterial strain that possesses high efficiency in accumulating toxic contaminants or biodegradation of persistent biodegradable matter has potential in the use of the treatment system to remove pollution such as oil and grease or heavy metals from any polluted aquatic effluent (Campere et al. 1993).

The application of microorganisms such as *Trichoderma viride* spores, *T. viride* mycelium, *Yarrowia lipolytica* and *Saccharomyces cerevisiae* for the treatment of POME have not been extended to the removal of oil and grease (Jameel and Olanrewaju, 2011) despite their high potential in removing COD from POME. This may be due to the fact that these microorganisms are not indigenous to POME. This therefore offer researchers a greater opportunity to investigate the removal of oily matter/oil residue(oil and grease) from POME using microorganisms isolated from POME (Jameel and Olanrewaju,2011).This is the focus and emphasis of the present study and it is design for this purpose. Hence, this has therefore attracted the interest of this study. The main aim of the present study was to evaluate the biodegradation potential of bacteria isolated from POME and to find the most suitable strain(s) for a biological treatment technology of POME.

II. MATERIALS AND METHODS

Sample collection/Sampling Source

Raw palm oil mill effluent (POME) was collected from the site of a palm oil mill industry in a sterile container and brought back to the laboratory. The sample was transported to the laboratory in an ice box and analyzed for microbiological and physicochemical properties within four (4) hours of collection. The physicochemical characteristics of the sample were determined in accordance with the standard methods published by American Public Health Association (APHA, 1995; APHA, 2005)

Sample Preservation.

The POME was preserved at a temperature less than 4 ^{0}C, but above the freezing point in order to prevent the wastewater from undergoing biodegradation due to microbial action (APHA, 1985).

Identification of Bacteria Isolates by sequencing of 16S rRNA gene

Preliminary identification of individual bacterial isolates was obtained by classical tests (Gerhardt *et al*., 1981; Bergey *et al*., 1994). Such identification included the shape of cells, Gram stain and colony morphology on solid nutrient media. Genetic identification of isolates was performed by determining nucleotide sequences of 16S rRNA genes using commonly used primers for amplifying the DNA between positions 27 and 1492 of bacterial 16S rRNA genes.

Sequence of Primers**:**
27F: 5'-AGAGTTTGATCMTGGCTCAG-3'
1492R: 5'-GGGTTACCTTGTTACGACTT-3'

Plate 1and 2 shown Genomic DNA and Purified PCR product of bacteria isolated from POME respectively. The bacteria were isolated from our previous study. Isolated bacteria from POME were investigated for their ability to produce lipase and cellulase on solid media in our previous work. Biodegradation potential and growth profile in mineral salt medium (MSM) was also investigated in our previous study.

Genomic DNA and Purified PCR product of bacterial isolated from POME

M 1 2 3 4 5 6

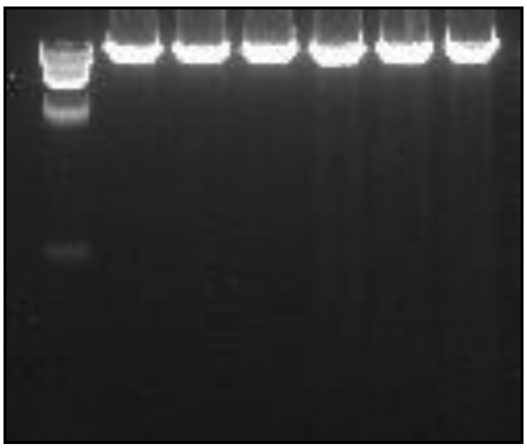

Plate 1: **Gel Picture of Genomic DNA:** Lane **1**: 101PB; **2**: 102PB; **3**: 103PB; **4**: 104PB; **5**: 105PB; **6**: 106PB; **M**: Lambda/HindIII marker.

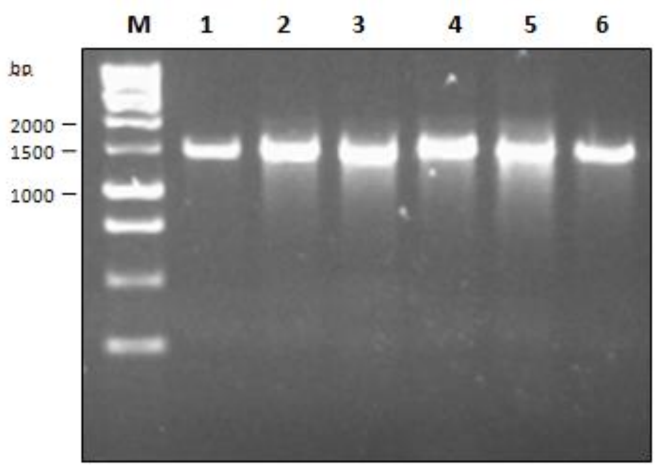

Plate 2: **Gel Picture of Purified PCR product:** Lane **1**: 101PB; **2**: 102PB; **3**: 103PB; **4**: 104PB; **5**: 105PB; **6**: 106PB; **M**: 1 kb marker (Fermentas)

Experimental procedure

Analytical methods and characterization of POME

Physicochemical parameters were also determined in order to characterize POME. These parameters included pH, total suspended solids (TSS), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD) and oil & grease (O&G. Characterization of the POME was carried out before and after the treatment to determine the efficiency of the treatment. Total suspended solids (TSS) were determined as dry weight (mg/L), pH was measured using pH meter, the organic strength (COD) of the wastewater was determined by spectrophotometer method, the biodegradability of the wastewater was measured in terms of BOD_5, and oil and grease was determined according to the partition-gravimetric method. All the methods were carried out according to the procedures described in the Standard Methods for the Examination of Water and Wastewater (Clesceri et al., 1999; APHA, 1995; APHA, 2005).

Oil-degradation rate (%) was defined as the amount of oil degraded versus the amount of initial oil. The COD degradation efficiency was defined as the amount of COD decreased versus the amount of initial COD. Biodegradability of the wastewater in terms of BOD_5 was defined as the amount of BOD decreased versus the amount of initial BOD and TSS degradation efficiency was defined as the amount of TSS decreased versus the amount of initial TSS. All the experiments were performed in triplicates.

Inoculation of POME with Bacterial Isolates

Sterile POME sample

Using single/individual bacterial strains.

*Micrococcus luteus*101PB, *Stenotrophomonas maltophilia*102PB,*Bacillus cereus*103PB, *Providencia vermicola*104PB, *Klebsiella pneumoniae*105PB and *Bacillus subtilis*106PB isolated from POME were used in the present study. Strains showed varying degrees of lipase and celllulase activity on solid media in our previous study. 101 PB, 102 PB, 103 PB and 106 PB were selected for POME inoculation based on the criteria that they were able to display good rate of growth and degradation/utilization of palm oil and cellulose as sole source of carbon and energy in MSM liquid medium in our previous study.

250 mL of raw POME sample was introduced into each conical flasks and sterilized at 121°C for 20 minutes. The sterilized raw POME was allowed to cool before inoculation. Eight percent of each inoculum (standard bacterial suspension) containing 10^4 cells/mL with an optical density (OD) of 1.2 at 600 nm was used to inoculate 250 mL of POME sample without addition of nutrients. They were incubated at 37°C and at 150 rpm shaking speed. Bacterial cultures were incubated under aerobic conditions at 37°C and agitated at 150 rpm. Samples were then aseptically drawn every 24 hours for 5 days and analyzed for BOD_5, COD, oil & grease, TSS and pH. Samples were also carried out for cell counting. Cell count was determined by plating serial dilutions of samples on nutrient agar plates and incubating at 37 °C for 24 h. Control flasks were not inoculated. All the experiments were performed in triplicates. The efficiency for organic load reduction and the percentage reduction was measured by using the following equation (Piro et al., 2011):

$$\text{Reduction (\%)} = \frac{C_{\text{raw POME}} - C_f}{C_{\text{raw POME}}} \times 100$$

Where $C_{raw\ POME}$ is the concentration of COD, BOD_5, TSS and oil & grease of raw POME and C_f the concentration of these parameters after treatment. Each set of these experiments was carried out three times.

Non- Sterile POME sample (Simulation Study)

Using single/individual bacterial strains.

The procedure for the simulation study was the same as forementioned only that the POME sample was not sterilized. The main aim was to investigate the maximum enhancement in the degradation abilities of the investigated bacteria using the natural conditions of the raw POME where they were originally isolated in order to get the most effective and economical treatment under the effluent's natural conditions in the field.

III. RESULTS AND DISCUSSION

Palm oil mill effluent (POME) characteristics

Raw POME collected from the palm oil mill was thick brownish in color, colloidal suspension, dark, oily and viscous with an obnoxious odour. The sampled effluent had a high COD content of 75,900 mg/L, BOD 34,393 mg/L, TSS 14,467 mg/L, oil & grease 190.6 mg/L and pH 4.74. The characteristics are presented in Table 1. This suggests increase potential for pollution of the effluent. In comparison, Chin et al. (1996) have reported that POME contains a high concentration of organic matter, COD concentration of 65,000 mg/L, BOD of 48,000 mg/l and oil and grease greater than 2000 mg/L. Other investigators have also reported values similar to the present study. BOD 25,545 mg/L, COD 55,775, TSS 18,479mg/L, oil & grease 8020 mg/L and pH 3.5 (Vijayaraghavan et al., 2007), BOD 43,750 mg/L, COD 51,000mg/L, TSS 18,000mg/L, oil & grease 130 mg/L, and pH 4.2 (Lam and lee, 2011), BOD 25,000mg/L, COD 50,000mg/L, TSS 18,000mg/L, oil and grease 4000mg/L (Ahmad et al., 2005).BOD 26,000mg/L, COD 55,700mg/L, TSS 16,500mg/L, oil and grease 4,900mg/L and pH 4.4 (Najafpour et al., 2006) and BOD 25,000mg/L, COD 50,000mg/L, TSS 18,000mg/L, oil & grease 4,000mg/L and pH 4.7 (AbdulKarim et al., 2011). The result for oil and grease in the present study was low when compared with results from other researchers who obtained higher values for oil and grease (Ahmad et al., 2003; Najafpour et al., 2006; Vijayaraghavan et al., 2007 and Wanna and Pornpan, 2007). Although, Lam and lee, (2011) also reported low values of 130 mg/L for oil and grease comparable to the present study which exceeded the discharge standard limit. The difference may be due to differences in species of oil palm, degree of oil extraction during milling and method of extraction, whether local or automated. The volume of water used during the milling process is also a factor to consider.

Table 1 Characteristics of raw palm oil mill effluent (POME) and Discharge Standard limits

Parameters	Raw POME (mg/L)	Discharge Effluent Standard
Chemical oxygen demand (COD)	75,900	-
Biochemical oxygen demand (BOD)	34,393	100
Total suspended solids (TSS)	14,467	400
Volatile suspended solids (VSS)	13,033	-
Oil and grease (O &G)	190.6	50
pH	4.74	5 - 9

All parameters are in mg/L except pH

Biodegradation study

Removal Efficiency (RE %) of oil and grease by bacteria isolates

Results showed that oil and grease decrease remarkably with treatments on the fifth (5) day. In POME sample, percentage removal efficiency of oil and grease for all the isolates are presented in figure 1.

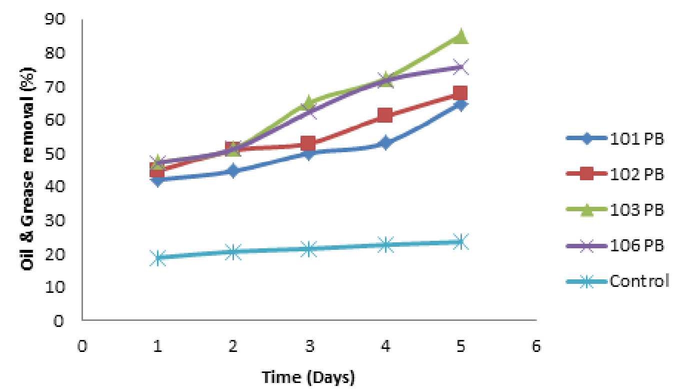

Figure 1 Percentage removal of oil and grease in sterile POME sample.

Results in figure 1 represent levels of percentage removal efficiency (RE %) of oil and grease from POME. Results revealed that removal efficiency of oil and grease for all the isolates are as follows: *Micrococcus luteus*101PB(64.76%), *Stenotrophomonas maltophilia* 102PB (67.65%),*Bacillus cereus*103PB(85.14%), *Bacillus subtilis*106PB (75.7%) and control (23.48%). It was observed that the pH of the POME increases as follows, 101 PB (7.02), 102 PB (7.22) 103 PB (8.01) and 106 PB (8.03) after 5 days of treatment Figure 1. The initial acidic pH of the raw POME (4.74) became alkaline except in the control sample (5.04) probably due to the utilization of fatty acids present in the raw POME by the bacteria isolates. The results presented here is comparable to those of Oswal et al. (2002) who reported increase from acidic pH to alkaline after treatment of POME with *Y lipolytica* NCIM 3589 indicating utilization of fatty acids present in the raw POME by the isolates. Bhumibhamon et al. (2002) has reported removal of fat and oil by *Bacillus* sp KUL39 (81.6%) from wastewater of palm oil and bakery industries. This is in consistent with the present study with 85.14% removal of oil and grease by *Bacillus cereus*103PB. Harikrishna et al. (2012) reported 71% removal of oil by *B subtilis*. Bujang, (2013) has also reported treatment of oily wastewater with *Bacillus cereus.*

Therefore, in the present scenario of POME treatment or study, as the pH increases for each selected strain, the maximum value of degradation activity was produced as also shown by other workers (Sztajer & Maliszewska 1988; Wang et al. 1988; Jaeger et al. 1994).

In the present study, it was observed that a clearer colour change from red of the solvent layer's colour according to the degradation extent of oil as compared to the control where no bacteria was inoculated (no colour change) indicates oil biodegradation during oil and grease analysis after treatment using standard methods. Chaudhry et al. (2012) also reported change in colour after treatment indicating oil degradation by their isolates in a similar study. April et al. (2000) reported similar findings.

The biodegradation of oil in the environment is a complex process, whose quantitative and qualitative aspects depend on the nature and amount of the oil present, the ambient and the seasonal environmental condition, and the constitution of the indigenous microbial community (Leahy and Colwell, 1990; Hinchee and Olfenbuttel, 1991).This is in compliance with the present study whereby constitution of indigenous bacterial isolated from POME were used for degradation oil present in POME sample.

The difference in percentage degradation rate by different isolates in various studies by researchers could be due to the difference in waste characteristics where each type of oily wastewater has its own characteristic composition (Ainon et al., 2010).

The ability of the bacterial isolates to degrade oil was demonstrated in terms of reduction in the quantity of oil. The percentage degradation was determined from the equation earlier aforementioned.

Generally, microbial oil degradation is considered to occur as a result of hydrolysis of oil by secretion of lipase (oil degradation enzyme), which degrades the oil to organic acids and volatile fatty acids (VFAs) or reduces it to a low molecule via beta oxidation (fatty acid degradation pathway). And finally, the oil is decomposed to CO_2 and H_2O (Koshimizu et al. 1997).

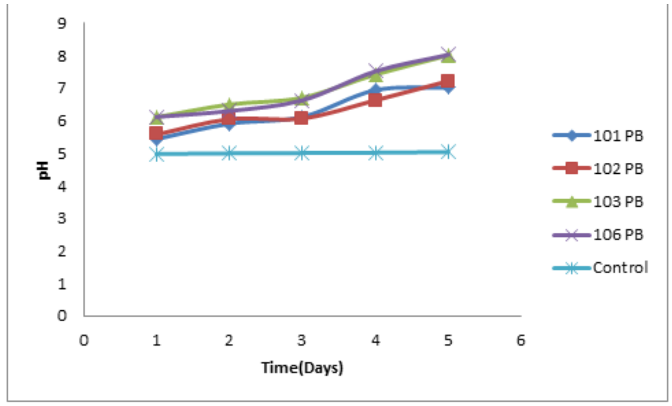

Figure 2: pH in sterile POME sample.

The strain 103PB with the oil and grease removal rate of 85.14% was the most effective followed by 106PB (75.7%), 102PB (67.65%) and 101PB (64.76%) Figure 1. The oil and grease removal were higher than the control sample where no strains were added. This suggests that our strains isolated from POME are effective in this present treatment technology for oil and grease removal. This study would help in understanding the role of bacteria in biological treatment of wastewaters such as those of oil processing.

In consistence with the present study, biological treatment of oil-containing wastes significantly removed organic load as well as oil and grease (ELGohary et al. 1987; Martine 1991; Martirani et al. 1996; Raj & Murthy 1999; El- Bestawy et al., 2005; El-Masry et al., 2004). Other investigators have also reported oil and grease removal which is comparable to the present study. Serikovna et al. (2013) reported fat, oil and grease removal by *P aeruginosa* G23 with the degradation rate of 62%-66%, *Acinetobacter* sp (60-65%) (Wakelin et al. (1997), *Rhodobacter shaeroide* S (74.2%) (Takeno et al. (2005). 96% of oil was also removed from wastewater as reported by Takeno et al. (2005), *Y lipolytica* W29 (93.3%) (Lan et al. (2009), *Acinetobacter* sp KUL8(88.8%), *Bacillus* sp KUL39(81.6%) for palm oil and bakery wastewater (Bhumibhamon et al. 2002). Hassan et al. (1997) has also reported the treatment of palm oil by *Rhodobacter sphaeroides.*

Our findings of the degradation of wastewater containing oil and grease such as POME with strains 101PB, 102PB, 103PB and 106PB were similar with those results in previous publications (Lanciotti *et al.*, 2005; Oswal *et al.*, 2002; Papanikolaou *et al.*, 2002; Scioli and Vollaro, 1997). However, they use organisms isolated from other sources, while in our study bacteria were isolated from POME for its treatment. This emphasizes the originality of our work. This was further elucidated by Jameel and Olanrewaju, (2011) who reported that the application of microorganisms such as *Trichoderma viride* spores, *T. viride* mycelium, *Yarrowia lipolytica* and *Saccharomyces cerevisiae* for the treatment of POME have not been extended to the removal of oil and grease (Jameel and Olanrewaju, 2011) despite their high potential in removing COD from POME. This may be due to the fact that these microorganisms are not indigenous to POME. This therefore offer researchers a greater opportunity to investigate the removal of oil and grease from POME using microorganisms isolated from POME (Jameel and Olanrewaju,2011).This is the focus and emphasis of the present study and it is design for this purpose. Hence, this has therefore attracted the interest of this study.

Results showed that higher oil and grease percentage removal was achieved in non-sterile POME sample with treatment after 5 days. This was due to the fact that bacteria already exist in consortium or mixed culture since this sample was not sterilize. This suggests that the addition or inoculation of more bacterial into the environment (POME) degrade the oil waste more efficiently. This is in agreement with Obire, (1988) who reported that biodegradation of oil polluted environment can be enhanced by inoculation with microbial species that will degrade the oil waste more efficiently.

The main aim of the non- sterile POME sample was to investigate the maximum enhancement in the degradation capabilities of the investigated bacteria using the natural conditions of the raw POME where they were originally isolated in order to get the most effective and economical treatment under the effluent's natural conditions in the field.

In non- sterile POME sample, percentage removal efficiency of oil and grease for all the isolates are presented in Figure 3.

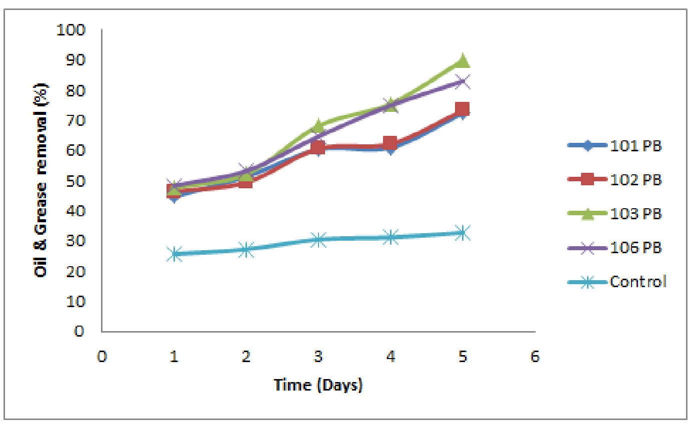

Figure 3: Percentage removal of oil and grease in non sterile POME sample

Results revealed that removal efficiency of oil and grease for all the isolates are as follows: 101 PB (72.50 %), 102 PB (73.30%), 103PB (89.87%), 106 PB (83.04%) and control (32.80%) Figure 3. It was also observed that the pH range for all the isolates were 7.04 – 8.37 after 5 days of treatment Figure 4. The initial acidic pH of the raw POME (4.74) became alkaline except in the control sample (6.27) probably due to the utilization of fatty acids present in the raw POME by the bacteria isolates (Oswal et al., 2002. In the non- sterile POME, pH increase was higher than in the sterile POME (non- sterile, 7.04-8.37 and sterile, 7.02- 8.03) probably because palm oil which is an inducer is more in the non sterile POME sample since no heat was applied and in the presence of some lipase producing organisms, hence raises extracellular lipase yield as much as 10-fold (Shabtai 1991; Shabtai & Daya-Mishre 1992; Sigurgisledottir et al. 1993; Woolley & Petersen 1994) with concomitant increase in pH to a more alkaline environment.

The strain *Bacillus cereus*103PB with the oil and grease removal rate of 89.87% was the most effective followed by *Bacillus subtilis* 106 PB (83.04%),*Stenotrophomonas maltophilia* 102 PB (73.30%) and *Micrococcus luteus*101PB(72.50%) Figure 3. The oil and grease removal were higher than the control sample where no strains were added to the already existing microbial population in order to increase their concentration and hence low removal rate was achieve as compare to POME samples where more strains were added to the microbial population already existing in the POME sample. This suggests that 103 PB and 106 PB and other strains were able to survive competition in the environment and together with other existing lipase producing

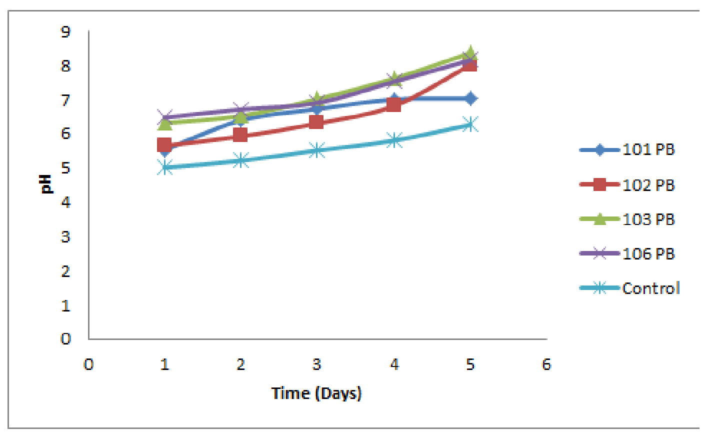

Figure 4: pH in non- sterile POME sample

organisms were able to achieve higher removal of oil and grease as compare to the sterile POME sample. This is in good agreement with Serikovna et al. (2013) who reported that, it is known that in order to reach more efficient removal of pollutant; bacterium must be pre-adapted in the medium containing the contaminant, which makes the bacteria more competitive. This suggest that if our isolates in the sterile POME sample are combine or mixed since they are already pre-adapted from POME, high removal rate will be achieve. This was evident in our previous study where mixed cultures provide high degradative activity than pure culture.

Since non- sterile POME s**a**mple in this present study represent mixed culture of organisms with the ability to remove higher organic load (oil and grease), cited literatures reported that using bacteria as mixed cultures produces higher degradation activity than pure/single cultures. El- Bestawy et al. (2005) and Wakelin et al. (1997) reported the investigation of pure and mixed cultures for microbial removal of fats, oils and greases. *Acinetobacter* sp. was the most effective among the pure cultures studied in the work, and hydrolyzed 60-65% of fatty material.

Removal of TSS in sterile and non sterile POME sample

In sterile POME sample, percentage removal efficiency of TSS for all the isolates is presented in figure 5. Results in figure 5 represent levels of percentage removal efficiency (RE %) of TSS from POME. Results revealed that removal efficiency of TSS for all the isolates are as follows: *Micrococcus luteus*101PB (45.85%), *Stenotrophomonas maltophilia* 102 PB (50.45%), *Bacillus cereus*103PB (71.63%), *Bacillus subtilis*106 PB (57.36%) and control (22.34%). The strain 103 PB with TSS removal rate of 71.63% was the most effective followed by 106 PB (57.36%), 102 PB (50.45%) and 101PB (45.85%) Figure 5. The TSS removal was higher than the control sample where no strains were added. This suggests that our strains isolated from POME are effective in this present treatment technology for TSS removal. This study would help in understanding the role of bacteria in biological treatment of wastewaters such as those of oil processing like POME.

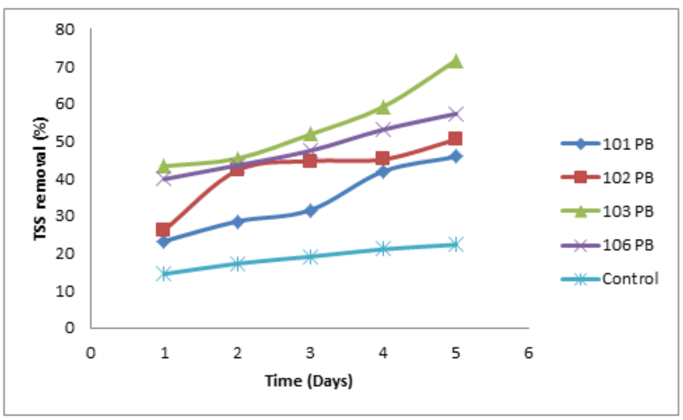

Figure 5: Percentage removal of TSS in sterile POME sample.

Comparisons of TSS removal efficiencies by bacteria isolated from POME revealed that percentage removal were similar with those results in previous publications. Abdul karim et al. (2011) reported 27.2% TSS removal by *Trichoderma harzianum* from POME. Maygaonkar et al. (2012) also reported TSS reduction by *Aspergillus nidulans* with significant change in TSS reduction from distillery effluent. Oswal et al.(2002) reported similar reduction of total dissolved solids (TDS) from POME.

In compliance with the present study, other workers have reported biodegradation of oily wastewaters with significant reduction in TSS and similar parameters (ELGohary et al. 1987; Martine 1991; Martirani et al. 1996; Raj & Murthy 1999; Odegaar et al. 1998; El- Masry et al., 2004; El-Bestwy et al., 2005).

Since palm oil mill effluent (POME) contains a very high organic matter, which is generally biodegradable, this facilitates the application of biological treatment based on aerobic process (Chin and Wong, 1983). This is evident by the present study in which biodegradation of POME using bacteria isolated from POME showed noticeable reduction in TSS and other parameters. The biological treatment depends greatly on active microorganisms, which utilizes the organic substances present in the POME as nutrients and eventually degrades these organic matters into simple by-products such as methane, carbon dioxide, hydrogen sulphide and water (Jameel and Olanrewaju, 2011; Jameel et al.,2011). Thus, the exploitation of these microorganisms isolated from POME for biodegradation and bioremediation purposes will offer a very efficient tool for purifying contaminated effluents water.The results presented in this study of simultaneous removal of TSS and oil and grease, appears useful for practical wastewater treatment as a compact treatment system for POME.

Results showed that higher TSS percentage removal was achieved in non-sterile POME sample with treatment after 5 days. The reasons are the same as earlier elucidated in non-sterile POME sample for oil and grease removal in our earlier study and the essence/aim of the step was clearly stated.

In non- sterile POME sample, percentage removal efficiency of TSS for all the isolates are presented in figure 6.

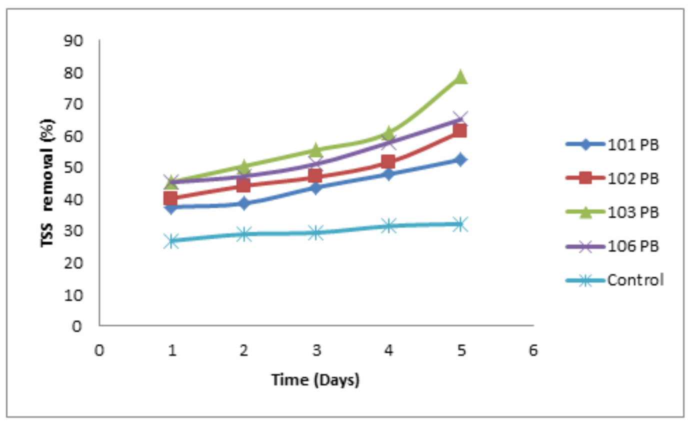

Figure 6 Percentage removal of TSS in non sterile POME sample.

Results revealed that removal efficiency of TSS for all the isolates are as follows: *Micrococcus luteus*101PB(52.53%),*Stenotrophomonas maltophilia* 102PB (61.28%),,*Bacillus cereus*103PB 78.57%), *Bacillus subtilis*106 PB (65.2%) and control (32.25%) Figure 6.The strain 103 PB with removal rate of 78.57% was the most effective followed by 106 PB (65.2%), 102 PB (61.28%) and 101 PB (52.53%) Figure 6. Strains were able to survive competition in the raw non- sterile POME sample because they were originally pre- adapted from the medium/niche (POME) which makes them more competitive (Serikovna et al., 2013). As earlier suggested, if our isolates in the sterile POME sample are combine or mixed as consortium, higher removal rate will be achieve in the cleaning technology of TSS and oil and grease polluted wastewaters. This is in good agreement with previous workers who used mixed/consortium/combination of organisms to achieve higher degradative activity in reducing the parameters. Higher biodegradative activity was achieved in our previous study when mixed cultures were used in biotreatment of POME. Oswal et al. (2002) has reported treatment of POME with a consortium of bacteria developed from garden soil. The sequential treatments brought about a significant reduction in organic matter in POME. El- Bestawy et al. (2005) reported the combination of *Pseudomonas* sp. and *P. diminuta* as mixed cultures which produced the highest activity in reducing organic matter from contaminated industrial effluents.

In comparison with other similar methodologies, the present treatment proposal manifested more advantages. Results in the present study confirmed the usefulness of our aerobic selection where besides avoiding the anaerobic conditions required for operating and maintain anaerobic strains (Fiestas 1984; Martine 1991), no primary treatment (Valenzuela 1986; Tsonis 1993) is needed and it is a one-step process.

IV. CONCLUSION

This study demonstrated that POME degrading microorganisms can be isolated from POME polluted area and the degrading ability of these microorganisms is a clear indicator that these bacteria can be applied in the bioremediation techniques for biodegradation of POME to enhance treatment.

The present treatment proposal using a selection of aerobic bacterial strains isolated from POME exhibited high efficiency for removal of oil and grease as well as organic load (TSS). The strains also produce extracellular lipase and cellulase in our previous study which stimulates better waste treatment. Besides the high efficiency, no additional physical or chemical treatment was required. Moreover, maximum removal of the contaminants was achieved bringing the wastewater to an environmentally accepted quality for discharging into surface water safely. The results presented in this study of simultaneous removal of TSS, oil and grease, appears useful for practical wastewater treatment as a compact treatment system for POME.

To this end, the study used known bacteria isolated from POME as compare to the anaerobic digestion techniques used by other investigators and in most palm oil mills in Malaysia which uses undefined/unknown microbial consortium/populations for their treatment technology. This emphasizes the originality of our work.

In a nutshell, the main aim of the present study was to evaluate the biodegradation potential of bacteria isolated from POME and to find the most suitable strain(s) for a biological treatment technology of POME. Thus the strain *Bacillus cereus*103PB is the most effective bacteria and the best candidate to use in biological treatment technology of POME having the highest TSS and oil and grease reduction rate. Hence from this study it could be concluded that *Bacillus cereus*103PB do possess the biodegradation ability, and is able to reduce the pollutants of the effluent sample.

ACKNOWLEDGMENT

The authors would like to thank Universiti Sains Malaysia for the financial support under Research Grant, No.: 1001/PTEKIND/814147.

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