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ORIGINAL PAPER

Reduction of Organic Load and Biodegradation of Palm Oil Mill Effluent by Aerobic Indigenous Mixed Microbial Consortium Isolated from Palm Oil Mill Effluent (POME)

J. D. Bala[1] · J. Lalung[1] · Adel A. S. Al-Gheethi[2] · H. Kaizar[1] · N. Ismail[1]

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Abstract

This study was designed to determine the potential of indigenous mixed microbial consortium isolated from palm oil mill effluent (POME) in reducing organic load and biodegradation of POME. 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 of the microbial strains suggests that they were identified 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. Results revealed that total percent reduction efficiency by the aerobic mixed microbial consortium for all bacteria–fungi combination (ABFC) gave a biochemical oxygen demand (BOD) reduction efficiency of about 90.23%, chemical oxygen demand (COD) 91.06%, and total suspended solids (TSS) 92.23% and bacteria–fungi stepwise (BFSW) recorded BOD reduction efficiency of 85.28%, COD 84.45%, and TSS 86.18% in 1000 mL of POME. The HPLC chromatogram results revealed increase in glucose level due to breakdown of cellulose which represents the cellulosic materials in POME by mixed microbial consortium signifying biodegradation of cellulose as a clean-up process for the tested POME sample. Therefore, the indigenous microbial strains are promising organisms for industrial applications. These microbes have direct applications in industrial process such as bioremediation and biodegradation of wastewaters.

Keywords Bacteria · Biodegradation · Fungi · Mixed microbial consortium · Organic load · POME · Wastewater

✉ J. D. Bala
jerrybrown316@yahoo.com; bala.jeremiah@futminna.edu.ng

J. Lalung
japareng@usm.my

Adel A. S. Al-Gheethi
adelalghithi@gmail.com

H. Kaizar
Kaizar.Kaizar@gmail.com

N. Ismail
drnorli@yahoo.com; norlii@usm.my

[1] Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia (USM), 11800 Pulau Penang, Malaysia

[2] Micro-pollution Research Centre (MPRC), Department of Water and Environmental Engineering, Faculty of Civil and Environmental Engineering, Universiti Tun Hussein Onn Malaysia, Batu Pahat, Johor, Malaysia

Introduction

The palm oil industry is identified as one of the agricultural industries in Malaysia that generate the highest pollution load into rivers throughout the country [81]. 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). POME is a non-toxic liquid waste with unpleasant smell; its chemical oxygen demand (COD) and biochemical oxygen demand (BOD) values are high enough to cause serious pollution and environmental problem to the rivers. COD and BOD of POME are very high and COD values greater than 80,000 mg/L are frequently reported. Incomplete extraction of palm oil from the palm nut can increase COD values substantially [60].

The treatment of POME usually involves cellulose- [69] and oil-degrading [60] microorganisms. However, the biological processes for wastewater treatment consist

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of mixed communities with a wide spectrum of microorganisms, including bacteria, protozoa, fungi, rotifers, and possibly algae [68].

Microbial degradation of organic wastes in wastewaters using microorganisms such as bacteria, molds, and yeasts had shown to be capable of completely degrading organic matter in wastewaters [32]. The use of a consortium of microorganisms is often desired since a simulation of the natural processes occurring in the environment is not possible with single-species populations but requires consortia activities [25]. The activities are mutual interactions between two or more populations in a given community, which enable the microorganisms to maximize their metabolic capabilities and to maintain community integrity and stability [35].

Very few studies have been conducted on aerobic digestion process for the treatment of organic pollutants present in POME [82]; as such, there seems to be dearth of information on the microbiology of POME in literatures [58]. The total suspended solids (TSS) which are the cellulosic material in POME are still present after treatment using various physical and chemical treatment processes. These remain a problem to be resolved further [2]. The use of microorganisms in biological treatment of wastewaters offers an alternative solution to reduce the COD, BOD, and TSS content of effluents [8]. Thus, the exploitation of microorganisms for biological treatment offers a very efficient tool for purifying contaminated effluents and water [39]. In addition, Maygaonkar et al. [54] reported that the physical and chemical treatment of industrial effluents is found to be insufficient whereas the biological treatment is most often found to be effective.

Therefore, this work represents one of the few studies on the aerobic biotreatment technology of POME-contaminated wastewater in Malaysia. Treatment of POME is essential to avoid environmental pollution. Biotreatment of POME by *Yarrowia lipolytica* NCIM 3589 has been studied [60]. Abass et al. [1], Soleimaninanadegani and Manshad [72], and Bala et al. [16] have also reported aerobic digestion of POME by selected microorganisms. Treatment of POME has been variously reported by other workers [3, 29, 56, 70].

The anaerobic digestion treatment of POME has employed, almost exclusively, uncharacterized microbial consortia to reduce the polluting power of wastes and wastewaters using various types of bioreactors by researchers and the ponding systems in the mills [55, 62]. This involves a consortium of unknown microorganisms.

This study seeks to avoid the anaerobic conditions required for operating and maintaining anaerobic strains, as aerobic condition requires less hassle compare to anaerobic conditions in terms of microbial strain sustainability. In the present study, microorganisms were isolated from POME and the best isolates capable of degrading organic wastes were further selected and used to inoculate the POME. The bioremediation and biodegradation potential of the microorganisms based on their efficiency for organic load reduction and the percent reduction was monitored with a goal to enhance industrial effluent treatment. Therefore, so far, to the best of our knowledge, no work has been done on the isolation of different aerobic microorganisms in POME and the cellulosic degradation in oil-contaminated wastewater such as POME.

It has been reported that one of the major sources of water pollution is suspended solids. Suspended solids in POME are considered as organic matter [47]. The TSS which represent the cellulosic materials in POME constitute about 50% of the POME sample [29] and still remain to be explored. Various physical and chemical treatment processes have been designed to treat agricultural and industrial effluents such as POME; however, the problem of chemical residues and TSS which is still present after the treatment process remains to be resolved further [2].

The application of microorganisms such as *Trichoderma viride* spores, *T. viride* mycelium, *Yarrowia lipolytica*, and *Saccharomyces cerevisiae* for the treatment of POME from other source was not that effective in organic load reduction [49]. This may be due to the fact that these microorganisms are not indigenous to POME [49]. This gap offered researchers a greater opportunity to explore the organic load reduction of POME by their indigenous (autochthonous) microbes. To this end, in this treatment proposal, effective indigenous mono or mixed culture of viable microorganisms is required to be selected. This is the focus of the present study and it is designed for this purpose. This has therefore attracted the interest of this study.

Thus, while enjoying a most profitable commodity, the adverse environmental impact from the palm oil industry cannot be ignored. Hence, serious measures have to be taken to prevent the growing pollution and ecological degradation related to POME while maintaining the sustainability of the economy. This study was designed to determine the potential of indigenous mixed microbial consortium isolated from POME in reducing organic load in POME, thereby decreasing COD, BOD, and TSS which represent the cellulosic materials in POME.

Methods

Sample Collection

Raw POME was collected aseptically from MALPOM Sdn. Bhd. Pinang Malaysia palm oil mill industry in a sterile microbiological container (20 L) and brought back to the laboratory. The raw POME was collected directly from the tap connected to the holding tank containing the raw POME before discharge. 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 order 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 the School of Industrial Technology laboratory, in Universiti Sains Malaysia. The physicochemical characteristics of the sample were determined in accordance with the standard methods published by the American Public Health Association [12].

Sample Preservation

The raw POME was preserved at 4 °C, in order to prevent the wastewater from undergoing biodegradation due to microbial action [12]. The sample was brought out from the refrigerator and left at room temperature before usage.

Experimental Design

A factorial complete randomized design (CRD) was used to determine the percentage reduction of the physicochemical parameters in 1000 mL POME sample experiment by all bacteria–fungi combination (ABFC) and bacteria–fungi in stepwise (BFSW). A $12 \times 2 \times 2$ CRD and a $5 \times 2 \times 2$ CRD for bacteria and fungi strains were used respectively. Analysis of variance (ANOVA) was carried out using SPSS for the data generated and means were separated using least significant difference (LSD) at 95% ($P < 0.05$) level of confidence.

Experimental Setup

The experimental setup consists of cultivation, isolation, and enumeration of total heterotrophic indigenous palm oil- and cellulose-utilizing bacteria and fungi from POME. Enzyme activity for the detection of lipase- and cellulase-producing bacteria and fungi on solid media by plate assay was also carried out in our previous studies [13–18]. One thousand milliliters (1000 mL) of POME sample was used to determine the percentage reduction of the physicochemical parameters by ABFC and BFSW. HPLC analysis for biodegradability of POME sample was carried out.

Microorganisms

Only four bacteria strains (*Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, and *Bacillus subtilis* 106PB) and two fungi strains (*Aspergillus fumigatus* 107PF and *Aspergillus niger* 109PF) were selected for POME inoculation and treatment based on the criteria that they were able to display good growth and degradation of oil and cellulose as sole source of carbon and energy in liquid mineral salt medium (MSM) from our previous work [14, 17, 18]. The bacteria and fungi strains were isolated from our previous studies [13–18]. Genetic identification of the isolated strains was performed by determining nucleotide sequences of 16S rRNA and 18S rRNA genes for bacteria and fungi strains respectively in our previous studies [13–18]. The isolated bacteria and fungi strains from POME in our previous work were investigated for their ability to produce lipase and cellulase on solid media by plate assay [18]. Biodegradation potential and growth profile in liquid MSM were also investigated in our previous study [13–18].

Sequences of primers used for bacteria strains are:
27F: 5′-AGAGTTTGATCMTGGCTCAG-3′
1492R: 5′-GGGTTACCTTGTTACGACTT-3′
Sequences of primers used for fungi strains are:
ITS1 F: 5′-TCCGTAGGTGAACCTGCGG-3′
ITS4 R: 5′-TCCTCCGCTTATTGATATGC-3′

Treatment of POME by Selected Mixed Microbial Consortium

In our previous study, batch culture experiment containing 250 mL of POME sample was used to evaluate the reduction efficiency potential of individual bacteria strains [16]. POME sample volume was increased in order to evaluate the mixed microbial strain percent reduction potential in 1000 mL POME sample. Reduction of organic load of POME by selected mixed microbial consortium using a 1000-mL plastic container containing both bacteria and fungi strains was carried out after successful reduction of organic load conducted in a 250-mL conical flask with individual bacteria strains from POME in our previous study [16]. The objective of this experiment in a 1000-mL plastic container was to mimic the practicability of the reduction efficiencies of the mixed microbial consortium in a 1000-mL plastic container as large amount of the effluent will be encountered during field application.

Three sets of experiment were carried out. The first three plastic containers in triplicates were inoculated with ABFC. These microbes comprised of *Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, *Bacillus subtilis* 106PB, *Aspergillus fumigatus* 107PF, and *Aspergillus niger* 109PF. The second three plastic containers in triplicates were inoculated with the isolates in a stepwise manner with the bacteria inoculated first (*Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, and *Bacillus subtilis* 106PB), then on the 25th day, fungi (*Aspergillus fumigatus* 107PF and *Aspergillus niger* 109PF) were inoculated (BFSW) and monitored for 50 days. Ten milliliters (10 mL) each of the

bacterial and 20 mL each of the fungal inoculums containing 10^6 cells/mL with an optical density (OD) of 1.2 at 600 nm were used to inoculate 1000 mL POME sample without addition of nutrients. The last experimental setup in triplicates was the control which no microbes were added. All the experimental setup was kept at ambient room temperature. This was maintained throughout the study. The aeration provided at interval of every 24 h was kept constant while the period (days) to evaluate samples for biodegradability of POME was varied at the 25th and 50th days. The aforementioned plastic containers were monitored for 50 days. This is to allow for maximum degradation of the organic load by the microbes as longer time would be required during field study and to mimic the practicability of environmental field application. Samples were aseptically withdrawn out at 5-day interval for physicochemical analysis. HPLC analysis for reducing sugars (glucose, sucrose, and fructose) was carried out before treatment in between the 25th and 50th days (after treatment) as an index for biodegradability of POME.

One thousand milliliters (1000 mL) was maintained as the working volume while at an interval of 24 h; 100 mL of the POME sample was withdrawn from the plastic containers and replaced with another 100 mL raw POME sample. The addition and withdrawal of 100 mL of POME in 1000-mL experiment were to assay the potential of the selected isolates to continuously reduce the organic load in the 1000-mL experiment for a long period of time and to maintain a semi continuous system. Aeration was provided by gradually stirring the POME sample in the plastic containers with a glass rod once at an interval of 24 h. The dissolved oxygen (DO) of the POME sample was measured to be 0.45–1.02 mg/L with a dissolved oxygen meter.

Analytical Methods and Characterization of POME

The following parameters BOD, COD, TSS, and oil and grease contents were analyzed by standard methods [12] for 50 days. These analyses were carried out according to the standard procedures [12]. Characterization of the POME was carried out before and after the treatment to determine the efficiency of the treatment. All the experiments were performed in triplicates. The efficiency for organic load reduction and the percent reduction was measured using the following Eq. 2.1 [61]. COD, BOD, TSS, and oil and grease contents were analyzed with a standard method [12] and presented as percent reduction.

$$\text{Reduction}\,(\%) = \left[\frac{C_{\text{raw POME}} - C_f}{C_{\text{raw POME}}} \times 100\right] \tag{2.1}$$

where $C_{\text{raw POME}}$ is the concentration of COD, BOD_5, TSS, and oil and grease contents of raw POME.

C_f is the concentration of these parameters after treatment. Each set of these experiments was done in triplicates.

All physicochemical parameters of the POME sample were determined in accordance with the standard methods published by the American Public Health Association [12]. The basic parameters that were analyzed for raw POME sample are as follows: chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), and oil and grease (O&G) contents.

BOD was determined according to the standard method 5210 B [12]. Dissolved oxygen meter (YSI) model 5000/5100 with membrane probe was used. COD was determined according to the standard method 5220 A [12] and digested by HACH COD reactor model 45600. TSS was determined according to the standard method 2540 D [12]. A Memmert Lab oven model U and a muffle furnace were used. O&G were determined according to the standard method 5520 B [12]. In the hexane-extractable partition-gravimetric method, hexane was evaporated by Buchi Rotavapor R-210/215.

Determination of Reducing Sugar Content in POME Sample by HPLC

High-performance liquid chromatography (HPLC) was performed on a Waters model equipped with Refractive Index Detector (RID) 2414 Waters model, Waters Sugar Pak™ chromatographic column 1; HPLC pump 515 Waters model and a temperature control module Waters model were used for sugar analysis (glucose, sucrose, and fructose) of POME. The analysis was conducted according to Takemoto et al. [75].

Preparation of Standard Solution

Standard of glucose, sucrose, and fructose was prepared by diluting 1.5 g of each into a 100-mL volumetric flask containing deionized water. Ten milliliters from the stock standard solution was taken and transferred into the 100-mL volumetric flask and topped up with deionized water to a 100-mL mark on the flask. Two milliliters (2 mL) of the diluted standard solution was withdrawn using a syringe and passed through a Sep-Pak disposable cartridge that has been previously activated with 2 mL of methanol and 4 mL of deionized water. The diluted standard solution was finally filtered through a nylon membrane syringe filter with 0.22 μm pore size before analysis.

Preparation of Mobile Phase for HPLC Analysis

Calcium-ethylenediaminetetraacetic acid (Ca-EDTA) was prepared by diluting 0.04 g Ca-EDTA into 1000 mL of deionized water. The resulting solution was further filtered using a vacuum membrane filter pump. The filtered solution was

Table 1 HPLC conditions for reducing sugar analysis

Model	Waters
Oven temperature	25 °C
Max temperature	90 °C
Pump flow	0.5 mL/min
Max pressure	2000 psi (13,789,514.6 Newton/square meter [N/m^2])
Detector	Refractive index detector
Detector temp	30 °C
Mobile phase	Calcium-ethylenediaminetetraacetic acid
Column	Waters Sugar Pak™ chromatographic column 1

transferred into a 1000-mL bottle and placed in an ultrasonic bath for 15 min in order to remove any air bubbles before usage.

Sample Preparation

Palm oil mill effluent (POME) sample was centrifuged and 2 mL of the centrifuged sample was slowly passed through the Sep-Pak disposable cartridge that has been previously activated with 2 mL of methanol and 4 mL of deionized water. Two milliliters (2 mL) of the collected sample was further filtered through a nylon membrane syringe filter with 0.22 μm pore size into a small vial bottle before analysis.

HPLC Standard Conditions

Reducing sugars (glucose, sucrose, and fructose) were analyzed by HPLC using the standard conditions as stated in Table 1. Samples were identified by comparing their retention time with those of known carbohydrate standards.

Results and Discussion

POME Characteristics

Raw POME collected from the palm oil mill was thick, brownish in color, in colloidal suspension, oily, and viscous with an obnoxious odor. The brown color of the raw palm oil mill effluent is composed of organic compounds such as anthocyanin and carotene pigments that were extracted from fresh fruit bunches in the sterilization process [56]. Furthermore, it included polyphenol compounds, tannin, polyalcohol, and melanoidin [4, 56]. The raw POME sample consists of high COD concentration up to 75,900 mg/L, BOD_5 34,393 mg/L, TSS 14,467 mg/L, oil and grease 191 mg/L, and pH 4.74 as presented in Table 2.

The high values of the parameters obtained from raw POME in the present study suggest the attendant-polluting potential of POME and the adverse environmental impacts. Researchers have reported alarming rise in environmental pollution due to the discharge of untreated POME into the environment [1, 7, 26, 38, 46, 51, 54, 56, 72, 82].

The value of pH recorded in this study is low (4.74), which is more acidic than the pH of 6.56 reported by Ohimain et al. [59]. The low pH of the POME indicates that it is acidic and should be treated to reach 7–7.5 pH as indicated level of plant compatibility. The acidic character of POME may have been influenced by the corrosion of iron used in processing [72]. Jameel et al. [48] and Din et al. [33] have reported that the acidic nature of POME is due to the presence of organic acids. Oswal et al. [60] in a similar study have reported the acidic nature of POME.

In comparison, Chin et al. [29] have reported that POME contains a high concentration of organic matter, COD concentration of 65,000 mg/L, BOD_5 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 with BOD_5 (25,000–43,750 mg/L), COD (50,000–55,775 mg/L), TSS (16,500–18,479 mg/L), oil and grease (130–8020 mg/L), and pH (3.5–4.7) ([6, 51, 57, 77]; and [2]). The result of

Table 2 Characteristics of raw palm oil mill effluent (POME) and discharge standard limits

Parameters	Raw POME (mg/L)	Discharge effluent standard	
		Malaysia	Thailand
Chemical oxygen demand (COD)	61,200–75,900	–	< 1000
Biochemical oxygen demand (BOD)	30,500–34,393	100	< 100
Total suspended solids (TSS)	12,800–14,467	400	< 150
Oil and grease (O&G)	145–191	50	< 25
pH	4.37–4.74	5–9	5–9

All parameters are in mg/L except pH

Source: Malaysia: Environmental Quality Act 1974 [37]; Thailand: Environmental Management Guideline [36]

oil and grease in the present study was low when compared with results from other researchers who obtained higher values for oil and grease [5, 56, 72, 78]. Although, Lam and lee [51] 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. In addition, Yacob et al. [83] have reported that the chemical properties of POME vary widely throughout the year due to mill operations and seasonal cropping.

Percentage (%) Reduction of BOD, COD, and TSS by Mixed Microbial Consortium of Microorganisms (ABFC and BFSW in 1000-mL Experiment)

One thousand milliliters (1000 mL) of POME was used to evaluate the reduction efficiency of all the microbial isolates in combination. Two sets of experiments were conducted which involved the inoculation of all the microbes into the 1000-mL experiment (ABFC) and secondly the inoculation of the microbes in a stepwise manner where bacteria was inoculated first followed by fungi (BFSW).

Percent reduction efficiency of BOD_5, COD, and TSS by consortium of microorganisms in 1000-mL experiment is presented in Figs. 1, 2, and 3 respectively. Results in Figs. 1, 2, 3 represent levels of percentage reduction efficiency (RE %) of the aforementioned parameters in POME. Results revealed that reduction efficiencies of BOD, COD, and TSS for ABFC and BFSW in 1000-mL experiment are as follows: total percent reduction efficiency for ABFC gave a BOD reduction efficiency of about 90.23%, COD 91.06%, and TSS 92.23; BFSW recorded BOD reduction efficiency of 85.28%, COD 84.45%, and TSS 86.18% and the control experiments for all the parameters showed reduction efficiency ranging from 23.47 to 31.58% (Figs. 1, 2, and 3).

ABFC reduction efficiency as compared with discharge effluent standard revealed that 3360 mg/L of BOD_5 after treatment is above the discharge effluent standard for Malaysia and Thailand (100 and < 100 mg/L respectively) (Table 2), 6785 mg/L of COD after treatment is above the discharge effluent standard for Thailand (< 1000 mg/L; no COD discharge effluent standard for Malaysia) (Table 2), and 1124 mg/L of TSS after treatment is above the discharge effluent standard for Malaysia and Thailand (400 and < 150 mg/L respectively) (Table 2).

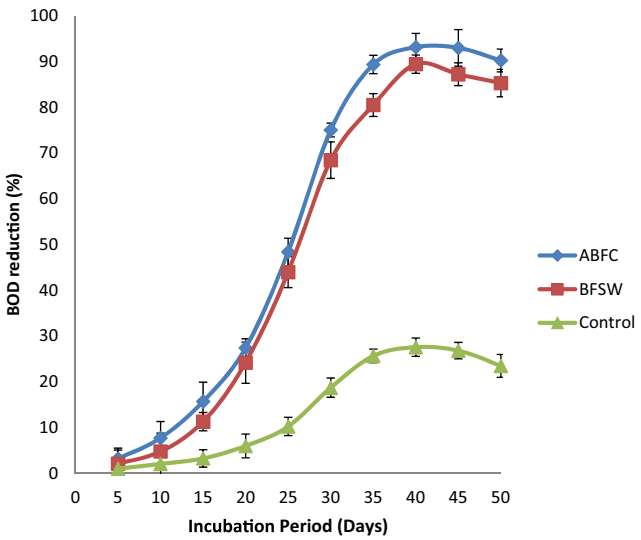

Fig. 1 Percentage reduction of BOD_5 by all bacteria–fungi combination (ABFC) and bacteria–fungi stepwise (BFSW) in POME sample

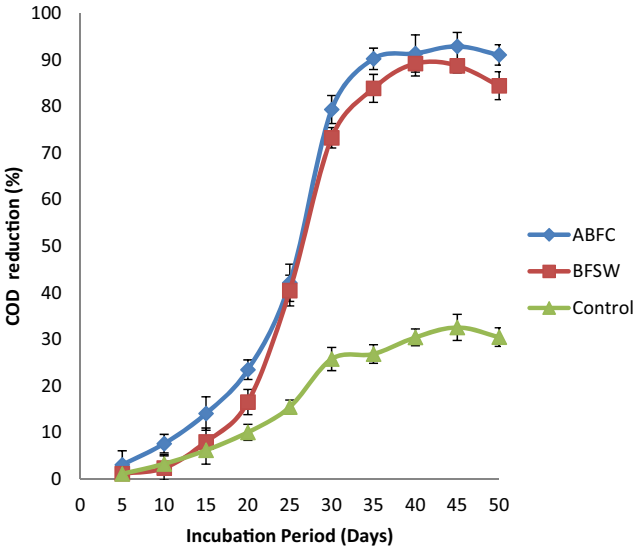

Fig. 2 Percentage reduction of COD by all bacteria–fungi combination (ABFC) and bacteria–fungi stepwise (BFSW) in POME sample

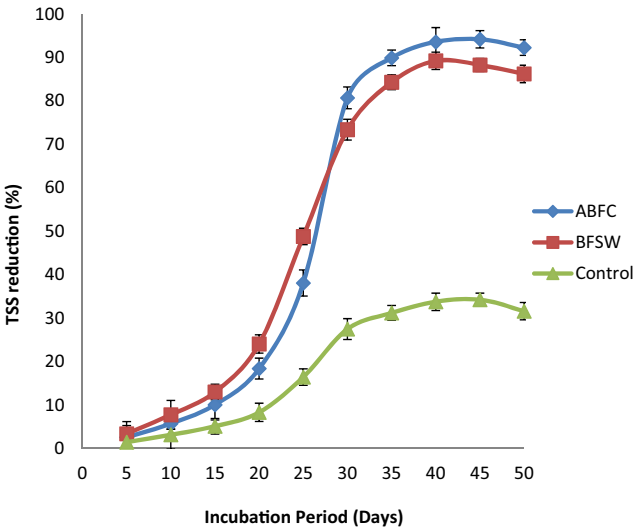

Fig. 3 Percentage reduction of TSS by all bacteria–fungi combination (ABFC) and bacteria–fungi stepwise (BFSW) in POME Sample

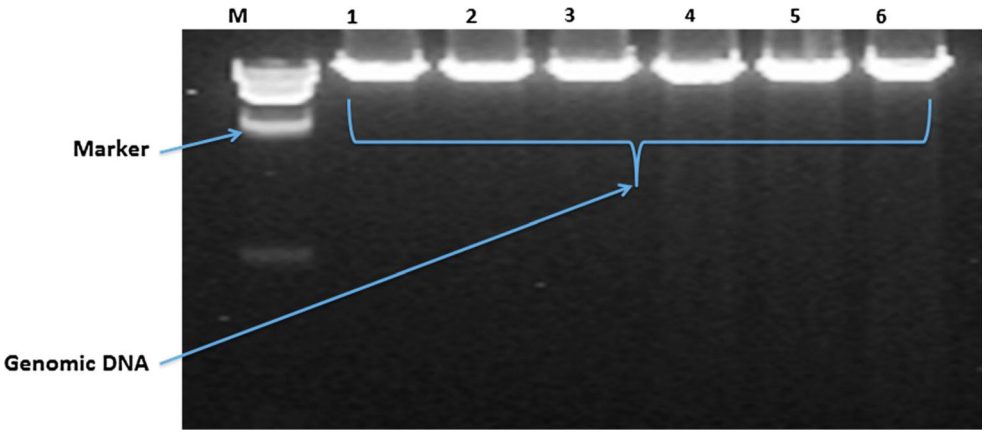

Plate 1 Gel picture of genomic DNA of bacteria isolated from POME. Lane 1: 101PB; 2: 102PB; 3: 103PB; 4: 104PB; 5: 105PB; 6: 106PB; M: Lambda/HindIII marker

BFSW reduction efficiency as compared with discharge effluent standard signifies that 5062 mg/L of BOD_5 after treatment is above the discharge effluent standard for Malaysia and Thailand (100 and < 100 mg/L respectively) (Table 2), 11,802 mg/L of COD after treatment is above the discharge effluent standard for Thailand (< 1000 mg/L; no COD discharge effluent standard for Malaysia) (Table 2), and 1999 mg/L of TSS after treatment is above the discharge effluent standard for Malaysia and Thailand (400 and < 150 mg/L respectively) (Table 2).

In order to meet the discharge effluent standards, successive treatment of the treated effluent for another consecutive time using the same microbial consortium in the present study will possibly achieve 100% reduction of the organic load (BOD, COD, TSS) since the same mixed microbial consortium of microorganisms was able to significantly reduce 84.45–92.23% of the organic load. This will further improve the present treatment technology as a clean-up process for the tested POME sample and the treated effluent.

ABFC showed significantly higher reduction of BOD_5, COD, and TSS compared to BFSW (Figs. 1, 2, and 3). This suggests that these indigenous microbial isolates are promising organisms for industrial applications. Hence, this mixed microbial consortium has direct applications in industrial process such as bioremediation and biodegradation of oily wastewaters. Control experiment showed the lowest reduction efficiency compared to treatment experiments (Figs. 1, 2, and 3). This revealed the potency and effect of our inoculated microbial isolates in this treatment technology. Qingwei et al. [63] have reported the effect of inoculation on organic matter biodegradation as compared to control experiment where no strains were inoculated. Plates 1, 2, 3, and 4 show genomic DNA and purified PCR product of bacteria and fungi strains isolated from POME while Figs. 4, 5, 6, 7, 8, and 9 revealed phylogenetic trees of the bacteria and fungi strains isolated from POME as members of the consortium used in this study.

In the present study, bacterial and fungal strains that were originally isolated and identified from POME in our previous study were used as consortium since a simulation of the natural processes occurring in the environment is not possible with single-species populations but requires consortium activities [25]. Such activities are typically interactions between two or more populations in a given community, which enable the microorganisms to maximize their metabolic abilities and to maintain community integrity and stability [35].

ABFC showed significantly higher reduction of the parameters compared to BFSW. This may be due to the combined synergistic effect of both bacteria and fungi combinations at the beginning or onset of the treatment process which was inoculated at the same time and proceeded throughout the treatment process compared to BFSW which involved the inoculation of bacteria combination first at the beginning followed by fungi combination on the 25th day in the treatment process. The synergistic effect of mixed microbial

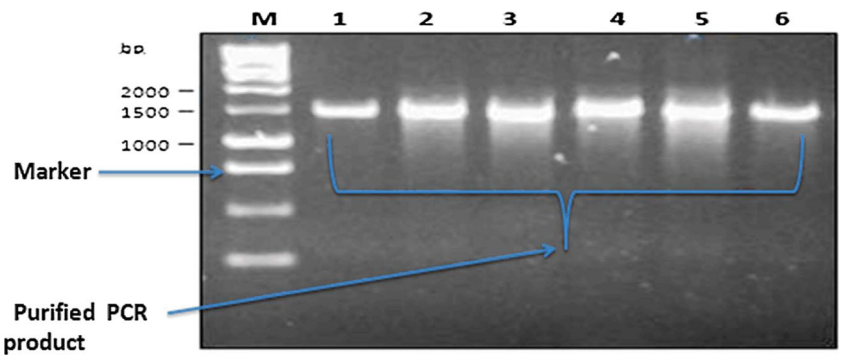

Plate 2 Gel picture of purified PCR product of bacteria isolated from POME. Lane 1: 101PB; 2: 102PB; 3: 103PB; 4: 104PB; 5:105PB; 6: 106PB; M: 1-kb marker (Fermentas)

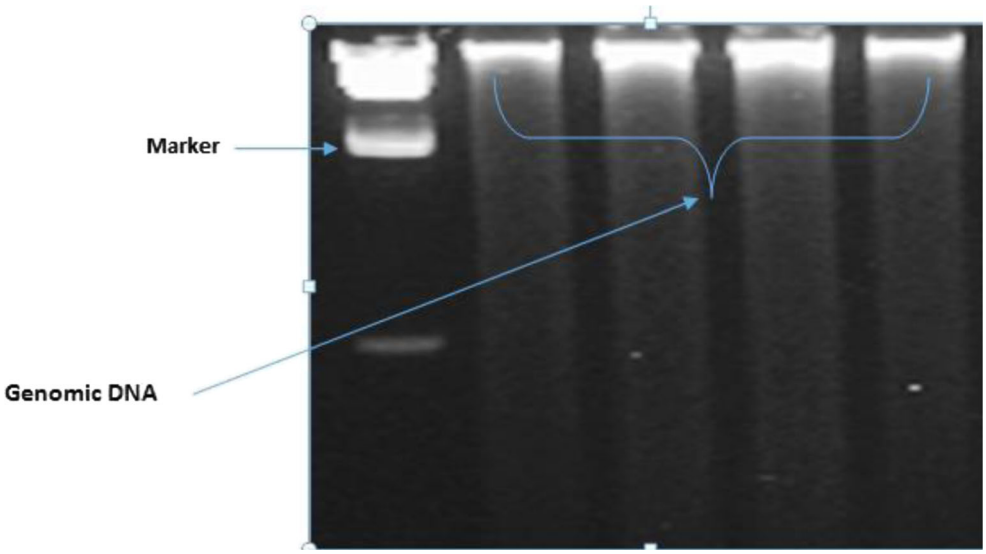

Plate 3 Gel picture of genomic DNA of fungi isolated from POME. Lane 1: 107PF; 2: 108PF; 3: 109PF; 4: 110PF; M: Lambda/HindIII marker

consortium for POME treatment brings about enhanced performance for effective biodegradation. However, synergism has been reported among mixed microbial consortium of organisms [28]. Benka-coker and Ekundayo [20] and Chigusa et al. [28] had reported that the performance of mixed microbial cultures could therefore be attributed to synergistic activities of the organisms.

Other studies have revealed the role of bacteria and fungi combination in bioremediation processes. De Felice et al. [30] used a combination of bacteria and yeast to degrade olive oil mill wastewater. The microbial combination reduced the COD of olive oil mill wastewater by 80%. Oswal et al. [60] have used the combination of *Yarrowia* with a consortium of bacteria and algae developed from garden soil, achieving COD reduction rate of 99% for the treatment of POME. The use of mixed cultures of bacterial strains has been previously reported by Bala et al. [13]. AbdulKarim et al. [2] in a similar study have also reported the use of *Trichoderma harzianum* and *Penicillium* in the treatment of palm oil mill effluent. Recently, Hernandez et al. [43] also reported the treatment of agro-industrial wastewaters using microalgae–bacteria consortium.

In a similar study, Sugiura et al. [73] and Sathishkumar et al. [67] reported that mixed microbial combination showed the maximum percentage reduction of organic load. Hamme et al. [41] noted that such mixed culture combination displays metabolic versatility and superiority to pure cultures. Consequently, Sathishkumar et al. [67] reported that a microbial consortium containing a number of microorganisms is considered to be well suited for the degradation of industrial wastewaters.

It has been reported that mixed microbial consortium cultures are better degraders compared to individual strain cultures ([27, 64, 73, 67]; Bala et al.[13]) because each member strain of the consortium acts in concert to effect the breakdown of all the different organic pollutants in wastewaters. A consortium culture also provides the necessary microenvironment, such as providing nutrients and being at the correct pH, thus stabilizing the culture for each of the members [42]. Thus, the use of a consortium of microorganisms is often desired since a simulation of the natural processes occurring in the environment is not possible with single-species populations but requires consortia activities [25]. The activities are mutual interactions between two or more populations in a given community, which enable the microorganisms to maximize their metabolic potential and to maintain community integrity and stability [35].

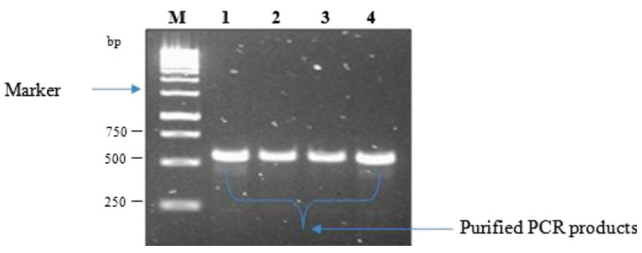

Plate 4 Gel picture of purified PCR product of fungi isolated from POME. Lane 1: 107PF; 2: 108PF; 3: 109PF; 4: 110PF; M: 1-kb marker (Fermentas)

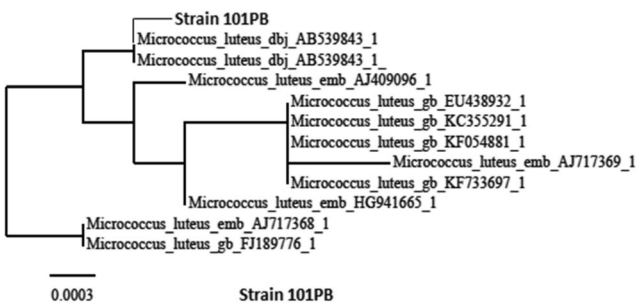

Fig. 4 Phylogenetic tree of *Micrococcus luteus* 101PB based on 16S rRNA gene sequence comparisons

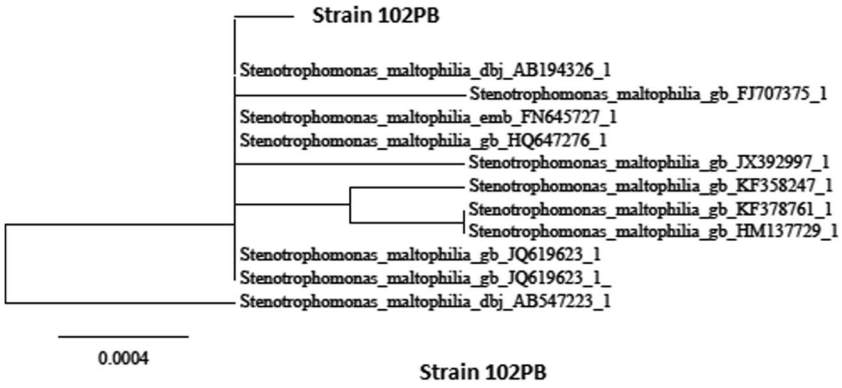

Fig. 5 Phylogenetic tree of *Stenotrophomonas maltophilia* 102PB based on 16S rRNA gene sequence comparisons

In the present study, it is worth noting that the achieved reduction of the parameters tested can further be treated in order to reduce the pollution load of POME into rivers, its attendant-polluting potential, and the adverse environmental impacts including land and aquatic ecosystem contamination and loss of biodiversity.

Moreover, the 1000-mL experiment in the present study resulted in a slower reduction process, even if similar final results were also obtained in the batch culture experiment containing 250 mL of POME sample in our previous study. The observed difference in the rate of the processes can possibly be ascribed to a reduced aeration of the larger volume system (1000-mL experiment) in respect to the shaken flask 250 mL POME sample experiment [50]. Thus, the indigenous microbial isolates are proven to be a good candidate for the effective reduction of organic load of POME. One of the major findings of the present study suggests that ABFC can be applied at the beginning of field bioremediation program in order to rid the environment of pollution load as a result of discharge of contaminated wastewaters since it shows higher reduction of most of the parameters (Figs. 1, 2, and 3).

Noteworthy in the present study also is the use of fungi in the biotreatment of POME. Fungi by nature are mostly aerobic and some cannot be used under anaerobic condition. The successful use of fungi in the present study provides an advantage over anaerobic treatment of POME which employed, almost exclusively, uncharacterized microbial consortia.

It has been reported that the presence of glucose could cause catabolite repression in the medium and result in the inhibition of the production of some hydrolytic enzymes [9, 10]. On the 25th day, there was a little increase in the level of residual glucose as detected by HPLC analysis (Figs. 12, 13, 14, and 15) and the production of enzyme continuous until the 30th and 35th days onward (Figs. 1, 2, and 3). On the 50th day, the concentrations of residual glucose increased significantly (Figs. 12, 13, 14, and 15), and hence, the residual glucose caused catabolite repression. Increasing of residual glucose in a medium will increase the occurrence of catabolite repression [9, 10]. As a result, there is inhibition in the cellulase and lipase enzymes produced by the microorganisms and a decrease in the reduction of the parameters (BOD_5, COD, and TSS) was evident as a decline in the graph (Figs. 1, 2, and 3), but a small amount of the enzyme already produced is still working, that is why the decline did not go down quickly.

Furthermore, lipase- and cellulase-producing microorganisms were prone to catabolite repression in the presence of residual glucose in the medium and thereby lead to the decrease in the trend of the percent reduction of the parameters (BOD_5, COD, and TSS) (Figs. 1, 2, and 3) in the POME sample which was evident by the decline in the graphs of all the parameters measured. Catabolite repression inhibits or represses the production of hydrolytic enzymes of an organism in the presence of residual glucose which will cause a decrease in the percent reduction of the organic load (parameters) since the enzymes

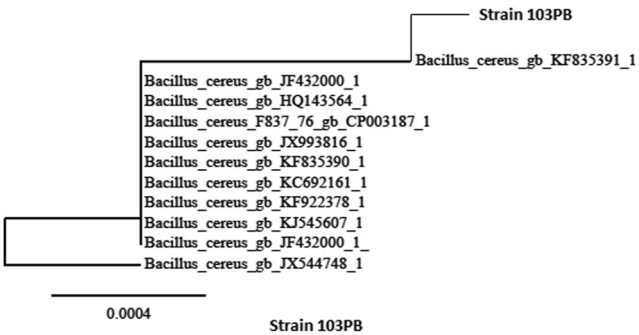

Fig. 6 Phylogenetic tree of *Bacillus cereus* 103PB based on 16S rRNA gene sequence comparisons

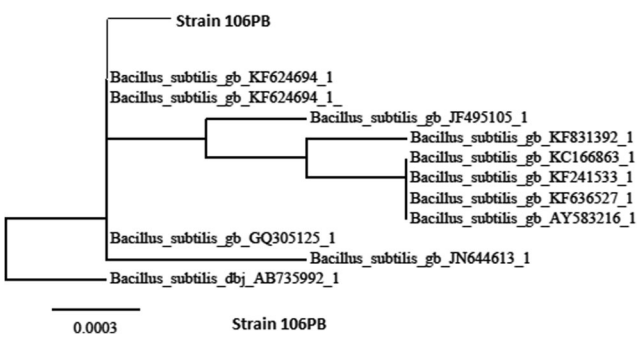

Fig. 7 Phylogenetic tree of *Bacillus subtilis* 106PB based on 16S rRNA gene sequence comparisons

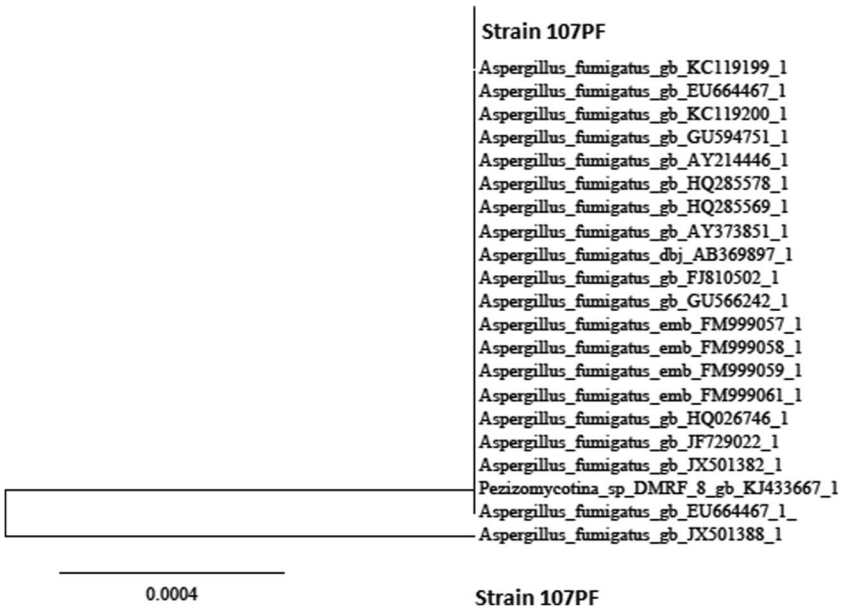

Fig. 8 Phylogenetic tree of *Aspergillus fumigatus* 107PF based on 18S rRNA gene sequence comparisons

involved in the degradation process are inhibited [10]. Boekema et al. [24] have reported that *Burkholderia glumae* strain PGI does not produce lipase in the medium containing glucose suggesting that lipase gene expression in the strain PGI was prone to catabolite repression. The presence of residual glucose inhibited or repressed the production of lipase in the medium, and hence, the degradative activity of the enzymes was inhibited which led to a decrease in the reduction of the organic load. In a similar study, Al-Gheethi [9] reported that *Sporosarcina pasteurii*, *B. subtilis*, *and Staphylococcus xylosus* lose the ability to produce cellulase in the presence of increased glucose. In addition, Lynd et al. [53] reported that the production of enzymes for the utilization of complex substrates, such as cellulose, is induced only in the presence of the substrate (or products thereof) but suppressed when easily utilizable sugars, such as glucose, are available in the medium.

Allcock and Woods [11], Al-Gheethi [9], Al-Gheethi et al. [10], and Bala [18] have reported that microbial strains can continually produce hydrolytic enzymes if they do not undergo catabolite repression to degrade their substrate (cellulose) and obtain the end-product (glucose) which can be detectable as long as the degrading microbes produce the enzymes. Even though microbes can utilize glucose for their survival (energy), it will be very less as compared to the production of the glucose in the medium.

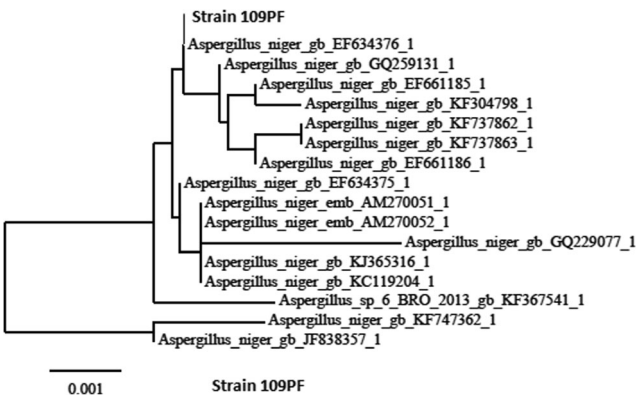

Fig. 9 Phylogenetic tree of *Aspergillus niger* 109PF based on 18S rRNA gene sequence comparisons

Biodegradation of Cellulose in POME Sample

HPLC Analysis

Biodegradability of cellulose was examined by high-performance liquid chromatography (HPLC) analysis. The sugars in POME were identified by using HPLC. Identification of the three sugars was performed by comparison of the retention time of the sugars in the POME sample and that of the known standards. The HPLC profile of the sugars in control and before treatment of raw POME revealed that glucose and fructose were found in POME (Figs. 10 and 11). Other retention time in the results denotes the presence of some sugars also found in POME that were not identified. Ohimain et al. [58] had reported the presence of sugars in POME.

Figures 12 and 13 and Figs. 14 and 15 revealed HPLC chromatogram results of after treatment at the 25th and 50th days for ABFC and BFSW respectively. The level of glucose in the tested sample after treatment was determined by comparing the total peak area of the chromatogram of the tested samples with that of the control [42]. If the peak level of glucose increased compared to the control, this indicated cellulose biodegradation in the tested sample (Beguin and Aubert [22], [52, 74]).

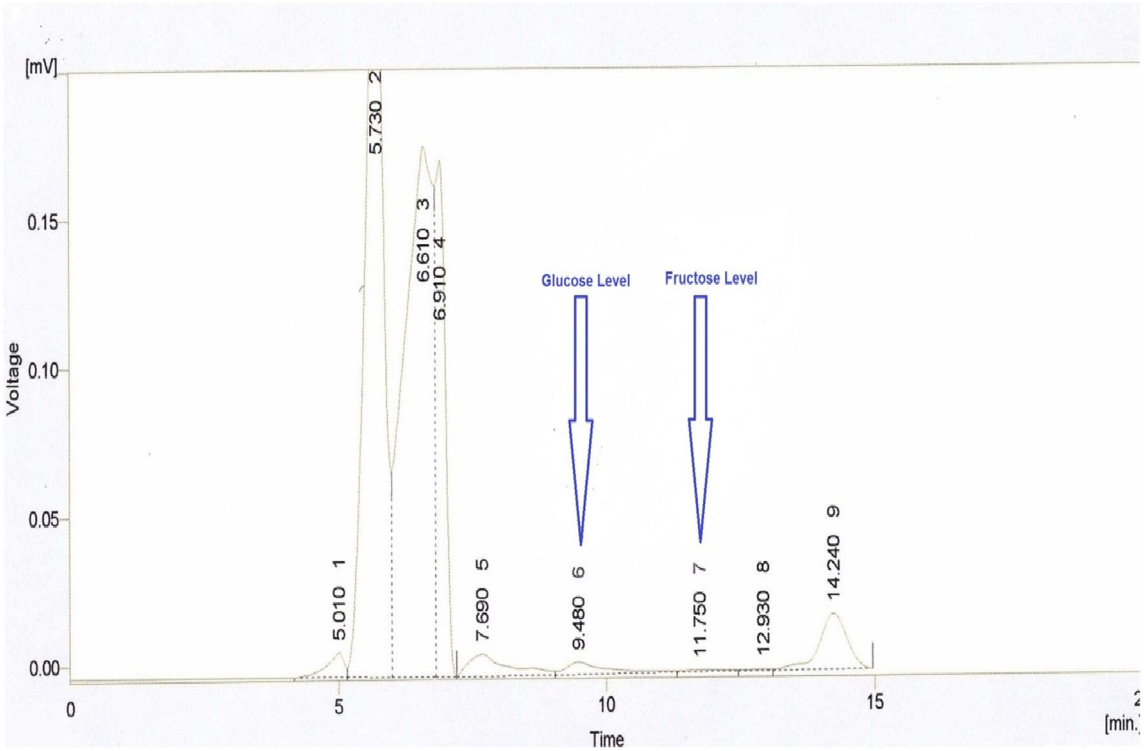

Fig. 10 HPLC chromatogram showing glucose and fructose with retention time of 9.480 (No. 6) and 11.750 (No. 7) respectively before treatment (raw POME). Residual glucose peak height (h) 4.128 mV (day 1)

The HPLC chromatogram results for the peak levels of glucose obtained in the tested sample as compared with the control and before treatment suggested that after treatment with ABFC and BFSW indicates enzymatic hydrolysis of cellulose in the POME by cellulases [40]. The bacteria and fungi in the present study were previously confirmed to

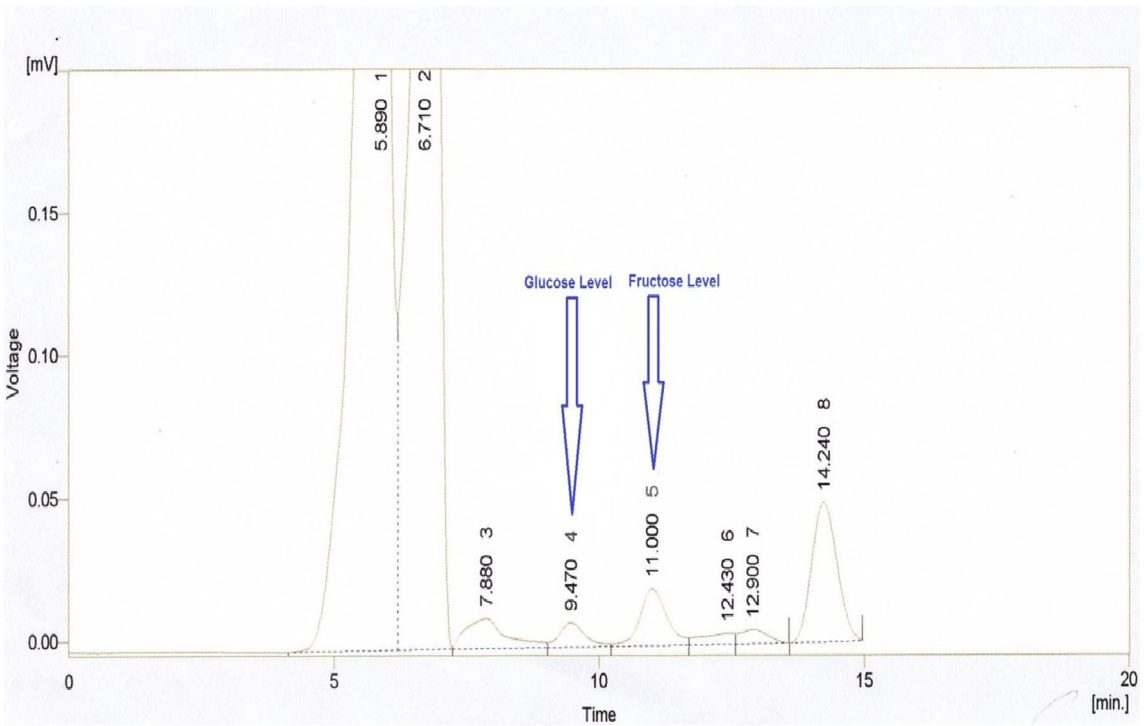

Fig. 11 HPLC chromatogram showing glucose and fructose with retention time of 9.470 (No. 4) and 11.000 (No. 5) respectively in control sample. Residual glucose peak height (h) 7.371 mV (50th day)

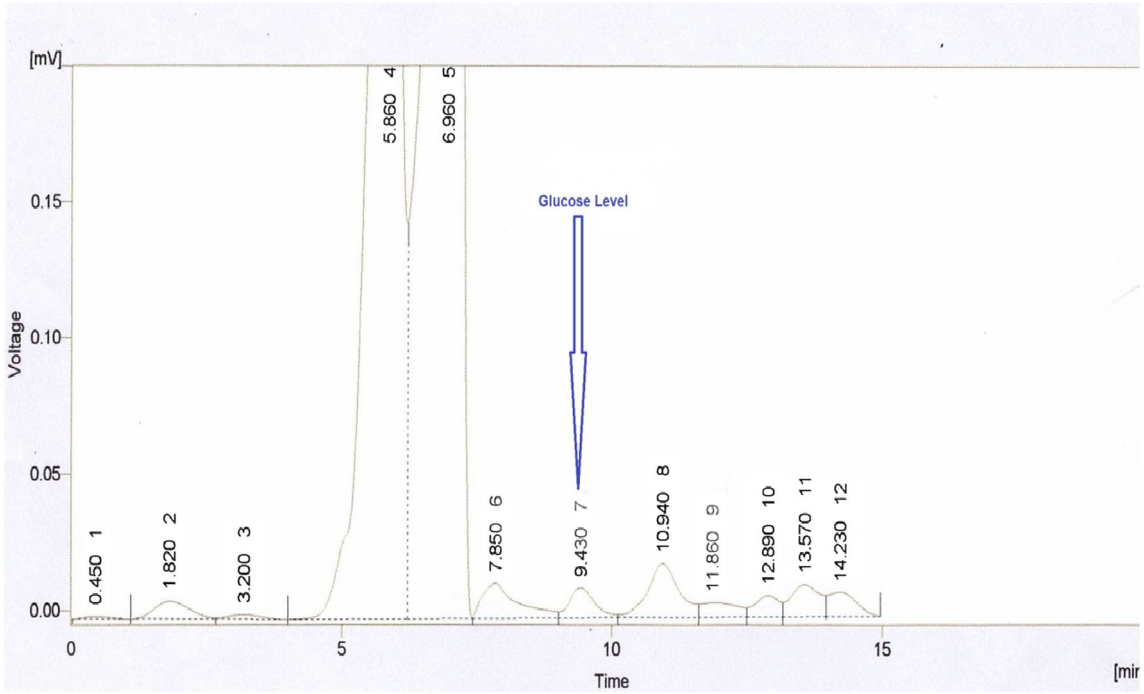

Fig. 12 HPLC chromatogram showing glucose peak level after treatment with ABFC at 25th day. Glucose retention time (9.430: No. 7). Residual glucose peak height (h) 11.007 mV

possess the ability to produce extracellular cellulase and lipase on solid media by plate assay in our previous study [13–18]. These enzymes (cellulase) are responsible for the breakdown of cellulose in POME [80]. The breakdown of carbon substrate such as cellulose by bacteria and fungi has been previously reported [60]. The identified bacteria and fungi in the present study are associated with cellulase production.

The composition of POME is mainly water, oil, suspended solid, dissolved solid and sand [46], and total suspended solids (TSS), as well as cellulose wastes [65], vegetative matter, colloidal slurry of water, and solids including about 2%

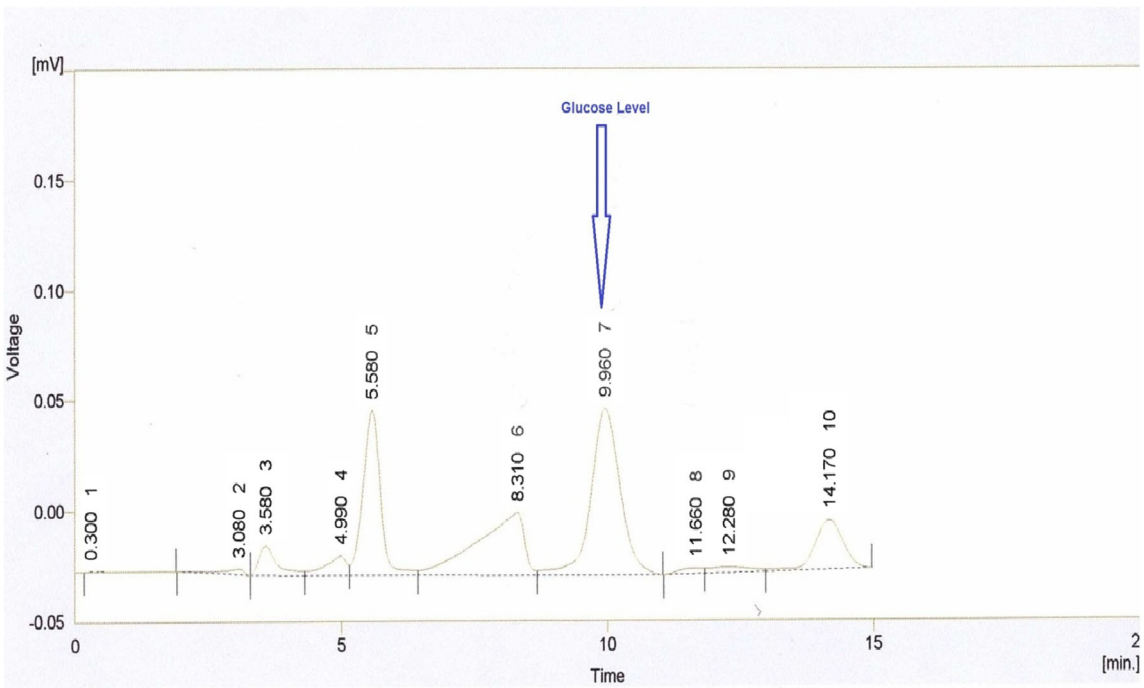

Fig. 13 HPLC chromatogram showing glucose peak level after treatment with ABFC at 50th day. Glucose retention time (9.960: No. 7). Residual glucose peak height (h) 75.601 mV

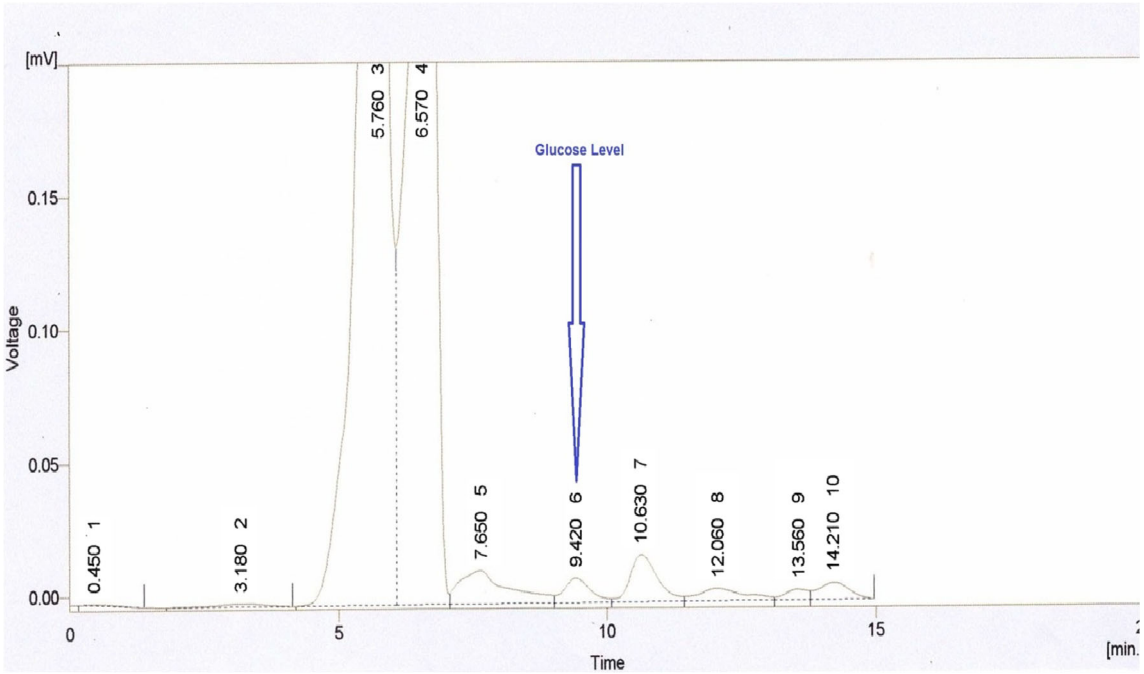

Fig. 14 HPLC chromatogram showing glucose peak level after treatment with BFSW at 25th day. Glucose retention time (9.420: No. 6). Residual glucose peak height (h) 9.188 mV

suspended solids originating mainly from cellulose fruit debris, that is, palm mesocarp [23]. The suspended solids in POME which are the cellulosic material derived from palm mesocarp are organic in nature [29] and constitute about 50% of the POME [45]. This, therefore, necessitates the degradation of cellulose in POME by cellulase-producing bacteria and fungi. During degradation, cellulose in the POME is broken down and mineralized [71].

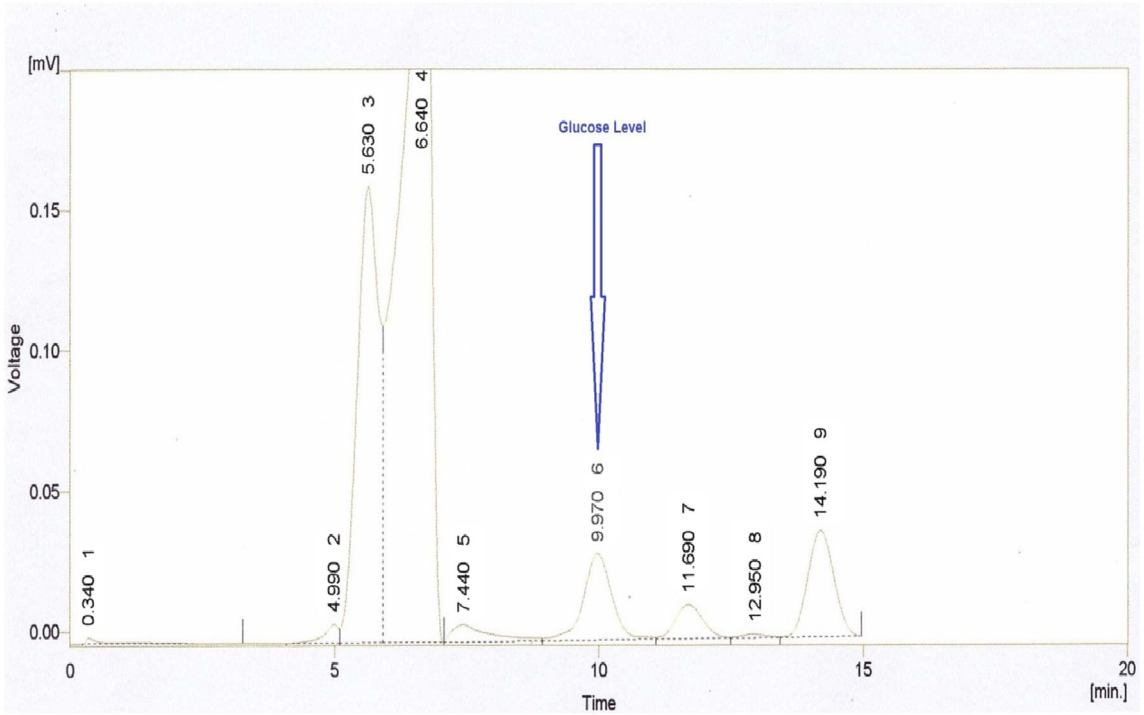

Fig. 15 HPLC chromatogram showing glucose peak level after treatment with BFSW at 50th day. Glucose retention time (9.970: No. 6). Residual glucose peak height (h) 30.913 mV

After treatment with ABFC and BFSW at the 25th and 50th days, glucose peak was evident as a result of biodegradation of cellulose in the POME sample as compared with the control and before treatment (Figs. 10, 11, 12, 13, 14, and 15). During the enzymatic hydrolysis, cellulose is degraded by the cellulases to reducing sugars primarily glucose that can be fermented by yeasts/fungi or bacteria to ethanol [74]. Cellulases refer to a class of enzymes produced mainly by fungi, bacteria, and protozoa that are capable of decomposing cellulose into small fragments, primarily glucose [44].

After treatment with ABFC at the 25th and 50th days, peak area levels of glucose increased from the 25th day to the 50th day (Figs. 12 and 13). It was also observed that higher glucose peak area was obtained in the ABFC than in BFSW on the 50th day indicating more degradative activities in ABFC. This was evident in the 1000-mL experiment where ABFC show significantly higher TSS reduction than BFSW to validate the glucose high peak level in ABFC during the HPLC analysis suggesting cellulose was degraded in both ABFC and BFSW. On the 50th day, higher glucose peak levels were observed in both ABFC and BFSW compared to that on the 25th day, control, and before treatment (Figs. 10, 11, 12, 13, 14, and 15) suggesting that cellulose was further degraded. Li and Ni [52] have reported that cellulose in *Dioscorea zingiberensis* processing wastewater was further and completely degraded by microorganisms based on the observation of the absorbance peaks of the wastewater sample after treatment during their study. Similarly, Lynd et al. [53], Ray [66], Hii et al. [44], and Dipasquale et al. [34] also reported that the enzymatic hydrolysis of cellulose requires the use of cellulase which converts cellulose into glucose. Based on the aforementioned results revealed in the present study by the HPLC analysis, microorganisms in this study were able to attack native cellulose in POME exhibited as TSS which represent the cellulosic materials in POME to glucose.

The evidence of the increased glucose peak level in the HPLC analysis (Figs. 12, 13, 14, and 15) suggests that the microorganisms in the present study are known to be strongly cellulolytic and are capable of solubilizing native cellulose. It is now established that at least three different types of enzyme known as cellulases are involved in the breakdown of native cellulose to glucose. The enzymes include endoglucanases, exoglucanases, and β-glucosidases and it has been shown that these enzymes are hydrolytic in character [79].

We intend to elucidate the specific function of these enzymes in the overall process of enzymatic cellulose degradation pathway. Figure 16 represents the pathway involved in enzymatic degradation of cellulose to glucose as corroborated by the HLPC results. Eq. 3.1 revealed the reaction equation for the enzymatic hydrolysis of cellulose to glucose.

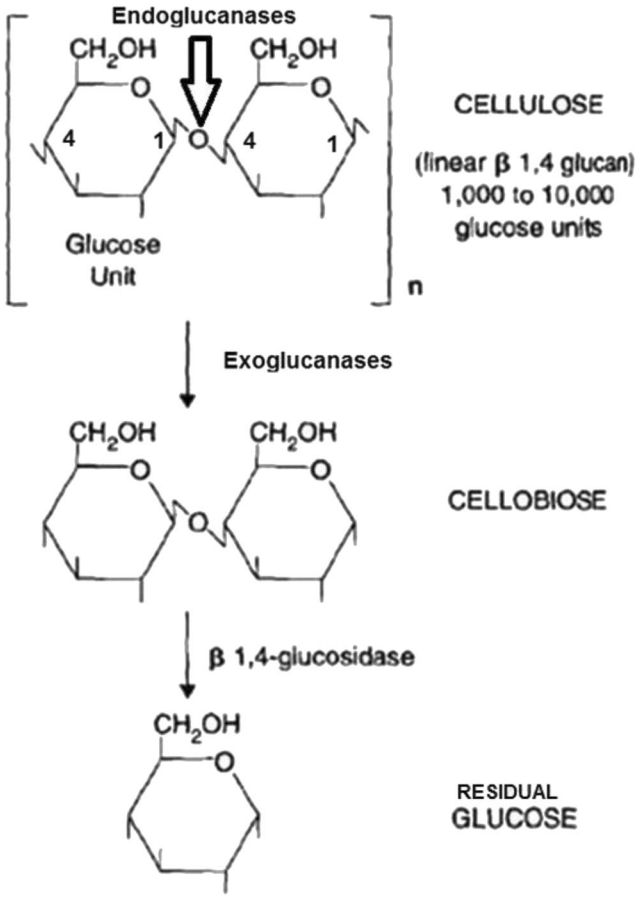

Fig. 16 Cellulose biodegradation pathway to glucose (modified from Béguin and Aubert [22], Tomme et al. [76], Deobald and Crawford [31], and Lynd et al. [53])

$$(C_6H_{10}O_5)_n + nH_2O \xrightarrow{H^+} nC_6H_{12}O_6 \quad (3.1)$$

cellulose — glucose — Cellulase

Reaction equation for the enzymatic hydrolysis of cellulose to glucose (modified from [21])

Endoglucanases (EG) cleave β-glucosidic bonds at random in the middle of cellulose molecules [53] (Fig. 16), exoglucanases (exo-cellobiohydrolases) (CBH), hydrolyze cellulose molecules stepwise either from the reducing or the nonreducing ends [19, 21], liberate cellobiose subunits [21] (Fig. 16) and β-glucosidases, finally hydrolyze cellobiose into glucose [21, 34] (Fig. 16) as detected by the HPLC analysis in our present study. The colour of the raw POME (brownish) was observed to change from brownish (before treatment) to dark (after treatment) after the treatment process. This could possibly be due to

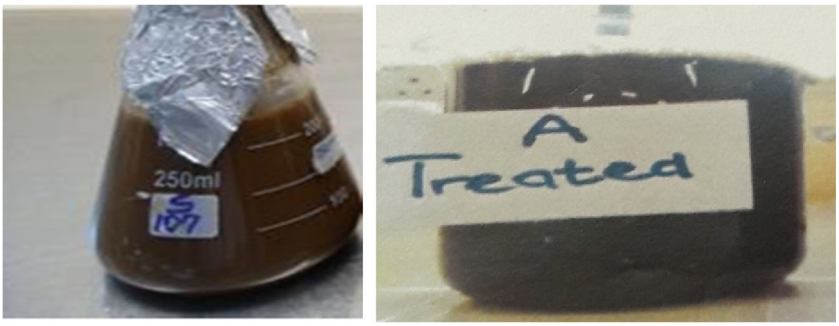

Fig. 17 Physical appearance of POME before (left: brownish) and after (right: dark) treatment process

biodegradation by the microbes. Physical appearance of POME before and after treatment process is presented in (Fig. 17).

Conclusions

Results revealed that the mixed microbial consortium containing bacterial and fungal strains isolated from POME possesses the ability to reduce organic load from POME. This reduction trend of the parameters (BOD_5, COD, and TSS) in the present study is positive to the biotreatment process of wastewater such as POME. The potentiality of the mixed microbial consortium in decreasing the parameters indicates the reduction in the environmental organic load on the receiving water stream.

The breakdown of cellulose-based polysaccharides by mixed microbial consortium into glucose suggests that microorganisms in the present study were able to attack native cellulose in POME exhibited as TSS which represent the cellulosic materials in POME as an indication of biodegradation of cellulose as a clean-up process of the tested POME sample. Therefore, it is suggested that the use of mixed microbial consortium will be an effective and eco-friendly technology for the reduction of organic load from POME. Besides the reduction potential efficiency of the mixed microbial consortium, the present treatment proposal showed more advantages since no additional physical or chemical treatment was required. The study would help in understanding the role of indigenous bacteria and fungi strains from POME in biological treatment technology of wastewaters such as those of oil processing-like POME. These results, however, indicate the prospect of isolating indigenous microorganisms in the POME for effective biotreatment of POME. Hence, the indigenous microbial strains are promising organisms for industrial applications. These microbes have direct applications in industrial process such as bioremediation and biodegradation of oily wastewaters.

In a nutshell, more technically advanced research efforts are required for searching, exploiting new bacterial and fungal strains having plasmid-linked degradation ability, and improving the practical application to propagate the use of bacterial and fungal strains for bioremediation of industrial wastewaters. Most research on bacterial and fungal potential to purify polluted wastewaters has been performed on a laboratory scale; hence, there is a need to extend such research to pilot scale and to apply it to industrial processes. Furthermore, the process should be scaled up to larger volume, in order to confirm the applicability of the system. Therefore, the work on pilot scale and the development of treatment plants are to be encouraged for biotreatment of raw wastewaters.

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References

1. Abass AO, Jameel TA, Muyibi AS, Abdul Karim IM, Alam Z (2012) Investigation of the viability of selected microorganisms on the biodegradation of palm oil mill effluents (POME). Int J Chem Environ Engineer 3(3):182–186
2. AbdulKarim MI, Daud NA, Alam MDZ (2011) Treatment of palm oil mill effluent using microorganisms. In: M.D.Z, Alam, A.T, Jameel and A, Amid, (eds). Current research and development in biotechnology engineering at International Islamic University Malaysia (IIUM) Vol. III. IIUM Press, Kuala Lumpur, p. 269-275. ISBN 9789674181444
3. Agamuthu P, Tan EL, Shaifal AA (1986) Effect of aeration and soil inoculum on the composition of palm oil effluent (POME). Agri Wast 15(2):121–132. https://doi.org/10.1016/0141-4607(86)90043-0
4. Agustin MB, Sengpracha WP, Phutdhawong W (2008) Electrocoagulation of palm oil mill effluent. Int J Environ Res Public Health 5(3):177–180. https://doi.org/10.3390/ijerph5030177
5. Ahmad AL, Ismail S, Bhatia S (2003) Water recycling from palm oil mill effluent (POME) using membrane technology. Desalination 157(1-3):87–95. https://doi.org/10.1016/S0011-9164(03)00387-4
6. Ahmad AL, Sumathi S, Hameed BH (2005) Residual oil and suspended solid removal using natural adsorbents chitosan, bentonite and activated carbon: a comparative study. Chem Eng J 108(1–2):179–185. https://doi.org/10.1016/j.cej.2005.01.016
7. Alade AO, Jameel AT, Muyubi SA, Abdul Karim MI, Alam MDZ (2011) Removal of oil and grease as emerging pollutants of concern (EPC) in wastewater stream. IIUM Engineer J 12(4):161–169

8. Alam MZ, Rashid SS, Karim MIA, Salleh MH (2009) Management of palm oil mill effluent through production of cellulases by filamentous fungi. World J Microb Biotechnol 25:2219–2226
9. Al-Gheethi AAS (2015) Recycling of sewage sludge as production medium for cellulase by a *Bacillus megaterium* strain. Int J Recycl Org Waste Agricult 4(2):105–119. https://doi.org/10.1007/s40093-015-0090-6
10. Al-Gheethi AAS, Efaq AN, Mohamed RMSR, Bala JD, Amir Hashim MK (2016) *Cellulase: Production, Applications and Health Benefits*. In: Stevenson M (ed) Microbial cellulase: production and application in enzymatic treatment of biosolids. Biochemistry Research Trends. Nova Science Publishers Inc, Hauppauge, New York ISBN: 978-1-63485-788-8; ISBN: 978-1-63485-802-1 pp. 45–62
11. Allcock ER, Woods DR (1981) Carboxymethyl cellulase and cellobiase production by *Clostridium acetobutylicum* in an industrial fermentation medium. Appl Environ Microbiol 41(2):539–541
12. APHA (2005) Standard methods for the examination of water and wastewater, in, 21st edn, American Public Health Association (APHA), Washington, DC
13. Bala JD, Lalung J, Ismail N, (2014a) Biodegradation potential and removal of oil and grease by bacterial isolated from palm oil mill effluent (POME). *Proceedings of the International Conference on Beneficial Microbes ICOBM 2014: Microbes for the Benefits of Mankind,* May 27–29, 2014, PARKROYAL Penang Resort, Penang, Malaysia. Editors: Liong M.T, Ahmad, R, Hena, S, Wan-Abdullah, W.N, Lee, C.K, Serri, N.A, Tajaruddin, H.A.B, Ong, J.S, Amy-Lau. S.Y. School of Industrial Technology, Universiti Sains Malaysia 2014. Pages 138–144. ISBN 978-967-394-186-5
14. Bala JD, Lalung J, Ismail N (2014b) Biodegradation of palm oil mill effluent (POME) by bacterial. Int J Sci Res Publ 4(3):502–511
15. Bala JD, Lalung J, Ismail N (2014c) Palm oil mill effluent (POME) treatment "microbial communities in an anaerobic digester": a review. Int J Sci Res Publ 4(6):2250–3153
16. Bala JD, Lalung J, Ismail N (2015a) Studies on the reduction of organic load from palm oil mill effluent (POME) by bacterial strains. Int J Recycl Org Waste Agric 4(1):1–10. https://doi.org/10.1007/s40093-014-0079-6
17. Bala JD, Lalung J, Al-Gheethi AAS, 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. Editors: Tow, TT, Yusup, Y, Fadhlullah, W. School of Industrial Technology, Universiti Sains Malaysia 2015. Pages 79–91. ISBN 978-967-394-211-4
18. Bala JD (2016) Aerobic treatment and biodegradation of palm oil mill effluent by indigenous microorganisms. PhD Thesis, Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia (USM), 11800 Pulau, Pinang, Malaysia
19. Barr BK, Hsieh Y-L, Ganem B, Wilson DB (1996) Identification of two functionally different classes of exocellulases. Biochemist 35(2):586–592. https://doi.org/10.1021/bi9520388
20. Benka-coker MO, Ekundayo JA (1997) Applicability of evaluating the ability of microbes isolated from an oil spill site to degrade oil. Environ Monit Ass 45:259–272
21. Beguin P (1990) Molecular biology of cellulose degradation. Annu Rev Microbiol 44(1):219–248. https://doi.org/10.1146/annurev.mi.44.100190.001251
22. Béguin P, Aubert JP (1994) The biological degradation of cellulose. FEMS Microbiol Rev 13(1):25–58. https://doi.org/10.1111/j.1574-6976.1994.tb00033.x
23. Bek-Nielsen C, Singh G, Toh TS (1999) Bioremediation of palm oil mill effluent. In: In: Proceedings of the Porim International Palm Oil Congress, 16th February 1999. Malaysia, Kuala Lumpur
24. Boekema BKHL, Beselin A, Breuer M, Hauer B, Koster M, Rosenau F, Jaeger KE, Tommassen J (2007) Hexadecane and Tween 80 stimulate lipase production in *Burkholderia glumae* by different mechanisms. Appl Environ Microbiol 73(12):3838–3844. https://doi.org/10.1128/AEM.00097-07
25. Cao SG, Yong H, Ma L, Guo SC (1996) Enzymatic properties by the immobilization method. Appl Biochem Biotechnol 59(1):7–14. https://doi.org/10.1007/BF02787853
26. Cheng J, Zhu X, Ni J, Borthwick (2010) A palm oil mill effluent treatment using a two-stage microbial fuel cells system integrated with immobilized biological aerated filters. Bioresour Technol 101(8):2729–2734. https://doi.org/10.1016/j.biortech.2009.12.017
27. Chhatre S, Purohit HJ, Shanker R, Khanna P (1996) Bacterial consortia for crude oil spill remediation. Water Sci Technol 34:187–193
28. Chigusa S, Hasegawa T, Yamamota N, Watanabe Y (1996) Treatment of waste water from oil manufacture plant by yeasts. Water Sci Technol 34:51–58
29. Chin KK, Lee SW, Mohammad HH (1996) A study of palm oil mill effluent treatment using a pond system. Water Sci Technol 34:119–123
30. De Felice B, Pontecorvo G, Carfagna M (1997) Degradation of waste waters from olive oil mills by *Yarrowia lipolytica* ATCC 20255 and *Pseudomonas putida*. Acta Biotechnol 17(3):231–239. https://doi.org/10.1002/abio.370170306
31. Deobald LA, Crawford DL (1997) Lignocellulose biodegradation. In: Hurst CJ, Knudsen GR, Stetzenbach LD, Walter MV (eds) Manual of Environmental Microbiology ASM Press. USA pp, Washington DC, pp 730–737
32. Dhouib A, Ellouz M, Aloui F, Sayadi S (2006) Effect of bioaugmentation of activated sludge with white rot fungi on olive mill wastewater detoxification. Lett Appl Microbiol 42(4):405–411. https://doi.org/10.1111/j.1472-765X.2006.01858.x
33. Din MF, Ujang Z, Muhd-Yunus S, Van-Loosdrecht MCM (2006) Storage of polyhydroxyalkanoates (PHA) in fed-batch mixed culture using palm oil mill effluent (POME). In: 4th Seminar on Water Management (jsps-vcc), Johor, pp 119–127
34. Dipasquale L, Romano I, Picariello G, Calandrelli V, Lama L (2014) Characterization of a native cellulase activity from an anaerobic thermophilic hydrogen-producing bacterium *Thermosipho* sp. strain 3. Ann Microbiol 64(4):1493–1503. https://doi.org/10.1007/s13213-013-0792-9
35. El-Masry MH, El- Bestaway E, El-Adi NI (2004) Bioremediation of vegetable oil and grease from polluted wastewater using a sand biofilm system. World J Microbiol Biotechnol 20(6):551–557. https://doi.org/10.1023/B:WIBI.0000043162.17813.17
36. Environmental Management Guideline (1997) Palm oil industry. German Technical Cooperation. Thailand, Bangkok, p 5
37. Environmental Quality (Prescribed Premises) (crude palm oil) Regulation 1977 (2014)
38. Francis AR, Masilamai D (2012) Removal of zinc (II) by non-living biomass of *Agaricus Bisporus*. Res J Recent Sci 1(9):13–17
39. Glazer AN, Nikaido H (1995) Microbial biotechnology: fundamentals of applied microbiology. USA: University of California, Berkley WH Fremanand Company. ISBN0-71672-608-4
40. Goyal AK, Eveleigh DE (1996) Cloning, sequencing and analysis of the ggh-A gene encoding a 1,4-beta-D-glucan glucohydrolase from *Microbispora bispora*. Gene 172(1):93–99. https://doi.org/10.1016/0378-1119(96)00076-5
41. Hamme JD, Odumeru JA, Ward OP (2000) Community dynamics of a mixed-bacterial culture growing on petroleum hydrocarbons in batch culture. Can J Microbiol 46(5):441–450. https://doi.org/10.1139/w00-013

42. Hazaimeh M, Mutalib SA, Abdullah PS, Kee WK, Surif S (2014) Enhanced crude oil hydrocarbon degradation by self-immobilized bacterial consortium culture on sawdust and oil palm empty fruit bunch. Ann Microbiol. 64(4):1769–1777. https://doi.org/10.1007/s13213-014-0821-3
43. Hernández D, Riaño B, Coca M, García-González MC (2013) Treatment of agro-industrial wastewater using microalgae–bacteria consortium combined with anaerobic digestion of the produced biomass. Bioresour Technol 135(2013):598–603. https://doi.org/10.1016/j.biortech.2012.09.029
44. Hii KL, Yeap SP, Mashitah MD (2012) Cellulase production from palm oil mill effluent in Malaysia: economical and technical perspectives. Eng Life Sci 12(1):7–28. https://doi.org/10.1002/elsc.201000228
45. Ho CC, Tan YK, Wang CW (1984) The distribution of chemical constituents between the soluble and the particulate fractions of palm oil mill effluent and its significance on its utilisation/treatment. Agric Wastes 11(1):61–71. https://doi.org/10.1016/0141-4607(84)90055-6
46. Ibrahim AH, Dahlan I, Adlan MN, Dasti AF (2012) Comparative study on characterization of malaysian palm oil mill effluent. Res J Chem Sci 2(12):1–5
47. Iwara AI, Ewa EE, Ogundele FO, Adeyemi JA (2011) Ameliorating effects of palm oil mill effluent on the physical and chemical properties of soil in Ugep, Cross River state, South-Southern Nigeria. Int J App Sci Technol 1(5):106–112
48. Jameel AT, Muyibi SA, Olanrewaju AA (2011) Comparative study of bioreactors used for palm oil mill effluent treatment based on chemical oxygen removal efficiencies In: M.D.Z, Alam, A.T, Jameel and A, Amid, (eds). Current research and development in biotechnology engineering at International Islamic University Malaysia (IIUM) Vol. III. IIUM Press, Kuala Lumpur, p.277-284. ISBN 9789674181444
49. Jameel AT, Olanrewaju AA (2011) Aerobic biodegradation of oil and grease in palm oil mill effluent using consortium of microorganisms In: M.D.Z, Alam, A.T, Jameel and A, Amid, (eds). *Current research and development in biotechnology engineering at International Islamic University Malaysia (IIUM)* Vol. III. IIUM Press, Kuala Lumpur, pp. 43-51. ISBN 9789674181444
50. Kissi M, Mountadar M, Assobhei O, Gargiulo E, Palmieri G, Giardina P, Sannia G (2001) Roles of two white-rot basidiomycete fungi in decolorization and detoxification of olive mill wastewater. Appl Microbiol Biotechnol 57(1-2):221–226
51. Lam MK, Lee KT (2011) Renewable and sustainable bioenergies production from palm oil mill effluent (POME): win-win strategies toward better environmental protection. Biotechnol Adv 29(1):124–141. https://doi.org/10.1016/j.biotechadv.2010.10.001
52. Li H, Ni J (2011) Treatment of wastewater from *Dioscorea zingiberensis* tubers used for producing steroid hormones in a microbial fuel cell. Bioresour Technol 102(3):2731–2735. https://doi.org/10.1016/j.biortech.2010.11.030
53. Lynd LR, Weimer PJ, Vanzyl WH, Pretorius IS (2002) Microbial cellulose utilization: fundamentals and biotechnology. Microbiol Mol Biol Rev 66(3):506–577. https://doi.org/10.1128/MMBR.66.3.506-577.2002
54. Maygaonkar PA, Wagh PM, Permeswaran U (2012) Biodegradation of distillery effluent by fungi. Biosci Dis 3(2):251–258
55. McHugh S, O'Reilly C, Mahony T, Colleran E, O'Flaherty V (2003) Anaerobic granular sludge bioreactor technology. Rev Environ Sci Biotechnol 2(2-4):225–245. https://doi.org/10.1023/B:RESB.0000040465.45300.97
56. Mohammed RR, Ketabachi MR, McKay G (2014) Combined magnetic field and adsorption process for treatment of biologically treated palm oil mill effluent (POME). Chem Eng J 243:31–42. https://doi.org/10.1016/j.cej.2013.12.084
57. Najafpour GD, Zinatizadeh AAL, Mohamed AR, Isa-Hasnain M, Nasrollahzadeh H (2006) High-rate anaerobic digestion of palm oil mill effluent in an upflow anaerobic sludge-fixed film bioreactor. Process Biochem 41(2):370–379. https://doi.org/10.1016/j.procbio.2005.06.031
58. Ohimain EI, Olukole CD, Izah SC, Eke RA, Okonkwo AC (2012) Microbiology of palm oil mill effluents. J Microbiol Biotech Res 2(6):852–857
59. Ohimain EI, Izah SC, Jenakumo N (2013) Physicochemical and microbial screening of palm oil mill effluents for amylase production. Greener J Biol Sci 3(8):307–318
60. Oswal N, Sarma PM, Zinjarde SS, Pant A (2002) Palm oil mill effluent treatment by a tropical marine yeast. Bioresour Technol 85(1):35–37. https://doi.org/10.1016/S0960-8524(02)00063-9
61. Piro P, Carbone M, Tomei G (2011) Assessing settleability of dry and wet weather flows in an urban area serviced by combined sewer. Water Air Soil Pollut 214(1–4):107–117. https://doi.org/10.1007/s11270-010-0408-y
62. Poh PE, Chong MF (2009) Development of anaerobic digestion methods for palm oil mill effluent (POME) treatment. Bioresour Technol 100(1):1–9. https://doi.org/10.1016/j.biortech.2008.06.022
63. Qingwei L, Mancl KM, Tuovinen OH (1998) Effect of inoculation on the biodegradation of butterfat-detergent mixtures in fixed-film sand columns. Bioresour Technol 64:27–32
64. Rahman KSM, Rahman JT, Lakshmanaperumalsamy P, Banat LM (2002) Towards efficient crude oil degradation by a mixed bacterial consortium. Bioresour Technol 85(3):257–261. https://doi.org/10.1016/S0960-8524(02)00119-0
65. Rashid SS, Alam MZ, Ismail M, Karim M, Salleh MH (2009) Management of palm oil mill effluent through production of cellulases by filamentous fungi. World J Microbiol Biotechn 25(12):2219–2226. https://doi.org/10.1007/s11274-009-0129-9
66. Ray, R.C., 2011. Solid-state fermentation for production of microbial cellulase: an overview. In: Golan AE (ed) Cellulase: types and action, mechanism, and uses. Nova Science Publishers Inc, New York
67. Sathishkumar M, Binupriya AR, Baik SH, Yun SE (2008) Biodegradation of crude oil by individual bacterial strains and a mixed bacterial consortium isolated from hydrocarbon contaminated areas. Clean 36(1):92–96
68. Sethupathi S (2004) Removal of residue oil from palm oil mill effluent (POME) using chitosan .Master Thesis, School of Chemical Engineering, USM, Malaysia
69. Shi J (2007) Microbial pretreatment of cotton stalks by *Phanerochaete chrysosporium* for bioethanol production. Biological and Agricultural Engineering, Raliegh, North Carolina
70. Sinnappa S (1978) Treatment studies of palm oil mill wastes water pollution. University Press Bangkok, Thailand, pp 21–25
71. Singh R, Ibrahim MH, Esa N, Iliyana M (2010) Composting of waste from palm oil mill: a sustainable waste management practice. Rev Environ Sci Biotechnol 9(4):331–344. https://doi.org/10.1007/s11157-010-9199-2
72. Soleimaninanadegani M, Manshad S (2014) Enhancement of biodegradation of palm oil mill effluents by local isolated microorganisms. Int Scholarly Res Notices 2014:1–8. https://doi.org/10.1155/2014/727049
73. Sugiura K, Ishihara M, Shimauchi T, Harayama S (1997) Physicochemical properties and biodegradability of crude oil. Environ Sci Technol 31(1):45–51. https://doi.org/10.1021/es950961r
74. Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 83(1):1–11. https://doi.org/10.1016/S0960-8524(01)00212-7
75. Takemoto H, Hase S, Ikenaka T (1985) Microquantitative analysis of neutral and amino sugars as fluorescent pyridylamino derivatives

by high-performance liquid chromatography. Anal Biochem 145(2):245–250. https://doi.org/10.1016/0003-2697(85)90357-4
76. Tomme P, Warren RA, Gilkes NR (1995) Cellulose hydrolysis by bacteria and fungi. Adv Microb Physiol 37:1–81. https://doi.org/10.1016/S0065-2911(08)60143-5
77. Vijayaraghavan K, Ahmad D, Abdul-Aziz ME (2007) Aerobic treatment of palm oil mill effluent. J Environ Manag 82(1):24–31. https://doi.org/10.1016/j.jenvman.2005.11.016
78. Wanna C, Pompan W (2007) Effect of temperature on the anaerobic digestion of palm oil mill effluent. Elec. J Biotechnol 10(3):376–385
79. Wilson DB (2011) Microbial diversity of cellulose hydrolysis. Curr Opinion Microbiol 14:1–5
80. Wong KM, Nor AA, Suraini A, Vikineswary S, Mohd AH (2008) Enzymatic hydrolysis of palm oil mill effluent solid using mixed cellulases from locally isolated fungi. Res J Microbiol 3(6):474–481
81. Wu TY, Mohammad AW, Md-Jahim J, Anuar N (2007) Palm oil mill effluent (POME) treatment and bioresources recovery using ultrafiltration membrane: effect of pressure on membrane fouling. Biochem Eng J 35(3):309–317. https://doi.org/10.1016/j.bej.2007.01.029
82. Wu TY, Mohammad AW, Jahim JM, Anuar N (2010) Pollution control technologies for the treatment of palm oil mill effluent (POME) through end-of-pipe processes. J Environ Manag 91(7):1467–1490. https://doi.org/10.1016/j.jenvman.2010.02.008
83. Yacob S, Hassan MA, Shirai Y, Wakisaka M, Subash S (2005) Baseline study of methane emission from open digesting tanks of palm oil mill effluent treatment. Chemosphere 59(11):1575–1581. https://doi.org/10.1016/j.chemosphere.2004.11.040