CHANGES IN PHYSICOCHEMICAL AND MICROBIAL COMMUNITY DURING CO-COMPOSTING OF OIL PALM FROND WITH PALM OIL MILL EFFLUENT ANAEROBIC SLUDGE

Mohd Najib Ahmad,[a,c] Mohd Noriznan Mokhtar,[a,*] Azhari Samsu Baharuddin,[a] Lim Siong Hock,[a] Siti Ramlah Ahmad Ali,[c] Suraini Abd-Aziz,[b] Nor Aini Abdul Rahman,[b] and Mohd Ali Hassan [a,b]

The aims of this study were to investigate the physicochemical changes and microbial population during co-composting of 1 ton oil palm frond (OPF) with 1,000 L palm oil mill effluent (POME) anaerobic sludge. In the first 30 days of composting, the temperature of the composting piles was observed in the thermophilic phase, within a range of 50 - 56°C. Meanwhile, the oxygen level, moisture content, and pH profiles of the compost were maintained at 2.0 to 12%, 60 to 70%, and 7.9 to 8.5, respectively, throughout the composting process. The total bacteria count was estimated to be about 55 x 10^{10} CFU/mL in the mesophilic phase, and then it increased up to 66 x 10^{10} CFU/mL in the thermophilic phase, and finally decreased to 9.0x10^{10} CFU/mL in the curing phase. The initial C/N ratio, 64, decreased to 18 after 60 days of composting process, indicating the maturity of compost product from OPF-POME anaerobic sludge. The diversity of the bacterial community was investigated using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis. The results suggested that the co-composting process of OPF with POME anaerobic sludge was dominated by *Pseudomonas sp*.

Keywords: Oil palm frond; Palm oil mill effluent (POME) anaerobic sludge; Composting; DGGE

Contact information: a: Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; b: Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; c: Microbial Technology Group, Plant Protection Unit, Biology Division, Malaysian Palm Oil Board (MPOB), 6, Persiaran Institusi, 43000 Kajang, Selangor, Malaysia.
** Corresponding author: noriznan@eng.upm.edu.my*

INTRODUCTION

Malaysia is one of the largest producers of palm oil, of which around 75.5 million metric tons (tons) of fresh fruit bunch (FFB) was processed in 2005 (MPOB 2005). Therefore, the palm oil industry is the major agriculture industry in Malaysia, estimated at around 17.7 million tons of palm oil on 4,500,000 hectares of land in 2008 (Malaysian Palm Oil Industry Performance 2008). Due to a great demand on palm oil in the food and oleo chemical industries, its production is expected to increase rapidly. The oil palm biomass, namely empty fruit bunches (EFB), oil palm fronds (OPF), and oil palm stems (OPS), are by-products that are produced at about 40 million tons per year (Astimar and Wahid 2006), and they have been of concern recently due to the significant impact on the

environmental issues. Astimar and Wahid (2006) predicted that about 7.14 million tons/year of OPF and about 2.86 million tons/year of EFB will be generated in 2020.

OPF is generated abundantly in fields, especially during the replanting process and pruning, and it's amount was estimated to be about 14.47 kg/ha and 10.40 kg/ha, respectively (Astimar and Wahid 2006). If the OPF is left without any treatment, it can create other environmental problems and global phenomenon due to high accumulated organic content on the ground (Kabbashi et al. 2006). In the normal practice, the conventional method of OPF disposal for replanting the oil palm through zero burning technique at the plantation can cause the problems of air pollution.

According to Baharuddin et al. (2009a), composting has been considered as one of the alternative methods to convert organic wastes into products that benefit plant growth and soil amendment. The major goal of composting is to provide a stable compost product that contains sufficient nutrients to be consumed by the plant and also can increase soil fertility. Two of the main components of agro-based biomass, i.e. cellulose and lignin, have been described as main sources of energy and humus formation, respectively, and their characteristics also contribute to air permeability, bulking, and water retention throughout the composting process (Hubbe et al. 2010). Although considerable research on composting has been conducted using EFB, mesocarp fibre and various organic wastes (Baharuddin et al. 2009a, Hock et al. 2009, Heerden et al. 2002, Khalil et al. 2001), there is less information regarding OPF composting at the field scale of operation. According to Baharuddin et al. (2010), POME anaerobic sludge was reported to be a main source for nitrogen and microbial seeding during co-composting process with empty fruit bunches (EFB).

Due to the increasing generation of OPF during harvesting and replanting it is crucial to find an alternative method for its disposal. In this article, composting methods have been considered for the utilization of abundant of OPF in the plantation, and the treatment was accomplished by the addition of POME anaerobic sludge. Therefore, the aims of this study are to investigate the physicochemical characteristics and microbial succession of prominent microbes during co-composting process of OPF with POME anaerobic sludge. Denaturing gradient gel electrophoresis (DGGE) fingerprint methods were used to detect the shift of microbes in the present study.

METHODS

Pilot scale composting site and raw materials

The co-composting of OPF-POME anaerobic sludge was performed at the composting site in the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia. Brick blocks with dimension at 2.1 m length, 1.5 m width, and 1.5 m height were used for the co-composting treatment under the shade and cement base.

Fresh OPF was collected from oil palm plantation at Malaysian Palm Oil Board (MPOB)-Universiti Kebangsaan Malaysia (UKM). It was chipped into slices with average length of 2 to 3 cm using conventional chipper machine at Biomass Pilot Plant, Agro Product Unit, MPOB-UKM. Chipped fronds were placed and segregated on the cement floor for drying. Then, the dried chipped frond was further ground by

conventional hammer-mill machine at the same place to obtain smaller size at length of 0.2 to 0.5 cm. Meanwhile, POME anaerobic sludge was obtained from a 500 m^3 closed anaerobic digesting tank system located at Felda Serting Hilir Palm Oil Mill, Negeri Sembilan. The thicken POME anaerobic sludge from the settling tank was used in the present study.

Co-Composting Process

One ton of the chipped-grounded OPF was loaded manually into the composting block. The POME anaerobic sludge, which consists of beneficial microorganism for co-composting, was sprayed to the composting pile every three days intervals using a centrifugal pump. The ratio of POME anaerobic sludge added onto OPF throughout the composting treatment was one to one. After POME anaerobic sludge was added, and the composting material was turned manually to provide sufficient aeration and to ensure good mixing of the composting materials. The addition of POME anaerobic sludge was stopped one week before maturity stage of the composting materials and followed by frequent turning. The maturity stage of the composting materials was determined every three days by C/N ratio analysis using CNHS 2000 analyzer (Leeco, USA).

Sampling and Analysis Method

Oxygen and temperature were analyzed using a Digital Temperature-Oxygen probe meter, Demista Instrument, (CM2006, USA). Moisture and pH were analyzed using a moisture analyzer, (MX-50, USA) and pH meter, (DELTA 320, Mettler Toledo, USA), respectively. These analyses were performed throughout the composting process. CNHS 2000 analyzer (Leeco, USA) and Inductively Coupled Plasma (ICP)-OES (Perkin Elmer, USA) were used to analyze carbon, nitrogen, nutrients and heavy metal elements in the composting material. Meanwhile, the total colony forming unit (CFU) of microbes was determined by serial dilution methods on nutrient agar plates. The plates were incubated at 30°C for 48 hours, and the development of bacteria colonies were counted and expressed in CFU/mL.

The analysis of oil-grease on POME anaerobic sludge was carried out according to the American Public Health Association (APHA 1998) method, as reported by Baharuddin et al. (2009a). BOD and COD were determined by using standard methods APHA (2005). Cellulose, hemicelluloses, and lignin content in OPF were determined using Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL), and Neutral Detergent Fiber (NDF) analysis (Gorring and Van Soest 1970). Proximate analysis was performed to analyze ash and crude protein in OPF according to APHA (2005) standard method using Fibertec I & M Systems (USA).

DNA Extraction

DNA microbes from composting material were extracted using a cell disruption method (Yeates et al. 1998). 2 g-wet basis composting material was added into 10 mL extraction buffer (100 mM Tris-HCl pH 8.0, 100 mM sodium EDTA pH 8.0, and 1.5 M NaCl). About 0.5 g of 2 mm glass bead (APS Finechem, Australia) was employed to disrupt the cell wall of microbes by applying vigorous vortex mixing for 2 minutes.

Polymerase Chain Reaction (PCR)

Prior to polymerase chain reaction (PCR), the DNA samples were diluted with sterilize ultra-pure water to minimize the inhibition effects of co-extracted contaminants. The 16S DNA was amplified by using a primer set of forward primer (341f) with a 40 bp GC clamp (First Base Laboratory, Malaysia), 5'-*CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG* -3‘ and reverse primer (518r), 5'-*ATT ACC GCG GCT GCT GG*-3'. PCR amplifications were carried out in 25 µL of PCR mixture, and diluted to 25 mL with sterilized ultra pure water. The PCR mixture was prepared as follows: 25 µL PCR mixture consists of 10 pmol of each primer, 200 µM of each deoxyribonucleoside triphosphate (Vivantis, Finland), 2.5 µL of 10X PCR buffer (Vivantis, Finland) containing 100 mM Tris–HCl, 15X mM MgCl , 500 mM KCl; pH 8.3, 1.25 U of Taq DNA polymerase (Vivantis, Finland) and 5 µL of DNA template, and diluted to 25 mL with sterilized water. The PCR cycling for 16S rDNA using 341f and 518r primers was performed using PCR Thermal Cycler (MasterEP Gradient, Eppendorf, Germany). The temperature used was as follows; 94^{o}C for 3 min followed by 30 cycles of 52^{o}C for 1 min, 72^{o}C for 1 min, 94^{o}C for 1 min and then continued at 52^{o}C for 1 min with final extension steps at 72^{o}C for 10 min.

Denaturant Gradient Gel Electrophoresis

Denaturant Gradient Gel Electrophoresis (DGGE) (DCode^{TM}, Biorad, USA) was conducted according to Muyzer et al. (1993). A 16S rDNA PCR product was separated in 1.0 mM of 6 % (w/v) polyacrilamide (37.5:1; acrilamide : bisacrilamide) (Bio-Rad, USA) with a denaturing gradient of 30 to 70 %, where 100 % denaturant correspondence to 7 M urea and 40 % (v/v) deionized formamide. An amount of 15 µL PCR products was pipetted into the individual lane, and DGGE was performed at 60ºC and 200V with 1X TAE buffer (Bio-Rad, USA) for 5 hours. Gel was stained with SYBR^{R} Green nucleic Acid Gel Stain (Invitrogen, USA) for 30 minutes and then rinsed with water and photographed on a UV transillumination table (Labnet, USA).

Band Excised and DNA Recovery

DNA bands from DGGE gels were excised with a sterile blade and placed in 1.5 mL eppendorf tube containing 50 µL ddH_2O. Tubes were incubated overnight at -20ºC to elute the DNA. Then, DNA was frozen and thawed for three times. Five µL of the supernatant was used as a template to re-amplify the DNA, using primers 314f (with no GC-clamp) and 518r. The PCR conditions were the same as described for DGGE amplifications. PCR products were purified using QIAprep spin columns (QIAGEN, Inc., Valencia, CA), and sequenced in both directions with the same primers as used in PCRs.

Sequencing and Band Characterization

The PCR products were sent for sequencing. Sequence similarity searches were conducted using a BLAST (Basic Local Alignment Search Tool) network service of the GenBank database through website (http://www.ncbi.nlm.nih.gov/) to identify the nearest relatives of the partially sequenced 16S rDNA genes and the excised dominant bands.

RESULTS AND DISCUSSION

Characteristics of Raw Materials and Final Compost

Physical changes such as color and texture of the compost were observed during composting treatment (Fig. 1). The final or matured compost was grayish in color, having a texture and earthy small close to that of natural. The analysis results shown in Table 1 revealed that raw OPF contained high cellulose (37.9 %), hemicellulose (46.0 %), and lignin (18.7 %), but low trace elements such as potassium and sulfur. As mentioned by Rosli et al. (2007), OPF are rich in holocellulose and also high in α-cellulose. At the same time, the lignin content of OPF is lower than commonly found in other hardwood such as aspen. OPF contains various sizes of vascular bundles, which is different from EFB and mesocarp fiber. In this study, hemicellulose content in OPF was higher than EFB and mesocarp fiber because they are from different groups relatively to hard and softwood characteristic, which are differ significantly (Lachke 2002). OPF hemicellulose contained highly acetylated heteroxylans, classified as 4-O-methylglucuronoxy, compared to EFB and mesocarp fiber. The lignin content of OPF was higher compared to EFB due to the characteristics of OPF which contained primary and secondary wall layers within the vascular bundles. Its characteristics such as rigidity and its role in the structural integrity of wood (Bobleter 1998) led to this result.

The results suggested that OPF could be used as a carbon source, whereas POME anaerobic sludge with opposite characteristics would complement the OPF during the co-composting process. POME anaerobic sludge used in the present study had high nitrogen and potassium content, and the results were almost the same as reported by other researchers (Baharuddin et al. 2009a; Hock et al. 2009). The high BOD level in the POME anaerobic sludge also indicated that the high organic matter that can be consumed by the microbes in the beginning of composting process with OPF. The high moisture content in the POME anaerobic sludge can also be used for water supply in the composting (Table 1). Interestingly, heavy metal elements such as cadmium, chromium, and lead were not detected in OPF as compared to EFB and mesocarp fibre.

(a) (b)

Fig. 1. Physical observation of compost at 10 (a) and 60 (b) day of composting

Table 1. Properties of EFB, Mesocarp Fiber, OPF, and POME Anaerobic Sludge

Parameters	EFB[a]	Mesocarp fiber[b]	OPF[c]	POME anaerobic sludge[c]
Moisture	24.0	33.7	42.7	95.4
pH	6.7	5.9	6.9	7.4
C (%)	53.0	42.7	42.1	32.5
N (%)	0.9	0.8	0.6	3.9
C/N	58.9	56.9	67.8	8.3
Oil and grease (mg/L)	-	-	-	183
Total solid (mg/kg)	-	-	-	55,884
COD (mg/L)	-	-	-	40.563
BOD (mg/L)	-	-	-	15,100
Cellulose (%)	52.5	21.3	37.9	-
Hemicellulose (%)	28.8	31.9	46.0	-
Lignin (%)	17.1	26.9	18.7	-
Ash (%)	-	-	3.4	-
Crude protein	-	-	1.2	-
Phosphorus (%)	0.6	0.1	0.1	1.2
Potassium(%)	2.4	0.5	1.1	2.0
Calcium (%)	1.0	0.2	0.6	1.6
Sulphur (%)	1.1	0.1	0.3	4.6
Magnesium (%)	0.6	0.1	0.1	0.9
Ferum (%)	1.0	0.2	0.6	1.9
Zinc (ppm)	17	10	25	158
Manganese (ppm)	230	nd	65	550
Copper (ppm)	14	27	13	243
Boron (ppm)	-	nd	9	180
Mobibdenum (ppm)	-	1	nd	nd
Cadmium (ppm)	1	nd	nd	nd
Chromium (ppm)	9	11	nd	23
Plumbum (ppm)	1	1	nd	nd
Nickel (ppm)	nd	4	nd	nd

[a] Baharuddin et al. (2009a), [b] Hock et al., (2009), [c] This work

According to Hock et al. (2009), the addition of POME anaerobic sludge into the oil palm mesocarp fibre (OPMF) compost would enrich and accelerate the composting process due to addition of nitrogen source and microbial seeding. It was reported that the N-P-K ratio of EFB-POME anaerobic sludge based compost was 2.2-1.3-2.8, whereas mesocarp compost was detected at 1.9-0.3-1.2. However, the N-P-K ratio of OPF compost with POME anaerobic sludge were found to be lower compared to EFB and mesocarp compost (1.8-0.1-0.9).

The lower N-P-K ratio might be attributed by the characteristics of feedstock materials and the process conditions. During replanting, the OPF was cut and then pre-treated using a hammer mill and a chipper machine. Meanwhile the EFB used in the composting as reported by Baharuddin et al. (2010) was obtained after the sterilization process of fresh fruit bunch (FFB) in the mill. The bunch of EFB was then physically treated and turned into press-shredded form.

Table 2. Properties of Compost Product from EFB-POME Sludge, Mesocarp Fiber-POME Sludge, and OPF-POME Sludge

Parameters	Compost from EFB-POME sludge[a]	Compost from Mesocarp fiber-POME sludge[b]	Compost from OPF-POME sludge[c]
Moisture	61.0	49.3	60.6
pH	8.1	7.5	8.2
C (%)	28.0	24.8	32.5
N (%)	2.2	1.9	1.8
C/N	12.7	12.6	18.0
Phosphorus (%)	1.3	0.3	0.1
Potassium (%)	2.8	1.2	0.9
Calcium (%)	0.7	0.9	0.6
Sulphur (%)	1.2	20.6	0.4
Magnesium (%)	1.0	0.3	0.2
Ferum (%)	1.2	1.0	0.2
Zinc (ppm)	91	190	38
Manganese (ppm)	250	151	72
Copper (ppm)	70	57	24
Boron (ppm)	-	7	9
Molibdenum (ppm)	-	nd	nd
Cadmium (ppm)	4	nd	nd
Chromium (ppm)	9	19	nd
Plumbum (ppm)	4	32	1
Nickel (ppm)	nd	3	nd
Day of composting	40	50	60

[a]Baharuddin et al. (2009a), [b]Hock et al. (2009), [c]This work

As for mesocap fiber, it was subsequently subjected to physical and thermal treatment, and the structure was more ruptured compared to OPF (Hock et al. 2009). The higher C/N ratio of raw OPF was the evident. It also observed that the structure of OPF was difficult to cut compared to EFB and mesocarp fibre. Besides that, the composition of carbohydrate in the OPF (total amount of cellulose and hemicelluloses) was higher than EFB and mesocarp fibre. Therefore, the condition of raw OPF may reflect the capability of microbes to utilize the substrate effectively during composting process. It can be seen that the OPF composting took about almost 60 days to achieve maturity with final C/N ratio of 18 (Table 2).

The particle size of OPF was concerned during substrate preparation for the composting process. In this study, OPF was cut using a chipper machine. The size of chipped OPF was 2 to 3 cm in length. Then, it was further ground using a hammer mill to produce finer structure of OPF and close to saw-dust appearance. According to Robert et al. (2000), there are three major concerns for physical characteristics; porosity, texture, and structure. In OPF composting, porosity of OPF compost pile likely affected the resistance to airflow and interfered with the continuity of air spaces. Fewer and larger particles were segregated in the pile, which could reduce surface area for microbial decomposition. Robert et al. (2000) suggested that the finer the texture, the greater the surface area exposed to microbial decomposition. Besides, the structure of OPF has low ability to resist compaction and settling. This condition contributed to a longer period of the OPF composting process.

PHYSICOCHEMICAL AND BACTERIAL CHANGES IN COMPOSTING

Temperature

Temperature is the main parameter to indicate the efficiency of a composting process (Li et al. 2008). According to Bazrafshan et al. (2006), temperature higher than 55°C could maximize sanitation, between 45°C and 55°C could maximize the biodegradation rates, and between 35°C and 40°C could maximize microbial diversity in the composting process.

In this study, at the initial stage of composting, the temperature observed was detected in the range of 34°C to 38°C. After 4 days of composting, the temperature increased, ranging from around 50°C to 56°C, and then it remained at a relatively steady value for 30 days (Fig. 2). In comparison, in composting of EFB (Baharuddin et al. 2009a), it was reported that the thermophilic phase temperature was maintained around 50 to 62°C from day 4 to day 40. In composting of mesocarp fiber, Hock et al. 2009 had reported that the thermophilic phase temperature obtained was around 50 to 68°C from day 1 to 40, indicating a long thermophilic phase. This condition suggested that the microbial activity was occurring in the thermophilic phase. These indigenous microbes were capable of oxidizing the degradable carbohydrate in OPF during an initial thermophilic phase, whereas more stable material such as lignin would be oxidized during a prolonged thermophilic stage. Most studies reported that the optimum temperature range for effective decomposing was occurring with the range of 50 to 70°C (Wong et al. 2001).

The maximum temperature of composting materials was not attained higher than 56°C during the thermophilic phase (Fig. 2). This might be due to the pre-treatment methods of OPF and the compaction of windrow piles that led to the low level of oxygen content (<5%). The fine texture of the OPF contributed to the slow air transportation within the compost pile and subsequently affected microbial activity in the composting process.

Fernández et al. (2008) had reported that high compaction in composting material and high environmental temperatures would result the formation of a larger proportion of solid. The formation could reduce the mass transfer for oxygen and evaporated water within composting material and thus limit heat generation by microbial activity. Therefore, composting temperature in this case is difficult to obtain higher than 60°C. However, microbial decomposition was still active in the compost pile because the temperature was maintained at around 53±3°C for 30 days.

According to Khalil et al. (2001), well aerated compost often attains temperatures of 50 to 65°C, and the temperature even can reach 80°C due to microbial activity in the decomposing process. On the 35th day of composting the temperature of the compost pile dropped to 47°C, indicating that the end of thermophilic phase. The process gradually turned to a curing phase until 60 days of composting, and the average temperature observed inside the pile was below than 39°C.

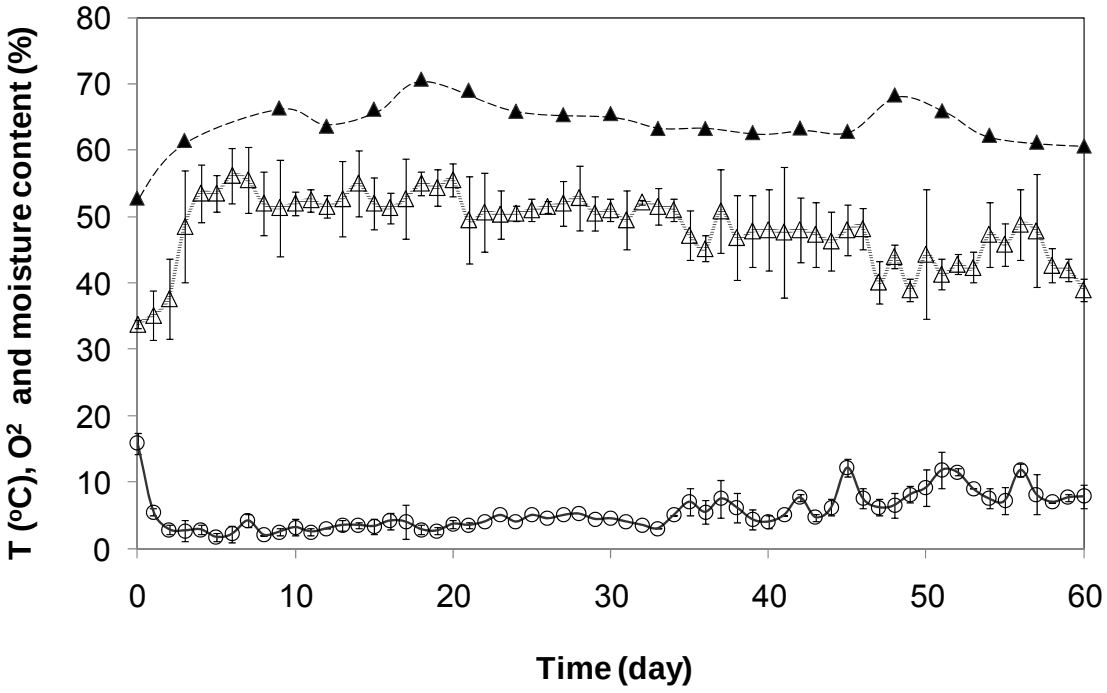

Fig. 2. Profiles of compost temperature, oxygen level and moisture content during co-composting of OPF and POME anaerobic sludge. Remarks: Δ = Temperature, O = Oxygen content and ▲ = Moisture content.

Moisture Content and Oxygen Level

The moisture content also is known to be a critical factor to determine the success of composting. POME anaerobic sludge was added during composting to maintain the optimum moisture content, resulting in a good environmental condition for bioactivity of the microorganisms. In this study, due to the condition of pre-treated OPF and compaction, the sludge addition led to water trapping and subsequently increased the moisture inside the pile, resulting in lower bacterial activity (if too high moisture content), and therefore lower thermophilic temperature was obtained. Even though POME was added to maintain the optimum condition between 50 and 70%, the OPE materials resisted moisture absorption, causing leachate to run off. Thus, this condition may reflect the low N-P-K ratio in the matured compost. The interval addition of POME anaerobic sludge and turning process are important to maintain the optimum metabolic heat generated by microbial activity during degradation process. In this study, the initial moisture content was detected at around 60 to 70 % throughout the composting. In comparison, the moisture content for EFB and mesocarp fiber composting was recorded at 65 to 75% (Baharuddin et al. 2009a) and 50 to 60% (Hock et al. 2009), respectively.

As shown in Fig. 2, the oxygen level in the piles was around 16% in the initial stage and dropped to around 2 to 7% due to compaction of materials during the thermophilic phase. The result also indicated that the microbes in the thermophilic phase were still active to oxidize the available organic matter in the composting materials. According to Baharuddin et al. (2009a), in composting of EFB, high remaining oxygen levels at least

10% will promote optimum the decomposition process during the thermophilic phase. In this study, because of low level of oxygen (below than 10%), the decomposition rate was slower during this phase. Furthermore, according to Hock et al. 2009, the rapid depletion of oxygen occurred during initial decomposition of mesocarp fiber composting due to active consumption of degradable material by microbes, oil degradation, and slow "burning" of compost materials. In the final stage of mesocarp fiber composting, the oxygen level increased after depletion of compost materials.

Effect of pH and Bacterial Population

As indicated in Fig. 3, the pH value was slightly increased on the 4th day of composting due to addition of POME anaerobic sludge into compost materials, which contributed to alkaline condition. Overall, the pH value was maintained in the range of 7.9 to 8.5. In EFB composting (Baharuddin et al. 2009a), the pH observed was 8.3 to 8.5 throughout the process, while in mesocarp fiber composting (Hock et al. 2009), the pH was reduced during the initial stage to 6.8 from 7.8. At 5 day of composting, the pH gradually increased and was maintained at around 7.5 to 8.2. This is attributed to rapid metabolic degradation of organic acid and intense proteolysis of liberating alkaline ammonia compound. In OPF composting, the increase of pH also attributed the increase of thermophilic bacteria. Moreover, this condition was due to the biochemical reactions of nitrogen-containing materials (Baharuddin et al. 2009a). At the end of composting the pH was stabilized at 8.2, which probably was due to the buffering nature of humic substances. A similar range of pH was also detected in EFB-POME anaerobic sludge-based compost at field scale operation (Table 2).

The initial number of colony forming units (CFU) was detected at 55 x 10^{10} CFU/mL (Fig. 3). When the composting process reached the thermophilic phase at temperatures of 50^{o}C and above, thermophilic bacteria increased drastically on the 5th day of composting, reaching a level of 66 x 10^{10} CFU/mL. This may be due to the availability of organic materials that can be consumed by the microbes easily. However, between the 10th and 60th day of composting, the microbial population decreased gradually. According to Baharuddin et al. (2009a), in EFB composting, the number of CFU at the initial stage was 6.4x10^{10} CFU/mL and it decreased gradually after day 20 until day 30. In mesocarp fiber composting, the initial number CFU observed was 4x10^{10} CFU/mL. The pH decreased gradually from day 15 to 30. This condition occurred due to the long period of the thermophilic phase, as well as compaction of composting materials that led to a low level oxygen and ash formation (Hock et al. 2009), which indirectly inhibited microbial growth during the process. In the latter stage of composting, mesophilic bacteria might become dominant with respect to the decrease in compost pile temperature.

C/N Ratio

As illustrated in Fig. 4, the nitrogen content slightly increased constantly, while carbon content slightly decreased (almost constant) throughout the composting process. The nitrogen content increased from 0.6 %, during the initial stage, to 1.8 % towards the end of composting. This was attributed by the activity of cellulolytic degrading microbes and their proliferation, which could retain nitrogen content (Hock et al. 2009), and increased microbial protein and humic substances (Thambirajah et al. 1995).

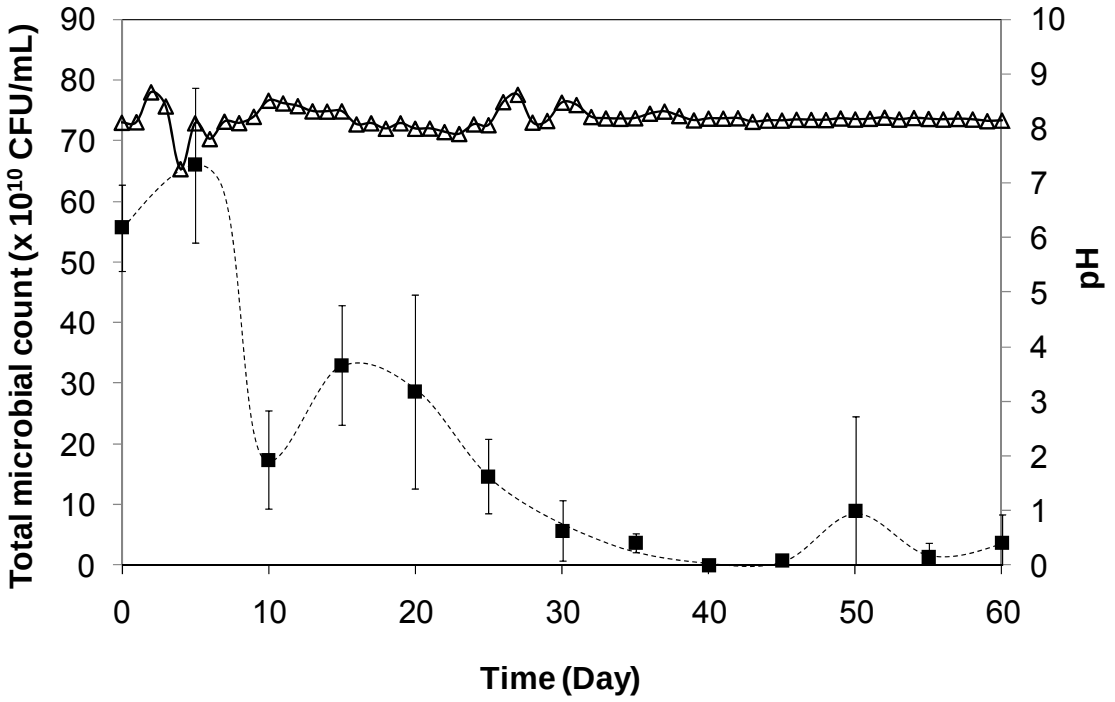

Fig. 3. Total microbial count and pH profiles throughout co-composting of OPF and POME anaerobic sludge. Remarks : ∆= pH and ■= total microbial count.

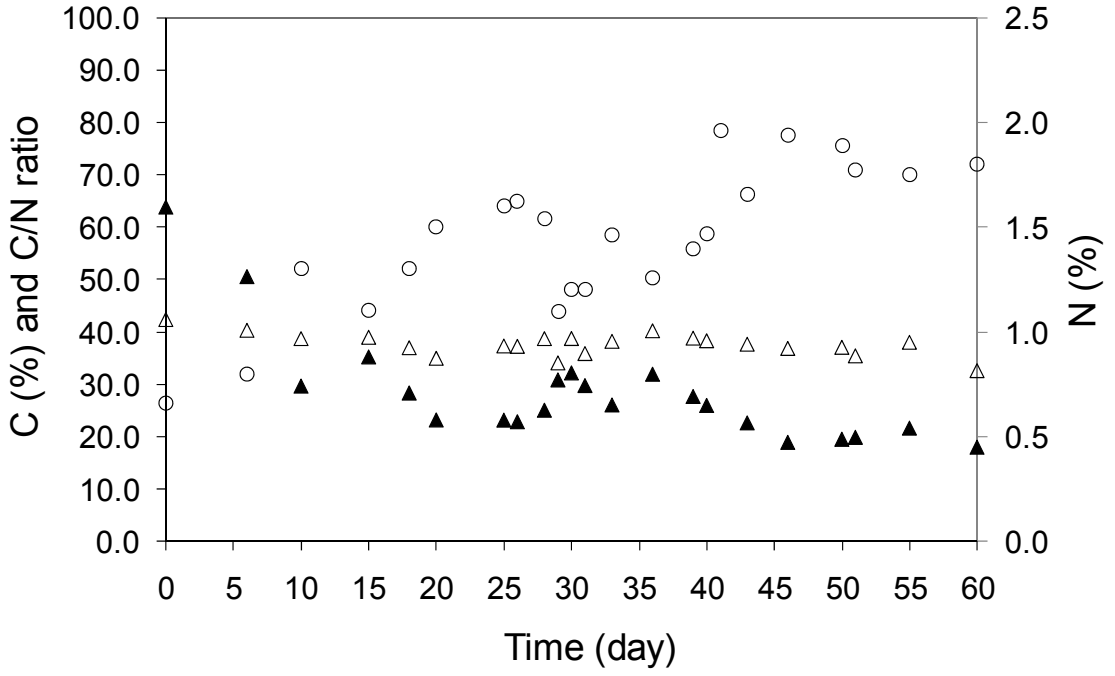

Fig. 4. Changes of carbon, nitrogen and C/N ratio throughout co-composting of CGOPF and POME anaerobic sludge. Remarks : ∆= Carbon, O= Nitrogen and ▲ = C/N ratio

The addition of POME anaerobic sludge was able to reduce the initial C/N ratio to an acceptable level. Initial C/N ratio, 64 dropped to 35 on the 15th day of composting, and finally to 18 at the end of composting. In this study, the C/N ratio of the matured compost was slightly higher than EFB and mesocarp fiber based composts, with a C/N ratio of 12.7 (Baharuddin et al. 2009a) and 13 (Hock et al. 2009), respectively. According to Heerden et al. (2002), a C/N ratio less than 20 could be considered as a satisfactory level of compost maturation.

Nutrients and Metal Elements

In general, a good compost product contains considerable amounts of nutrients such as phosphorus (P), potassium (K), calcium (Ca), iron (Fe), and magnesium (Mg). As illustrated in Table 2, the critical nutrient elements in final compost such as P (0.1 %) and K (0.9 %) were slightly lower compared to compost product obtained from mesocarp fibre (Hock et al. 2009). The content of calcium, magnesium, iron, and sulphur were slightly increased due to addition of POME anaerobic sludge throughout the composting process. The final concentration of Ca (0.6 %) was comparable to the results obtained from EFB compost (Baharuddin et al. 2009a). The micronutrient elements (zinc, manganese, copper, boron) that are crucial elements for plants re-growth, plant health, and development of microorganism (Hock et al. 2009) were also detected in the compost sample.

As shown in Table 2, the heavy metals content such as Cr , Pb, Cd, and Ni in the final compost was low (<20 ppm) and met the USEPA standards to be used as fertilizer and soil amendment. Interestingly, the heavy metals elements in OPF-based compost were lower than mesocarp fibre compost (Hock et al. 2009). According to US EPA (Moldes 2007), the maximum allowable level in exceptional quality of compost are 1200 ppm (Cr), 300 ppm (Pb), and 420 ppm (Ni), respectively.

DGGE Analysis Using 16S rDNA Universal Primers

The result of DGGE analysis showed that the position of most bands did not change significantly during the composting (Fig. 5). The bands shown indicated that microbial community did not change much throughout composting process. Detailed DGGE analysis of each sample showed the predominance of different microbial species in each community (Table 3). Different environmental conditions and substrate characteristics had contributed to the colonization of certain dominant microbes throughout composting process.

As reported by Baharuddin et al. (2009b) (EFB composting), the major microbial communities were mainly uncultured and unidentified bacteria when using shredded EFB materials with partially treated POME for co-composting treatment. In this study, the major member was detected as *Pseudomonas sp*. and very close to the phylogeny of *Gammaproteobacteria*.

The recovered sequences were mainly derived from four phylogenetic groups: *Gammaproteobacteria* (17 sequences), *Bacteria* (6 sequences), *Bacillales* (2 sequences), and *Bacteroidetes* (2 sequences) (Table 3). Most of the bands were high in similarity, which were greater than 90%.

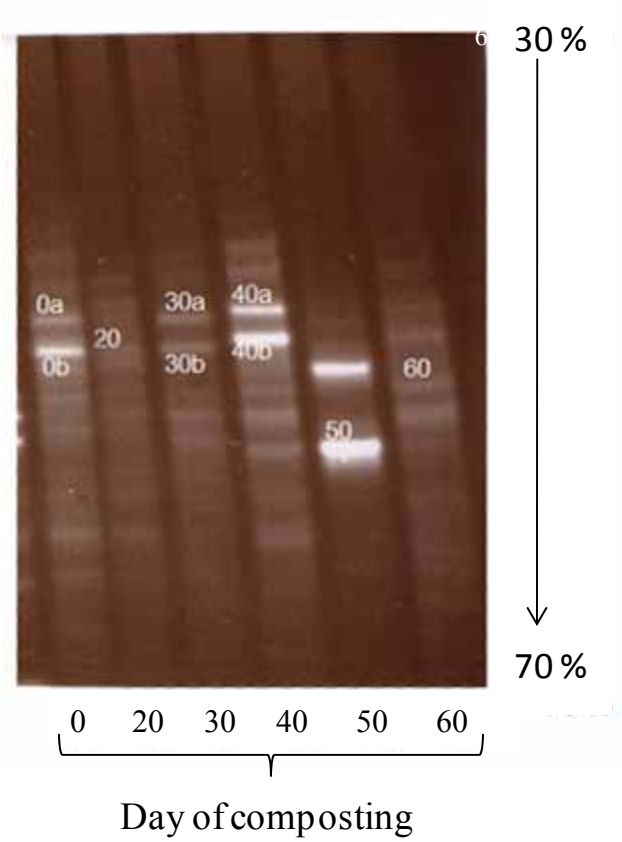

Fig. 5. Comparison of the DGGE banding patterns of microbial communities of the substrates at various composting time. The arrow on the right indicates the gradient of DNA denaturants.

At the initial stage of composting, one major group consisted of *Gamma-proteobacteria*, which was identified as the dominant microorganism, having sequence similarity greater than 95%. In the previous study (Baharuddin et al. 2009b), three major groups known as *cyanobacteria*, *delta-proteobacterium*, and *firmicutes* were detected at initial stage of co-composting of EFB and POME. This study reveals that the use of OPF as a compost substrate led to the widespread presence of *Gammaproteobacteria* such as *Pseudomonas sp.* (Fig. 6), with sequence similarity in the range of 96 to 99 %. When the temperature increased to 50^{o}C within 10 to 20 days of composting, not only *Gammaproteobacteria* was found, but also *Bacteria* species. The *Bacteria* species that was grown on 20th day of composting were uncultured bacterium clone B11.5.29. In EFB composting (Baharuddin et al. 2009b), the major band detected at day 10 and 20 was uncultured bacterium clone biogas and closest to the phyla *proteobacterium* bacteria. On the other hand, the *Pseudomonas sp*. and *Pseudomonas anguilliseptica* were the prominent microbes detected in the thermophilic stage at 20 days composting process with similarity greater than 95% and 97%, respectively (Table 3).

Within 30 to 40 days of composting, the major groups, *Gammaproteobacteria* and *Bacteria* were still detected during the curing phase. On the 30th day of composting, the phyla *Bacteri*a, which is known as uncultured bacterium clone R2J7C4 F12, was detected

at a sequence similarity of 98%. Bacterial species of soil bacterium 5V-07 was detected on the 40th day of composting with a sequence similarity of 95%. In EFB composting, the major bands present from day 10 to 45 were uncultured compost bacteria, closest to *proteobacterium* and isolated from hot compost sample. On the 50th day of composting, the phyla *Bacillales*, consisting of *Bacillus sp.* KAR28 and *Bacillus psychrodurans* strain 1Sf92 with a sequence similarity of 99%, were detected. Furthermore, the phylum *Bacteroidetes*, consisting of *S. mizutae* and *Pedobacter solani* strain N1db8 were found on the 60th day of composting. The phylum *Bacteroidetes* consists of three large classes of bacteria that are widely distributed in the environment, including in soil, in sediments, sea water, and in the guts and on the skin of animals (Baharuddin et al. 2009b).

The different prominent microbes detected in this study in comparison to EFB-POME anaerobic sludge based compost might be due to the different carbon source and efficiency of composting condition such as temperature, moisture content, and oxygen level throughout composting treatment. Thus, the results also suggested that the POME anaerobic sludge comprised many microbes that are capable of accomplishing the composting process within 60 days, and the different conditions of composting process may reflect the appearance of the different prominent microbes throughout the treatment.

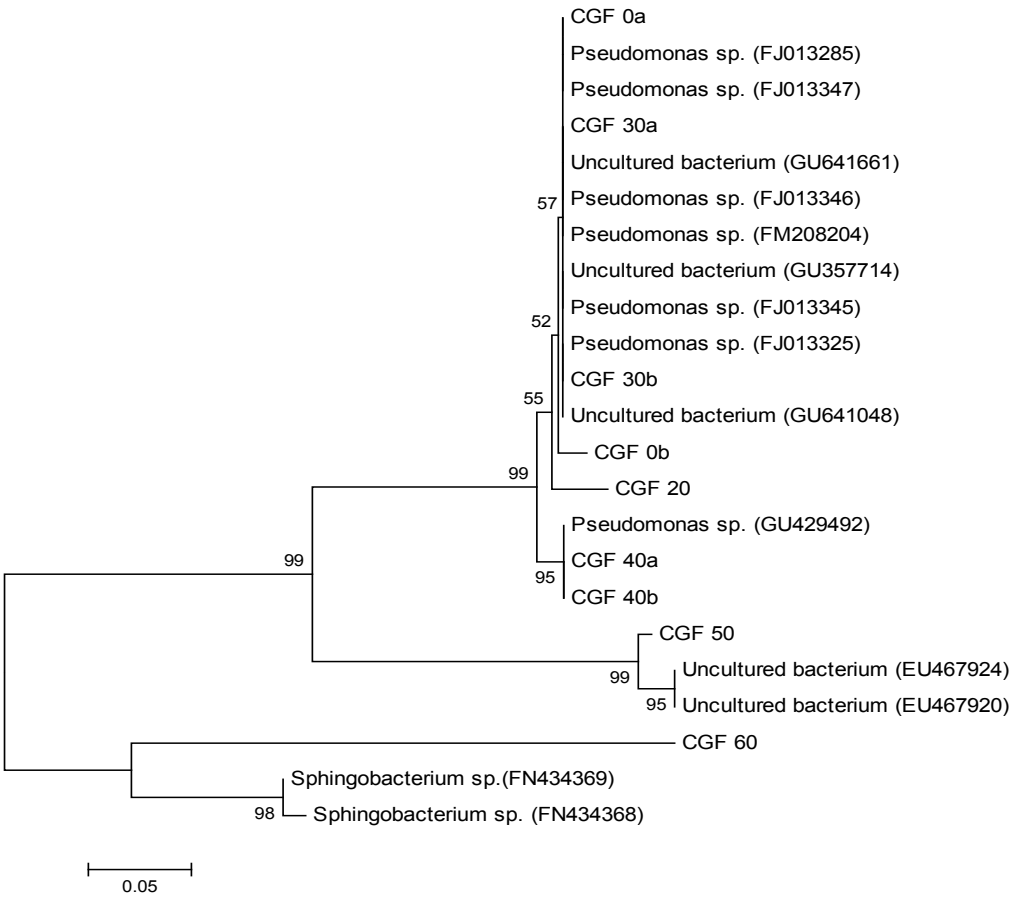

Fig. 6. Neighbor-joining tree representing the phylogenetic relationship of the most abundant 16S rDNA sequences from chipped ground OPF compost samples to various closely related sequences obtained from BLAST searches

Table 3. Phylogenetic Affiliation of Excised DGGE Bands from Compost Source

Band	Nearest relative (accession)	Similarity (%)	Phylogeny
0a 1	*Pseudomonas* sp. ITRI53 16S ribosomal RNA gene, partial sequence	99%	*Gammaproteobacteria*
0a 2	*Pseudomonas* sp. B2-67 partial 16S rRNA gene, strain B2-67	99%	*Gammaproteobacteria*
0a 3	*Pseudomonas anguilliseptica* partial 16S rRNA gene, strain KB3	99%	*Gammaproteobacteria*
0b 1	*Pseudomonas* sp. ITRI73 16S ribosomal RNA gene, partial sequence	96%	*Gammaproteobacteria*
0b 2	*Pseudomonas* sp. MIXRH13 16S ribosomal RNA gene, partial sequence	96%	*Gammaproteobacteria*
0b 3	*Pseudomonas* sp. 01WB03.3-1 partial 16S rRNA gene, strain 01WB03	96%	*Gammaproteobacteria*
20 1	Uncultured bacterium clone B11.5.29 16S ribosomal RNA gene, partial sequence	95%	*Bacteria*
20 2	*Pseudomonas sp*. T4 (2009) 16S ribosomal RNA gene, partial sequence	95%	*Gammaproteobacteria*
20 3	*Pseudomonas anguilliseptica* strain D4029 16S ribosomal RNA gene, partial sequence	97%	*Gammaproteobacteria*
30a 1	Pseudomonas sp. MIXRI75 16S ribosomal RNA gene, partial sequence	99%	*Gammaproteobacteria*
30a 2	Pseudomonas sp. MIXRI74 16S ribosomal RNA gene, partial sequence	99%	*Gammaproteobacteria*
30a 3	Uncultured bacterium clone R2J7C4_F12 16S ribosomal RNA gene, partial sequence	98%	*Bacteria*
30b 1	*Pseudomonas sp.* RF-122 16S ribosomal RNA gene, partial sequence	98%	*Gammaproteobacteria*
30b 2	*Pseudomonas sp.* BSw21399B 16S ribosomal RNA gene, partial sequence	98%	*Gammaproteobacteria*
30b 3	*Pseudomonas sp.* K3B-3 16S ribosomal RNA gene, partial sequence	98%	*Gammaproteobacteria*
40a 1	*Pseudomonas sp.* ITSI26 ribosomal RNA gene, partial sequence	100%	*Gammaproteobacteria*
40a 2	*Pseudomonas sp.* 01WB04. 1-29 partial 16S rRNA gene, strain 01WB04. 1-29	100%	*Gammaproteobacteria*
40a 3	*Pseudomonas sp. pah3* 16S ribosomal RNA gene, partial sequence	100%	*Gammaproteobacteria*
40b 1	Uncultured bacterium clone ribosomal RNA gene, partial sequence	97%	*Bacteria*
40b 2	Soil bacterium 5V-07 16S ribosomal RNA gene, partial sequence	95%	*Bacteria*
40b 3	*Pseudomonas sp.* R-32553 16S rRNA gene, strain R-32553	95%	*Gammaproteobacteria*
50 1	*Bacillus sp.* KAR28 16S ribosomal RNA gene, partial sequence	99%	*Bacillales*
50 2	*Bacillus psychrodurans* partial 16S rRNA gene, strain 1Sf92	99%	*Bacillales*

50 3	Uncultured bacterium clone POROKL1D04 16S small subunit ribosomal RNA gene, partial sequence	99%	*Bacteria*
60 1	Bacterium enrichment culture clone HQ-2 16S ribosomal RNA gene, partial sequence	94%	*Bacteria*
60 2	*S. mizutae* (ATCC 33299T) gene for 16S rRNA	94%	*Bacteroidetes*
60 3	*Pedobacter solani* strain N1d-b8 16S ribosomal RNA gene, partial sequence	94%	*Bacteroidetes*

CONCLUSIONS

1. The final C/N ratio for compost obtained using OPF-POME anaerobic sludge was 18, and there were considerable amounts of macro and micronutrients.
2. The number of mesophilic and thermophilic microorganisms fluctuated or changed with respect to the changes in temperature or composting phase.
3. The final compost contained a low level of heavy metals.
4. The DGGE analysis revealed that phylum *Gammaproteobacteria* was present on most of major bands, and a group of *Gammaproteobacteria* related to *Pseudomonas sp.* was the dominant species found during the composting process.
5. The N, P, and K levels observed in the final compost were 1.8%, 0.1%, and 0.9%, respectively.

ACKNOWLEDGMENTS

The authors would like to thank Malaysian Palm Oil Board (MPOB) and FELDA Palm Industries for the financial (67001) and technical support of this study.

REFERENCES CITED

APHA. (1998). *Standard Methods for Examination Water and Wastewater*, 20th edition. American Public and Health Association, Washington D.C. American Public Health Association.

APHA, AWWA, and WEF. (2005). *Standard Methods for the Examination of Water and Wastewater*, 21st ed. American Public Health Association, Washington, D.C.

Astimar, A. A., and Wahid, M. B. (2006). "Supply outlook of oil palm biomass in Malaysia," *Proceedings of the Seminar on Ecomat Research and Promotion; Towards Enrichment of the Environment*, July 24-25, Beijing, China, pp. 13-26.

Baharuddin, A. S., Wakisaka, M., Shirai, Y., Abd Aziz, S., Abdul Rahman, N. A., and Hassan, M. A. (2009a). "Co-composting of empty fruit bunches and partially treated palm oil mill effluents in pilot scale," *Int. J. Agri. Res.* 4(2), 69-78.

Baharuddin, A. S., Kazunori, N., Abd-Aziz, S., Tabatabaei, M., Rahman, N. A. A., Hassan, M. A., Wakisaka, M., Sakai, K., and Shirai, Y. (2009b). "Characteristics and microbial succession in co-composting of oil palm empty fruit bunch and partially treated palm oil mill effluent," *Open Biotechnol. J.* 3, 87-95.

Baharuddin, A. S., Hock, L. S., Yusof, M. Z. M, Rahman, N. A. A, Shah, U. K. M., Hassan, M. A., Wakisaka, M., Sakai, K., and Shirai, Y. (2010). "Effects of palm oil mill effluent (POME) anaerobic sludge from 500 m^3 of closed anaerobic methane digested tank on pressed-shredded empty fruit bunch (EFB) composting process," *Afr. J. Biotechnol.* 9(16), 2427-2436.

Bazrafshan, E., Zazouli, M. A., Bazrafshan, J., and Bandpei, A. M. (2006). "Co-composting of dewatered sewage sludge with sawdust," *Pak. J. Biol. Sci.* 9(8), 1580-1583.

Bobleter, O. (1998). "Hydrothermal degradation and fractionation of saccharides and polysaccharides," *ChemInform*. Volume 29, Issue 52.

Fernández, F. J., Sánchez-Arias, V., Villaseñor, J., and Rodríguez, L. (2008). "Evaluation of carbon degradation during co-composting of exhausted grape marc with different biowastes," *Chemosphere* 73(5), 670-677.

Goering, H. K., and Van Soest, P. J. (1970). *Forage Fiber Analyses (apparatus, reagents, procedures and some applications)*. US Agricultural Research Service. 20 p.

Heerden, I. V., Cronjé, C., Swart, S. H., and Kotzé, J. M. (2002). "Microbial, chemical and physical aspects of citrus waste composting," *Biores. Tech.* 81(1), 71-76.

Hock, L. S., Baharuddin, A. S., Ahmad, M. N., Shah, U.K.M., Abdul Rahman, N. A., Abd-Aziz, S., Hassan, M. A., and Shirai, Y. (2009). "Physicochemical changes in windrow co-composting process of oil palm mesocarp fiber and palm oil mill effluent anaerobic sludge," *Aust. J. Basic Appl. Sci.* 3(3), 2809-2816.

Hubbe, M. A., Nazhad, M., and Sánchez, C. (2010). "Composting as a way to convert cellulosic biomass and organic waste into high-value soil amendments: A review," *BioResources* 5(4), 2808-2854.

Kabbashi, A. N., Alam, M. Z., and Othman, A. (2006). "Biocomposting process for utilization agro-industrial wastes," *Proceedings of the 1st International Conference on Natural Resources Engineering & Technology*, July 24-25. Putrajaya, Malaysia, pp. 176-181.

Khalil, A. I., Beheary, M. S., and Salem, E. M. (2001). "Monitoring of microbial populations and their cellulolytic activities during the composting of municipal solid wastes," *W. J. Microbiol. Biotechnol.* 17(2), 155-161.

Lachke, A. (2002). "Biofuel from D-xylose – the second most abundant sugar," *Resonance*. 50-58.

Malaysian palm oil industry performance 2008 (2009). *Global Oils and Fats Business Magazine* Vol. 6 (1) (Jan-March), Malaysian Palm Oil Board.

Moldes, A., Cendón, Y., and Barral, M. T. (2007). "Evaluation of municipal solid waste compost as a plant growing media component, by applying mixture design," *Biores. Tech.* 98(16), 3069-3075.

MPOB. (2005). *Review of the Malaysian oil palm industry*. Ministry of Plantation Industries and Commodities. Malaysian Palm Oil Board, pp. 1-9.

Muyzer, G., De Waal, E.C., and Uitterlinden, A.G. (1993). "Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA," *Appl. Environ. Microbiol*. 59(3), 695-700.

Robert, E. G., Gwendolyn, M. H., and Donald, S. (2000). "Chapter 2: Composting," Part 637, *Environmental Engineering, National Engineering Handbook*, National Resources Conservation Service. The United States Department of Agriculture (USDA). pp 1-67.

Rosli, W. D. W., Law, K. N., Zainuddin, Z., and Asro, R. (2007). "Pulp from oil palm fronds by chemical processes," *Industrial Crops and Product.* 25, 89-94.

Thambirajah, J. J., Zulkafli, M. D., and Hashim, M. A. (1995). "Microbiological and biochemical changes during the composting of oil palm empty fruit bunches. Effect of nitrogen supplementation on the substrate," *Biores. Tech.* 52(2), 133-144.

Wong, J. W. C., Mak, K. F., Chan, N. W., Lam, A., Fang, M., Zhou, L. X., Wu, Q. T., and Liao, X. D. (2001). "Co-composting of soybean residues and leaves in Hong Kong," *Biores. Tech.* 76(2), 99-106.

Li, X., Zhang, R., and Pang, Y. (2008). "Characteristics of dairy manure composting with rice straw," *Biores. Tech.* 99(2), 359-367.

Yeates, C., Gillings, M. R., Davison, A. D., Altavilla, N., and Veal, D. A. (1998). "Methods for microbial DNA extraction from soil for PCR amplification," *Biol. Proced. Online* 1(1), 40-47.

Article submitted: June 25, 2011; Peer review completed: August 7, 2011; Revised version received and accepted: September 30, 2011; Published: October 2, 2011.