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Full Length Research Paper

Evaluation of pressed shredded empty fruit bunch (EFB)-palm oil mill effluent (POME) anaerobic sludge based compost using Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) analysis

Azhari Samsu Baharuddin[1], Nor' Aini Abdul Rahman[2]*, Umi Kalsom Md Shah[2], Mohd Ali Hassan[2], Minato Wakisaka[3] and Yoshihito Shirai[3]

[1]Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[2]Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[3]Department of Biological Functions and Engineering, Graduate School of Life Science and System Engineering, Kyushu Institute of Technology, 2-4 Hibikino, Wakamatsu-ku, Kitakyushu, Fukuoka 808-0916, Japan.

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Pressed-shredded empty fruit bunches (EFB) and palm oil mill effluent (POME) anaerobic sludge from a 500 m^3 closed anaerobic digester system was utilized for the co-composting treatment. Scanning electron microscopy (SEM) analysis showed that the shredding-pressing treatment on EFB gave better results in removing the debris and silica bodies as compared to only shredding treatment. However, similar characteristics were detected in both physically-treated EFB samples by Fourier transform infrared (FTIR) analysis, mainly in the regions of 900 to 1740 and 2800 to 3400 cm^{-1}. After the anaerobic digestion of fresh raw POME, the protein origin (Amide I) band appeared in the POME anaerobic sludge. Besides, the band intensities at 2925 and 2855 cm^{-1} which attributed to the composition of fat and lipid was decreased. The maturity of the composting material after 40 days of treatment was detected by the appearance of the nitrate band at 1376 cm^{-1} and the results corresponded to the final C/N ratio of 12.4. Solid state ^{13}C CP/MAS nuclear magnetic resonance (NMR) was also used to reveal the characteristic changes of pressed-shredded EFB-POME anaerobic sludge based compost.

Key words: Empty fruit bunch, palm oil mill effluent, compost.

INTRODUCTION

The co-composting of empty fruit bunches (EFB) and palm oil mill effluent (POME) is one of the alternative ways to reduce the abundance of biomass generated at palm oil mills (Baharuddin et al., 2009). Composting is a biological process by which organic material undergoes decomposition and transformation processes. Mineralization and humification of organic matter take place during the composting process. Normally, a compost pile has two stages: an active composting stage and a curing period stage. When the temperature rises above 60°C, many microorganisms begin to die or become dormant, and after that the temperature starts to stabilize or decrease. In the curing stage, the materials degrade at a much slower rate. The composting process continues until the remaining nutrients are consumed by the remaining microorganisms and almost all the carbon is converted to carbon dioxide (Kale et al., 2006).

*Corresponding author. E-mail: nor_aini@biotech.upm.edu.my. Tel: +603-8946 6699. Fax: +603-8946 0913.

Abbreviations: EFB, Empty fruit bunches; **POME,** palm oil mill effluent; **SEM,** scanning electron micrscope; **FTIR,** Fourier transform infrared; **NMR,** nuclear magnetic resonance; **CPO,** crude palm oil.

EFB contains a high cellulosic matter which is easily decomposed by a combination of physical, chemical and biological processes. In general, the EFB consists of 30% solid with holocellulose content of 65.5%, lignin 21.2%, ash 3.5%, hot-water-soluble substances 5.6% and alcohol-benzene soluble fraction 4.1% (Thambirajah et al., 1995). It was reported that lignin provides plant strength and resistance to microbial degradation (Shibata et al., 2008). However, many microorganisms were capable of degrading cellulose and hemicellulose as carbon and energy sources. During composting treatment, the capacity of thermophilic microorganisms to assimilate organic matter depends on their ability to produce the enzymes for substrate degradation (Tuomela et al., 2000). It was reported that the total composting process of shredded EFB and partial treated POME was accomplished for about 60 to 70 days. However, the composting period reduced to 40 days of treatment when the raw materials was replaced by pressed-shredded EFB and treated POME anaerobic sludge. This result was attributed to the characteristics of POME anaerobic sludge which was considerably consistent and lower C/N ratio as compared to partially treated POME (Baharuddin et al., 2010).

The composting process involves changes of the chemical composition and physico-chemical parameters in the composting mixture. Many methods have been suggested to establish the degree of compost maturity such as physico-chemical changes, microbiological methods, studies of humidified organic matter and germination index (Grigatti et al., 2004). Humic substance is a parameter for organic matter quality determination, but it is a time consuming method. Besides, the ratio of carbon and nitrogen content of composting materials is often used as indicator for the compost maturity and stability. On the contrary, Fourier transform infrared (FTIR) spectroscopy is a fast, cheap and robust laboratory method in which the infrared spectrum shows chemical composition of the composting materials (Grube et al., 2006).

This study was conducted to reveal the suitability of using pressed-shredded EFB and POME anaerobic sludge for the co-composting process. Thus, physico-chemical characteristics of raw EFB (shredded and pressed-shredded) and POME (fresh raw POME and POME anaerobic sludge) were evaluated. Furthermore, the characteristic changes of pressed-shredded EFB-POME anaerobic sludge based compost were also determined.

MATERIALS AND METHODS

Raw materials and pre-treatment

The EFB was obtained from the Seri Ulu Langat Palm Oil Mill, Dengkil, Selangor whereas POME anaerobic sludge was obtained from a 500 m^3 semi-commercial closed anaerobic digester system at Felda Serting Hilir, Negeri Sembilan, Malaysia. Fresh raw POME from palm oil mill was used for biogas generation in a 500 m^3 semi-commercial closed anaerobic digester tank. The thicken POME anaerobic sludge from the bottom part of the settling tank after anaerobic digestion was used for the composting treatment in this study. Meanwhile, the EFB material was physically treated either by the shredding or the pressing-shredding methods at the palm oil mill for the recovery of the remaining crude palm oil (CPO). The pressed-shredded EFB was further used in the co-composting treatment.

Composting site and process

This research was conducted at Universiti Putra Malaysia under the shade and cement base area. Brick blocks with length of 2.1 m, width of 1.5 m and height of 1.5 m each were used as composting blocks. One ton of pressed-shredded EFB was loaded into the composting block. About 50 kg of recycled EFB compost was added to facilitate the composting process. POME anaerobic sludge was added at three day intervals using a motor pump in order to maintain the moisture content of the pile within 55 to 65%. Windrow turning was conducted one to three times a week. Each composting cycle was completed within 40 days. The total of POME anaerobic sludge added into the pile throughout the process was about one ton (1:1 ratio) (Baharuddin et al., 2010).

Analysis

The temperature and oxygen of the heaps was monitored by using a compost monitoring device. Inductively Coupled Plasma (OES, Perkin Elmer, USA) was used to determine carbon, nitrogen and nutrients elements. The analysis for oil and grease, and pH were conducted according to the American Public Health Association (APHA) methods (1998). Meanwhile, analysis of cellulose, hemicellulose and lignin content were determined according to Gorring and Soest (1970). Scanning electron microscopy (Philips XL 30 ESEM, Holland) was used to view the structure of the EFB sample. All analysis was done in triplicates.

Structural characterization {FTIR and nuclear magnetic resonance (NMR)}

FTIR analysis was conducted to evaluate the infrared spectrum that shows chemical composition of raw materials and compost samples. The FTIR analysis was performed using a Perkin Elmer FTIR spectrophotometer (Uberlingen, Germany). KBr pellets were made by weighing 2 mg of dried samples and 300 mg of KBr and pressing the mixture under vacuum for 10 min. The samples were measured at the range of 4000-400 cm^{-1} wavelength. Data collection and processing were performed by using the GRAMS/386 version 3.02 software.

Meanwhile, the solid state ^{13}C CP/MAS NMR spectrum was recorded at room temperature on a Bruker Avance 400 MHz NMR operating at a static magnetic field of 9.4T. Magic angle spinning was operated at 5.0 kHz using 4 mm ceramic Si3N4 rotor. The ^{13}C CP/MAS experiment was performed using a 3.8 microsecond 90° pulse with a delay time of 5 s, a contact time of 1 ms and spinning rate of 5 kHz and 2000 transients. Chemical shifts were measured with respect to tetramethylsilane. The Bruker Win NMR software was used to measure peak areas of the following chemical shift regions: 0 to 47 ppm (aliphatic alkyl C), 47 to 90 (alkyl-O or C-O, C-N bonds as in carbohydrates, alcohols), 90 to 102 ppm (acetal), 102 to 145 ppm (aromatic C), 145 to 167 ppm (phenolic C), 67 to 187 ppm (carboxyl, ester and amide) and 187 to 220 ppm (carbonyl

Table 1. Characteristics of shredded EFB, pressed-shredded EFB, fresh raw POME and POME anaerobic sludge.

Parameter (% dry weight)	Shredded EFB	Pressed-shredded EFB	Fresh raw POME	POME anaerobic sludge
Total carbon	53.5	45.4	34.3	36.1
Total nitrogen	1.0	0.8	2.5	4.1
Phosphorus	0.6	0.1	0.9	1.2
Potassium	2.3	1.9	2.3	4.6
C/N ratio	53.5	56.7	13.7	8.8
Oil and grease*	1.6	0.1	2861.0	150.0
Cellulose	50.3	52.5	36.7	15.3
Hemicellulose	26.1	20.6	21.5	10.1
Lignin	18.0	14.7	28.0	43.8

*Oil and grease content in POME (mg/L).

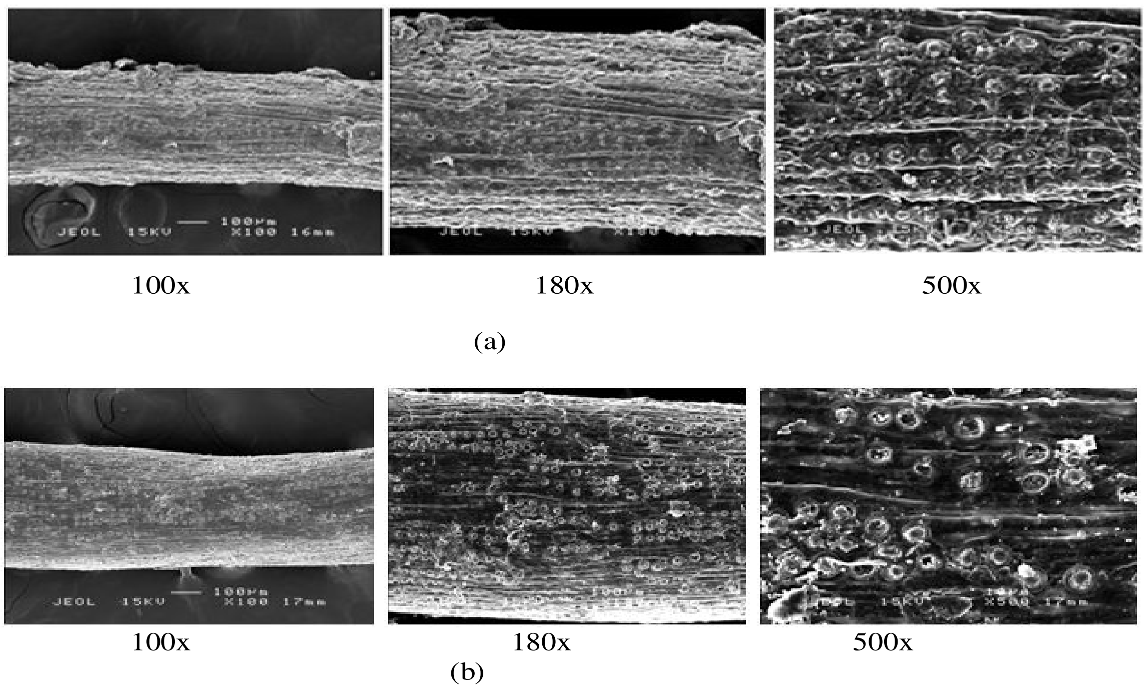

Figure 1. SEM micrograph of (a) shredded and (b) pressed-shredded EFB at 100x, 180x and 500x magnifications.

C). Replicates of three raw materials and compost samples were collected and analyzed.

RESULTS AND DISCUSSION

Characteristics of shredded EFB and pressed-shredded EFB

The physical treatment of EFB materials was conducted by using shredding and the combination of pressing-shredding methods at palm oil mill. The oil and grease content of the shredded EFB was about 1.6% of dry matter. After the pressing-shredding method, about 0.1% of oil and grease content was detected in the pressed-shredded EFB (Table 1), indicating that over 90% of remaining CPO can be recovered from the EFB materials. Although, the pressing-shredding method was efficient for CPO recovery, the composition of N, P and K content in the pressed-shredded EFB was found to be lower as compared to shredded EFB (Table 1). In addition, the composition of cellulose, hemicellulose and lignin content of both pre-treated EFB also had no much difference. Both pre-treated EFB showed a similar range of C/N ratio of about 50 to 60. Figure 1 shows the electron micrographs of shredded and pressed-shredded EFB. It can be seen that the outer structure of both

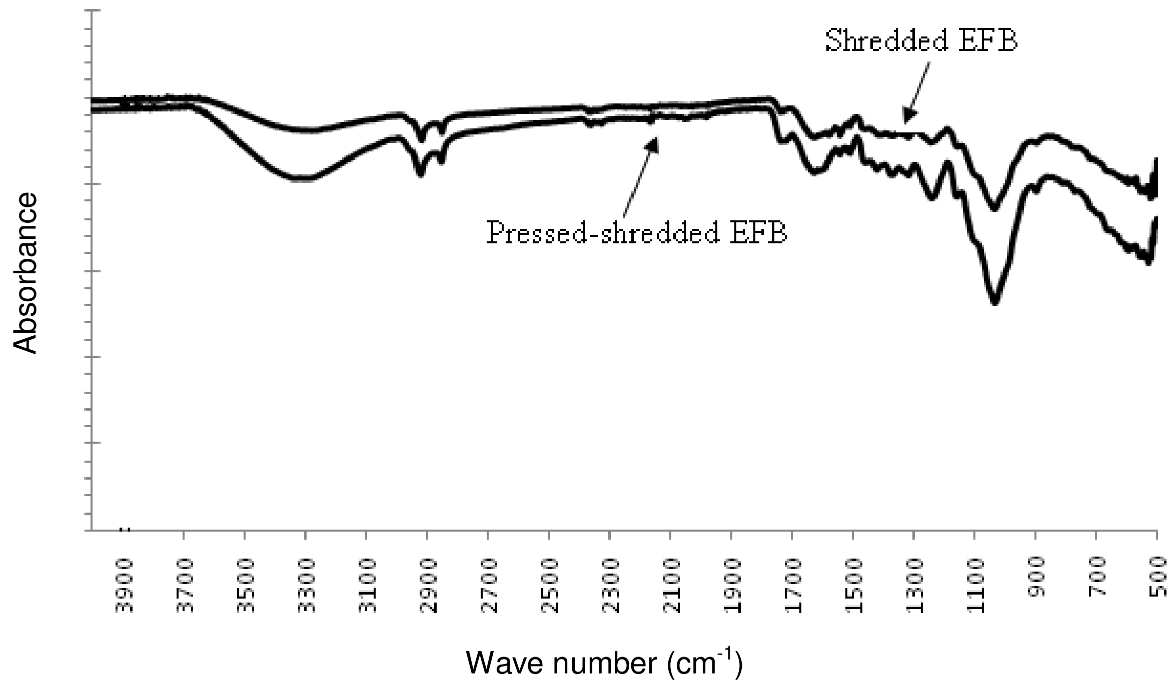

Figure 2. FT-IR analysis of shredded and pressed-shredded EFB.

samples were still intact after undergoing the physical treatment. It is suggested that the surface of the plant cells of both samples were relatively undamaged. However, some silica bodies on the outer surface of pressed-shredded EFB material were being removed (Figure 1b). Therefore, pressed-shredded EFB was selected as the main carbon source for the co-composting treatment based on higher CPO recovery and better physical structure as compared to shredded EFB.

The FTIR spectra of shredded and pressed-shredded EFB showed a strong similarity of the main absorption bands in the regions of 900 to 1740 and 2800 to 3400 cm^{-1} (Figure 2). Similar IR spectra were also obtained by Grube et al. (2006) for wood chips. The band at 950 to 1200 cm^{-1} region corresponds to the valent of C-O, C-C and deformation vibrations of ring structures of CH_2OH origin. In this study, the most intensive broad absorption band appeared in the carbohydrate region with a maximum value at 1034 cm^{-1} and assigned to the vibrations C_3HO_3H and $C_6H_2O_6H$ of cellulose and pyranosyl-ring. Meanwhile, the bands in the region of 1500 to 1600 cm^{-1} can be assigned to the aromatic rings of lignin and 1738 cm^{-1} was attributed to the stretching vibrations of esters in pectins (C=O). Besides, peaks around 1510, 1460, 1270 and 1130 cm^{-1} was typical for lignin component as reported by Grube et al. (2006). Shredded and pressed-shredded EFB also showed similar absorption bands at 2922 and 2852 cm^{-1}. These absorption peaks were attributed to the asymmetrical and symmetrical stretching of methylene ($-CH_2-$) groups (Giovanela et al., 2004). The bands were also found in waste samples and related to the fat and lipid components (Reveille et al., 2003). The band at 3400 cm^{-1} corresponds to the vibration of O-H stretch and attributed to the component of hydroxyl groups and water.

Characteristics of fresh raw POME and POME anaerobic sludge

The characteristics of fresh raw POME and POME anaerobic sludge used in this study are shown in Table 1. POME anaerobic sludge is referred to as the thicken sludge after the digestion process of fresh raw POME in a 500 m^3 of closed anaerobic digester system (Sulaiman et al., 2009). POME anaerobic sludge showed high VSS value (about 21110 mg L^{-1}) and this might be due to the presence of beneficial microbes after the completion of anaerobic digestion treatment. The interval addition of POME anaerobic sludge which consisted of high nitrogen content (4.1%) was contributed to the low level of initial C/N ratio of the composting mixture (Table 2). In this study, POME anaerobic sludge was used as the main nitrogen source and microbial seeding for the co-composting treatment with pressed-shredded EFB materials

The FTIR spectra of fresh raw POME and POME anaerobic sludge are shown in Figure 3. The main absorbance in the IR spectra of POME anaerobic sludge

Table 2. Profile of temperature, pH and C/N ratio of the co-composting process.

Compost sample	Temperature (°C)	pH	C/N ratio
Day 2	47.8	7.8	40.1
Day 5	58.5	8.2	38.0
Day 10	62.7	8.5	32.1
Day 20	57.2	8.6	25.4
Day 30	41.3	8.3	17.5
Day 40	35.2	8.1	12.4

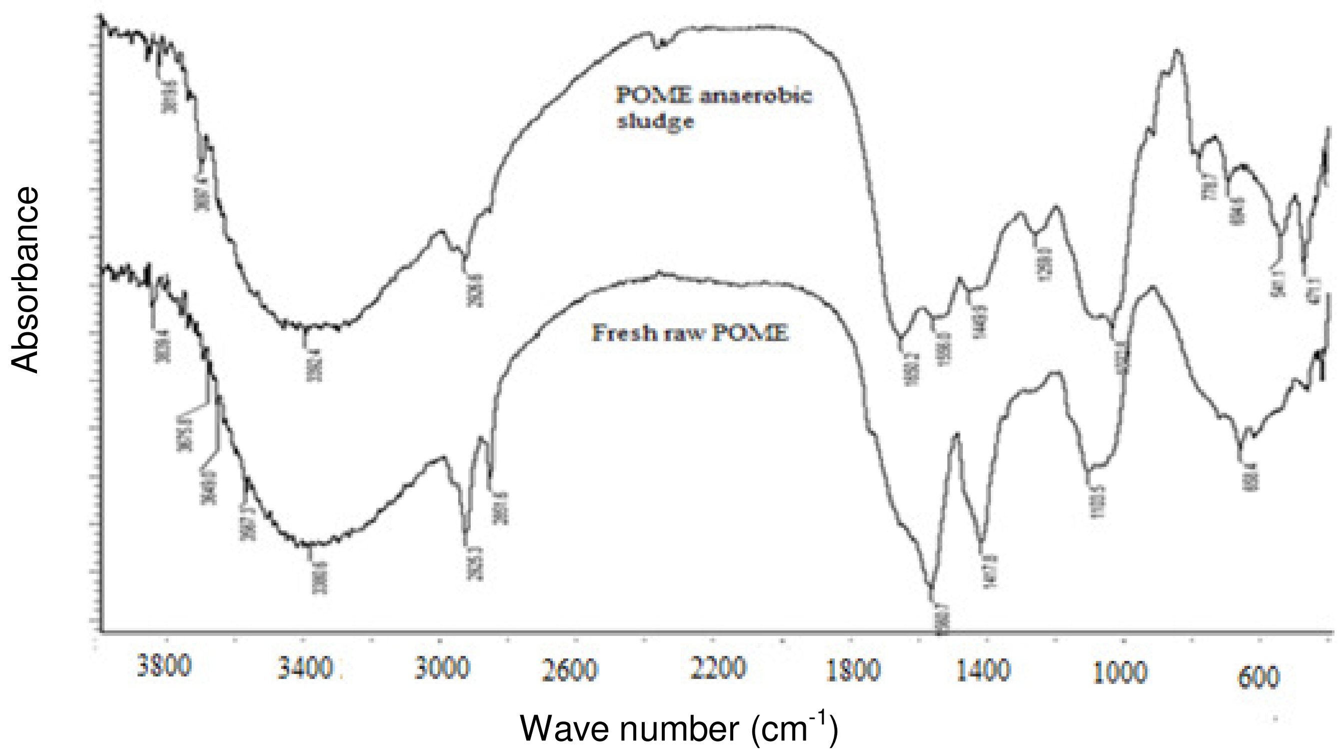

Figure 3. FT-IR analysis of fresh raw POME and POME anaerobic sludge.

was detected at a peak around 1033 cm^{-1} and assigned to C-O stretching of polysaccharides, Si-O of silica impurities and clay minerals possibly in a complex with humic acids (Filip and Bielek, 2002). A similar IR spectrum was also obtained by Grube et al. (2006) in sewage sludge characteristics. Peaks around 1130 and 1260 cm^{-1} detected in both POME samples were typical for lignin (Matias et al., 2000). The methyl asymmetric C-H bending was observed around 1449 and 1417 cm^{-1}. Two broad absorptions band with peaks around 1560 and 1650 cm^{-1} were protein in origin (amide II and amide I correspondingly) but the amide I band was not present in the fresh raw POME spectra. Giovanela et al. (2004) suggested that the IR spectrum of nitrogen band was mostly present in the form of amide group.

In the IR spectra of fresh raw POME, the absorption bands at 2925 and 2855 cm^{-1} were attributed to aliphatic methylene groups and said to be fat and lipid (Grube et al., 2006). However, the broad absorption band around 2925 cm^{-1} was reduced after the anaerobic digestion was completed. In addition, the shoulder peak of 2855 cm^{-1} almost disappeared in the POME anaerobic sludge. It could be suggested that the component of fat and lipid were consumed by the microorganisms in the anaerobic digestion process, catalyzing a complex series of biochemical reactions that mineralize organic matter for producing methane and carbon dioxide. The results were also supported by the reduction of the oil and grease contents in the fresh raw POME from 2861 to 150 mg L^{-1} after it underwent the anaerobic digestion process (Table 1). The band attributed to the hydroxyl group and water (3400 cm^{-1}) was also detected in the fresh raw POME

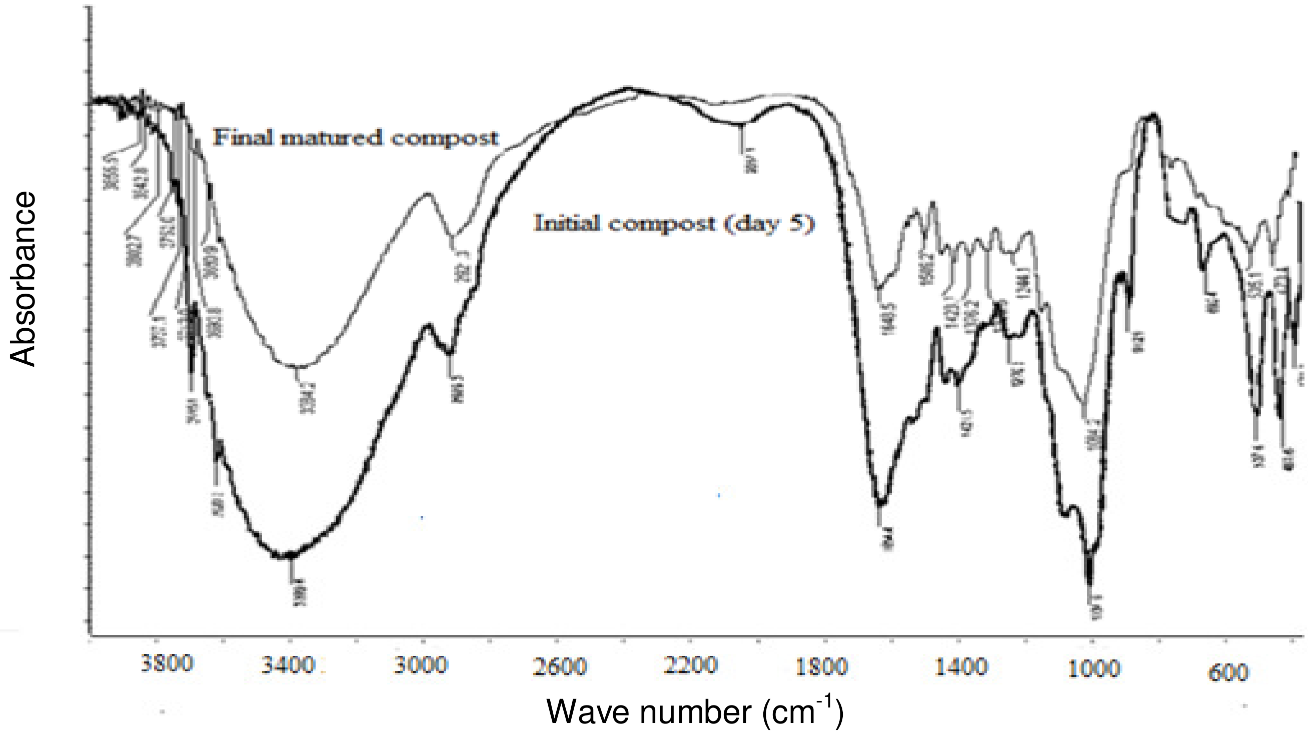

Figure 4. FTIR spectra of composting mixture at the initial and final stage of composting process.

and POME anaerobic sludge. The moisture content of POME before and after anaerobic digestion process was found to be higher than 90% (dry matter).

Physicochemical properties of EFB-POME anaerobic sludge compost by FTIR analyses

The IR spectroscopic of initial and final matured compost is illustrated in Figure 4. It is suggested that the characteristics of compost sample was mainly derived from the component mixture of pressed-shredded EFB and POME anaerobic sludge (Figures 2 and 3). The most intense broad absorption band in the compost sample was detected at 1034 cm^{-1}. Grube et al. (2006) reported that peaks around 1034 cm^{-1} could be attributed to C-O stretching of alcohol, sulfoxides, carbohydrates or polysaccharides-like substance, or Si-O of silicates. It can be seen that similar absorption band were also detected in EFB and POME anaerobic sludge. According to Smidt et al. (2002), the intensity changes of bands at 1034 to 1040 cm^{-1} can also be used to evaluate the decomposition of organic components in the composting process. In this study, the intensity of this band (1034 to 1040 cm^{-1}) was decreased towards the end of composting treatment (Figure 4). A peak around 1270 cm^{-1} in both composting mixtures could be attributed to C-O stretching of aryl ethers. Meanwhile, a shoulder peak around 1123 cm^{-1} was also decreased considerably. The band was attributed to the aromatic ring bends, symmetric bending of aliphatic CH_2, OH and C-O stretching of various groups.

The most obvious differences in both composting mixture was detected in the region of 1350 to 1400 cm^{-1}, where a nitrate band appeared (Figure 4). Usually the nitrate band is detected in the latter stage of composting process (Smidt et al., 2002), indicating the decomposition state and oxidation of nitrogen compound. The appearance of the nitrate band around 1376 cm^{-1} was the evidence of compost maturity for pressed-shredded EFB-POME anaerobic sludge based compost. It is also suggested that the structure of EFB was degraded or disintegrated substantially during composting treatment. The band at 1376 cm^{-1} can be assigned to the vibration of N-O stretching (Figure 4). The nitrate component in the final matured compost was generated from the POME anaerobic sludge addition on the EFB piles throughout the treatment. Meanwhile, the sharp peak detected around 1509 and 1421 cm^{-1} in the latter stage of the composting treatment can be assigned to the accumulation of remaining lignin from the composting mixture. Similar result was also obtained in sewage sludge based compost (Grube et al., 2006). The reduction of cellulose content was attributed to the microbial consumption, whereas the increment of lignin content could be due to the accumulation of nondegradable compost material. It is assumed that humus is formed mainly from lignin and

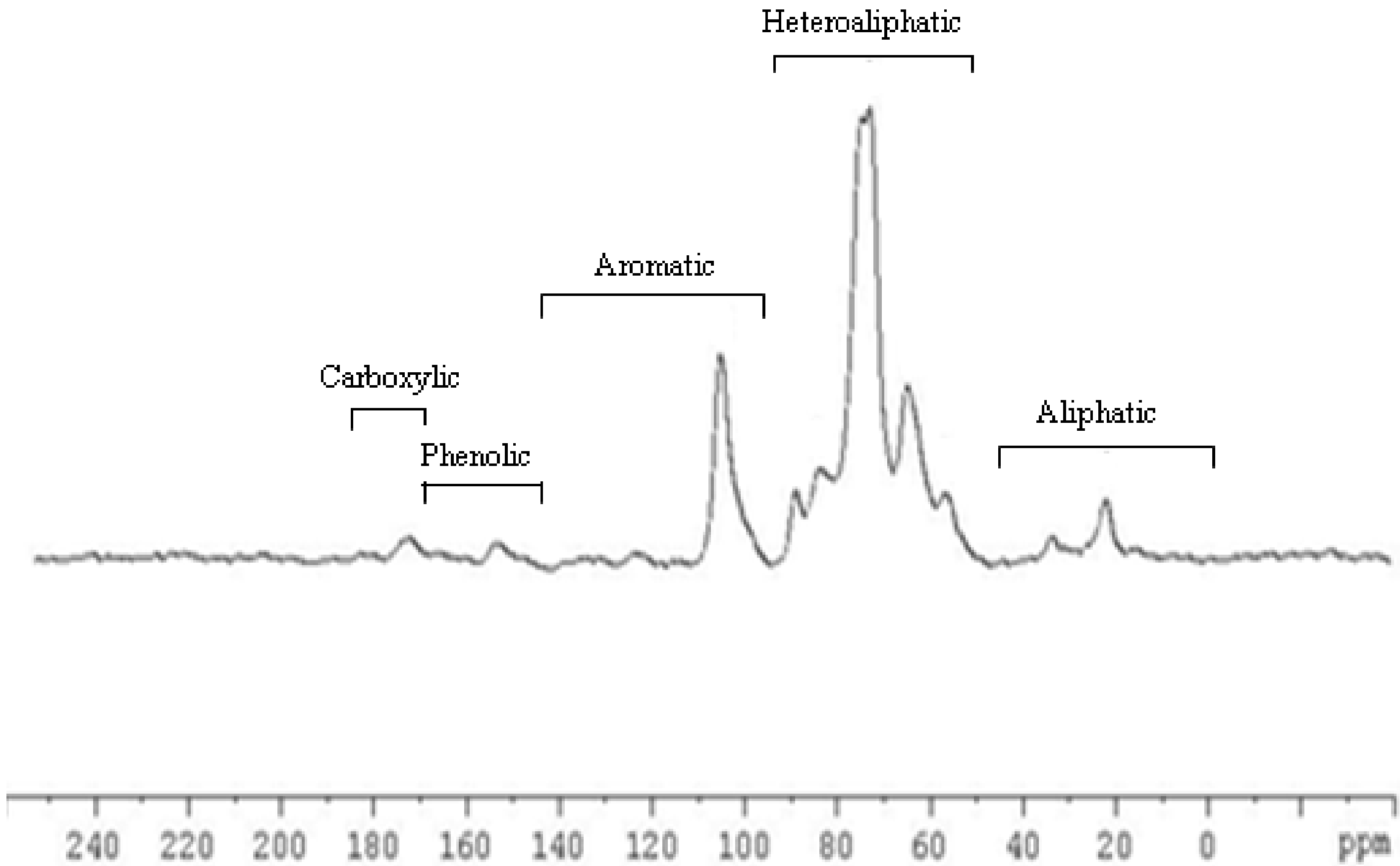

Figure 5. NMR-CP/MAS ^{13}C analysis of the composting mixture at day 5 of treatment.

is not totally mineralized during composting (Tuomela et al., 2000).

The strong band at 1650 cm^{-1} in the composting mixture (Figure 4) can be assigned to amide I, carboxylates and C=C from aromatic and alkenes. A similar peak was also detected in POME anaerobic sludge. The appearance of an absorption band around 2921 cm^{-1} in the composting mixture is attributed to the humid acid component. According to Giovanela et al. (2004), the absorption range of 2940 to 2850 cm^{-1} indicated the saturation of humic acid. According to Madson and Marco (2003), the absorption region of 3000 to 2800 cm^{-1} in the compost sample was mainly assigned to the C-H bond from aliphatic groups. The compost materials contain a substantial amount of organic matter with a significant amount of humic substance. Humic acid constitutes the stable fraction of carbon, thus, regulating the carbon cycle and the release of nutrients, including nitrogen, phosphorus and sulphur in the soil (Baddi et al., 2004). A broad range of O-H and N-H stretching vibration band was obtained around 3384 cm^{-1} for intermolecular hydrogen bonding (H-bonded OH groups) which is attributed to the phenolic group's component.

NMR analysis of the initial composting mixture at the thermophilic stage

The ^{13}C CP/MAS NMR spectrum of initial stage of composting process is presented in Figure 5. The compost sample was collected at day 5 of treatment with temperature of 58.5°C, indicating that thermophilic stage occurred (Table 2). The ^{13}C CP/MAS NMR spectra exhibited major peaks at δ 19 and 32 ppm (aliphatic C), δ 58 and 64 ppm (N-alkyl C), δ 74 and 82 ppm (carbohydrates or aliphatic alcohols), δ 90 ppm (acetal), δ 104 ppm (C-substituted aromatic C), δ 153 ppm (O-substituted aromatic or phenolic) and δ 174 ppm (carboxyl and amide C). No peak of carbonyl group was detected. During the degradation of organic matter by thermophilic microorganisms, the cell wall was ruptured and the component molecules from one organelle were mixed with molecules from other organelles. The reaction takes place during the mixing process which constitutes the first step in the humification process. Since the NMR spectra measured the component of EFB after the degradation has occurred, the spectra could therefore provide an insight into the first reactions of the mobile components during humification. It can be seen that the NMR spectra detected in the early stage of the composting process did not contradict the results obtained in the FTIR analysis (Figure 4).

Conclusion

SEM, FT-IR and ^{13}C CP/MAS NMR analysis revealed that the pressed-shredded EFB and POME anaerobic sludge was suitable substrate for the co-composting process based on its physicochemical properties as compared to the shredded EFB and raw POME. FTIR analysis also proved that the pressed-shredded EFB-POME anaerobic sludge based compost was stabilized after 40 days of treatment and the results corresponds to the final C/N ratio of 12.4.

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