# Effect of Seed Sludge Quality using Oil Palm Empty Fruit Bunch (OPEFB) Bio-Char for Composting

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In this study, a comparison between oil palm empty fruit bunch (OPEFB) composting using palm oil mill effluent bio-char solution (POMEBS) aerobic sludge and palm oil mill effluent (POME) anaerobic sludge was reported. A set of experiments was designed by central composite design (CCD) using response surface methodology (RSM) to statistically evaluate the POMEBS aerobic sludge as microbial seeding. The bacteria count of POMEBS aerobic sludge ($3.7\times10^6$ CFU/mL) at the optimum point was higher than that of POME anaerobic sludge ($2.5\times10^5$ CFU/mL). Denaturing gradient gel electrophoresis (DGGE) and Fourier transform infrared spectroscopy (FTIR) were also performed. A rotary drum composter was then used to compost OPEFB with POMEBS aerobic sludge and POME anaerobic sludge, separately. Thermogravimetric analysis (TGA) showed that composting OPEFB with POMEBS aerobic sludge had a higher degradation rate compared to composting OPEFB with POME anaerobic sludge. In addition, the final N:P:K values for composting OPEFB with POMEBS aerobic and POME anaerobic sludge were 3.7:0.8:6.2 and 1.5:0.3:3.4, respectively. POMEBS aerobic sludge improved the composting process and compost quality.

*Keywords: Palm oil mill effluent (POME) anaerobic sludge; Palm oil mill effluent bio-char solution (POMEBS) aerobic sludge; Response surface methodology (RSM); Denaturing gradient gel electrophoresis (DGGE); Rotary drum composting; Fourier transform infrared (FTIR); Thermogravimetric analysis (TGA)*

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## INTRODUCTION

Oil palm is currently the most productive oil crop in the world, more productive even than soybean and corn. The oil can either be obtained from its mesocarp or its kernel. This fact also means that oil palm produces the greatest amount of biomass. In Malaysia, it is estimated that the industry generates at least 30 million tonnes of biomass per year, including the fronds, trunks, empty fruit bunches, and leaves (Hashim *et al.* 2012). In addition to this solid biomass, the industry also produces a large amount of liquid waste known as palm oil mill effluent (POME). For every tonne of oil palm fresh fruit bunch (OPFFB) processed, about 0.7 tonne of POME will be generated, which comprises 26.3 kg of biological oxygen demand (BOD), 53 kg of chemical oxygen demand (COD), 19 kg of suspended solids (SS), and 6 kg of oil and grease (Lorestani *et al.* 2006). One of the most widely used techniques to utilize these solid and liquid wastes

is the production of compost. This involves the use of microbes to degrade the wastes, which can later be used to improve the quality of degraded soils.

In Malaysia, composting OPEFB with POME anaerobic sludge is a popular method of waste treatment that has been reported in a previous study using an in-vessel composter and windrow system (Wan Razali *et al.* 2012). The compost was completed in 40 days with a N:P:K ratio of 2.8:0.4:2.8. Composting is a process whereby degradable organic matter is converted into stable matter containing a humic-like substance (Ishii *et al.* 2000). The existing composting technology requires a long decomposition time because microbes need to adapt to temperature changes from mesophilic to thermophilic. Thus, in order to improve the existing composting time, POME anaerobic sludge needs to be modified so that its characteristics are suitable for the aerobic composting process. In order to induce microbial proliferation during the composting process, higher surface area material such as bio-char can be added. Bio-char's natural characteristics allow it to act as a host for microbes to colonize (Fischer and Glaser 2012). In addition, urea can be added to improve the final N content. Hence, palm oil mill effluent bio-char solution (POMEBS) was formulated using response surface methodology (RSM) to improve the composting process and the final compost quality.

In order to identify the microbes present in POMEBS, denaturing gradient gel electrophoresis (DGGE) was used. DGGE is a useful tool for revealing microbial succession because it can separate DNA fragments amplified by polymerase chain reaction (PCR) according to the differences in base-pair sequences and visualize the bacterial community as a band finger print (Baharuddin *et al.* 2009).

Compost quality is generally defined on the basis of two criteria, which are stability and maturity. Compost stability refers to the resistance of organic compost matter to further degrade, whereas compost maturity is related to suitability for plant growth and humification (Som *et al.* 2009). Thermogravimetric analysis (TGA) can be used to characterize compost stability (Melis and Castaldi 2004). Thermogravimetry (TG) is a technique in which weight changes are measured during incremental heating of a sample, while the first derivative of TG trace (DTG) shows the steps by which the reactions take place. DTG does not contain any new information; however, the temperatures at which mass loss is at a maximum and superimposed transformation are clearly shown as DTG peaks (Dell' Abate *et al.* 1998). The suitability for plant growth is usually referred to as N:P:K values, where nitrogen is important to promote the growth of leaves; phosphorus is important for photosynthesis, energy transfer within plants, and fruit growth; and potassium is important for the manufacture and movement of sugars, cell division, and root development (Yadav and Garg 2011; Tairo and Ndakidemi 2013).

Therefore, the main objective of this study is to evaluate POMEBS aerobic sludge as microbial seeding for the OPEFB composting process in terms of degradation performance and final compost quality.

## EXPERIMENTAL

### Raw Material Preparation

Pressed, shredded OPEFB was obtained from Seri Ulu Langat Palm Oil Mill (Dengkil, Selangor, Malaysia). The OPEFB was dried in an industrial oven at 60 °C before it was ground with a ring knife flaker (Pallmann, Germany) to an average size of 0.5 to 2.0 mm. A small particle size is important to improve the accessibility of carbon

sources for microorganisms (Bernal *et al.* 2009). POME anaerobic sludge was obtained from FELDA Serting Hilir Palm Oil Mill (Serting, Negeri Sembilan, Malaysia). The POME anaerobic sludge was stored at 4 °C prior to use. OPEFB bio-char (2.0 to 5.0 mm) was obtained from Nasmech Technologies Sdn Bhd and was produced *via* slow pyrolysis (300 to 400 °C) in a batch process at atmospheric pressure (Salleh *et al.* 2010). It was ground into particles (0.25 mm) using a universal cutting mill Pulverisette 19 (Fritsch, Germany). Urea beads N46 (Malaysia) were used as nitrogen sources.

## Experimental Procedure

POMEBS aerobic sludge was prepared by mixing POME anaerobic sludge with ground OPEFB bio-char and urea beads. The mixture was mixed in conical flasks according to the composition generated by Design Expert software. The mixture was aerated with oxygen at a constant flow rate (1.05 mL/s) through a tube. To prevent nitrogen losses to the atmosphere by vaporization, the flask was covered with aluminum foil before it was incubated in a water bath for 24 h. Then, total solids (TS), total suspended solids (TSS), and volatile suspended solids (VSS) were determined according to the standard method (APHA 2005). TS, TSS, and VSS were chosen as dependent variables because they can be correlated with the number of bacteria in the sludge (Otero *et al.* 2002). Composting experiments using OPEFB with POMEBS aerobic sludge and POME anaerobic sludge were run using a rotary drum composter (Jora JK400, SWEDEN). Ten kg of OPEFB were mixed with 30 L of POMEBS aerobic sludge and 30 L of POME anaerobic sludge, separately. The drum was manually rotated every day for 10 days and continued once every two days until the 40-day composting process was complete.

## Sampling and Analysis

The presence of viable bacteria in POMEBS aerobic sludge and POME anaerobic sludge was determined by the plate counting method (Brock *et al.* 2012). Temperature and oxygen concentration in the composter were measured using a portable temperature and oxygen detector manufactured by Demista Instrument USA (Model CM 2006, USA). Compost samples were collected every five days throughout the composting process and stored at -20 °C prior to analysis.

Moisture content was determined using an AND MX90 Moisture Analyzer (MX90, JAPAN), and the pH value was measured using a pH meter (model DELTA 320, Mettler Toledo, USA). Carbon and nitrogen were determined using an elemental analyzer (Thermo Finnigan, Italy). Nutrients and heavy metal elements were analyzed using Inductively Coupled Plasma (ICP)-OES, (Perkin Elmer, USA).

## Central Composite Design (CCD)

To determine the optimum mixture conditions, a series of experiments were carried out with POME anaerobic sludge, OPEFB bio-char, and urea beads as independent process variables. The design was carried out using a $2^3$ factorial with six axial points ($\alpha = 0.5$) and six replicate center points, according to the CCD.

To optimize the effective process parameters, the CCD method chosen as the experimental design was appropriate for fitting a quadratic surface with a minimum number of experiments and helped analyze the interaction between the parameters (Arami-Niya *et al.* 2012).

**Table 1.** Experimental Design Matrix and Response Results

| Run | Independent variables | | | Dependent variables | | |
|---|---|---|---|---|---|---|
| | Factor 1 | Factor 2 | Factor 3 | Response 1 | Response 2 | Response 3 |
| | A:Biochar g/L | B:Urea g/L | C:Temperature °C | TS g/L | TSS g/L | VSS g/L |
| **1** | 10.00 | 30.00 | 62.62 | 91.90 | 70.50 | 57.00 |
| **2** | 2.38 | 30.00 | 55.00 | 72.70 | 61.00 | 45.30 |
| **3** | 10.00 | 30.00 | 55.00 | 134.10 | 89.70 | 69.00 |
| **4** | 15.00 | 50.00 | 50.00 | 77.00 | 59.40 | 43.00 |
| **5** | 17.62 | 30.00 | 55.00 | 126.80 | 83.40 | 57.10 |
| **6** | 5.00 | 10.00 | 60.00 | 86.20 | 70.50 | 68.00 |
| **7** | 10.00 | 0.00 | 55.00 | 139.50 | 105.20 | 86.00 |
| **8** | 10.00 | 30.00 | 55.00 | 111.30 | 83.20 | 54.30 |
| **9** | 10.00 | 30.00 | 55.00 | 113.70 | 84.90 | 55.00 |
| **10** | 10.00 | 30.00 | 47.38 | 99.00 | 80.20 | 57.30 |
| **11** | 5.00 | 50.00 | 50.00 | 119.40 | 86.10 | 49.10 |
| **12** | 10.00 | 30.00 | 55.00 | 107.40 | 83.40 | 54.90 |
| **13** | 15.00 | 10.00 | 60.00 | 92.00 | 71.40 | 51.30 |
| **14** | 10.00 | 60.49 | 55.00 | 93.90 | 68.80 | 53.10 |
| **15** | 5.00 | 10.00 | 50.00 | 80.40 | 76.90 | 58.40 |
| **16** | 10.00 | 30.00 | 55.00 | 113.00 | 85.40 | 55.20 |
| **17** | 5.00 | 50.00 | 60.00 | 155.60 | 82.00 | 65.40 |

Table 1 shows the designed level and range of the variables investigated in this study. The quadratic equation model for predicting the optimal point is expressed by Equation 1.

$$Y_i = x + x_2A - x_3B - x_4C - x_5AB + x_6AC + x_7BC - x_8A^2 + x_9B^2 + x_{10}C^2 \quad (1)$$

where $Y_i$ is the response (dependent variable); $x$ is constant coefficient; $x_2$, $x_3$, and $x_4$ are linear coefficients; $x_5$, $x_6$, and $x_7$ are interactive coefficients; $x_8$, $x_9$, and $x_{10}$ are quadratic coefficients; and A, B, and C are code-independent variables. Design Expert software (Version 7.0, Stat-Ease Inc., Minneapolis, MN, USA) was used for regression and graphical analysis of the data obtained. An analysis of variance (ANOVA) was used to estimate the statistical parameters. RSM was chosen as the method to calculate the optimum value. The variability in dependent variables was explained by the multiple coefficients of determination ($R^2$), while the model equation was used to predict the optimum values (Amouzgar *et al.* 2010).

## Scanning Electron Microscopy

The morphological structure of the materials (bio-char, POME anaerobic sludge, and POMEBS aerobic sludge) was analyzed by scanning electron microscopy (SEM) (S-3400N, Hitachi, Japan). SEM images of all the samples were taken at 1000× and 10000× magnifications.

## Fourier Transform Infrared Analysis

Fourier transform infrared spectroscopy (FTIR) was used to evaluate the changes between chemical bonds in functional groups of POME anaerobic sludge and POMEBS

aerobic sludge. This was carried out using a Perkin Elmer GX2000R infrared spectrophotometer by subjecting the sample to wave numbers within the range of 500 to 4000 $cm^{-1}$ at a resolution of 4 $cm^{-1}$.

## DGGE Analysis of Partial 16S rDNA Genes

Microbe DNA was extracted from approximately 2.0 mL of POMEBS aerobic sludge in optimal conditions. The extraction was replicated twice. The sludge sample was poured 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 beads were consumed and then vigorous vortex mixing was applied for 2 min to disrupt the microbe's cell wall.

The DNA samples were diluted with sterilized ultra-pure water to minimize the inhibition effects of co-extracted contaminants. The 16S rDNA was amplified by using a primer set, consisting of forward primer (341f) with a 40 bp GC clamp (First Base Laboratory, Malaysia), 5'-*CGC CCG CCG CGC 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 cycling for 16S rDNA using 341f and 518r primers was performed with a PCR Thermal Cycler (MasterEP Gradient, Eppendorf, Germany) (Ahmad *et al.* 2011).

The DGGE was performed according to Muyzer and Smalla (1998). The 16S rDNA PCR products were separated in 1.0 mM of 6% (w/w) polyacrylamide with a denaturing gradient of 30 to 70% (100% denaturing gradient correspondence to 7 M urea and 40% (v/v) deionized formamide). The gel was allowed to polymerize for at least 2 h. Five microliters of PCR product was loaded into each individual well. The DGGE was performed in 1× TAE buffer at 60 °C under a constant volume of 200 V for 5 h. After electrophoresis, the DGGE gel was stained using SYBR nucleic acid gel stain for 30 min and then rinsed with distilled water and photographed on a UV transillumination table (Labnet, USA). The DNA bands from the DGGE gel were excised with Pasteur pipettes and placed in 1.5-mL Eppendorf tubes. The band DNA was eluted in 50 µL of TE buffer, and the tubes were incubated overnight at -20 °C to extrude the DNA. Then, the DNA was frozen and thawed three times. Approximately 5 µL of the supernatant was used as the template to re-amplify the DNA. The re-amplified PCR product was further purified using QIAprep spin columns (Qiagen Inc., Valencia, CA).

The PCR products were sent for sequencing. The Gen-Bank database (www.ncbi.nlm.nih.gov) with BLAST (basic local alignment search tool) was used as a reference to identify the nearest relatives of partially sequenced 16S rDNA genes and the excised dominant bands.

## Thermogravimetric Analysis (TGA)

Compost samples from day 1 and day 40 (dried at 60 °C for 24 h, then ground (0.25 mm particle size) using a universal cutting mill Pulverisette 19 (Fritsch, Germany)) were thermogravimetrically analyzed. TGA was carried out with a Mettler TG20 Thermobalance, TA3000 system. The following conditions were used for all TGA analyses: heating rate 10 °C/min from 25 to 500 °C, under a constant nitrogen flow of 10 mL/min, and sample weight of about 10 mg. Measurements were repeated twice.

## RESULTS AND DISCUSSIONS

### Regression Analysis

Optimization of the POMEBS aerobic sludge was achieved with CCD. Data were analyzed using the Design Expert software to yield analysis of variance (ANOVA), regression coefficients, and regression equations. The polynomial equations describing the TS, TSS, and VSS as simultaneous functions of bio-char (A), urea (B), and temperature (C) are presented as Eqs. 2, 3, and 4.

$$Y_1(\text{Total solids}) = 112400 + 6315.35A - 14954.26B + 3650.45C - 15425AB - 3375A + 16425BC - 4084.72A^2 + 8825ABC + 26629.26A^2B \quad (2)$$

$$Y_2(\text{Total suspended solids}) = 83458.96 + 2122.85A - 11937.17B - 639.50C - 3437.5AB + 3462.5AC + 6112.5BC - 5121.24A^2 + 5537.5ABC + 13674.67A^2B \quad (3)$$

$$Y_3(\text{Volatile suspended solids}) = 58121.08 - 973.12A - 10789.37B - 1758.43C - 987.5AB - 3637.5AC + 3587.5BC - 4058.53A^2 + 3835.46B^2 + 6826.87A^2B \quad (4)$$

For TS, the amount of bio-char (A), urea (B), and two-level interactions of AB, BC, $A^2B$, and $AB^2$ were the significant terms reduced from insignificant parameters. For TSS, it was found that the significant terms were the amount of bio-char (A), urea (B), temperature (C), and two-level interactions AB, AC, BC, $A^2$, $C^2$, ABC, $A^2B$, $A^2C$, and $AB^2$. The quadratic model of VSS showed the less significant terms involved, which were the amount of bio-char (A) and two-level interactions $A^2$, $B^2$, and $AB^2$. These regressions were statistically significant at 93.45%, 98.39%, and 84.49% for TS, TSS, and VSS, respectively. The impact of significant terms was defined by $R^2$; an increase in the significance terms led to the models being more accurate. The model can give a predicted value that is near the actual value of the response when the regression coefficient value ($R^2$) is close to 1 (Arami-Niya *et al.* 2012), while a high $R^2$ value shows that the model obtained is able to give a good estimate of the response of the system within the range of study (Kang *et al.* 2012). Therefore, from the statistical results obtained, it can be verified that the models are accurate enough to predict the optimum conditions for producing POMEBS aerobic sludge.

### Model Analysis

The three-dimensional (3-D) response surfaces using Eqs. 2, 3, and 4 are shown in Fig. 1. These 3-D graphs illustrate the relationship between factors and their effects in order to find the optimum point for each response. In order to show the interactive effects of independent variables on responses, two variables were well distributed in certain ranges while another one was kept constant. The 3-D response surfaces in Fig. 1 (a) and (b) show the effects of bio-char and urea on TS and VSS; Figs. 1 (c) and (d) show the effects of temperature and bio-char on TS and VSS; and Figs. 1 (e) and (f) show the effects of temperature and urea toward TS and VSS. In Figs. 1 (a) and (b), the optimum points for TS and VSS are at bio-char contents of 10.0 to 10.6 g/L and urea contents of 10.2 to 10.6 g/L. In Figs. 1 (c) and (d), the optimum points of TS and VSS are at temperature of 50.0 to 50.6 °C and bio-char contents at the range of 11.4 to 12.2 g/L for

TS and 9.8 to 10.6 g/L for TSS. In Figs. 1 (e) and (f), the optimum points are at 50 °C and 10 g/L urea for both TS and VSS.

**Table 2.** Analysis of Variance (ANOVA) for Regression Equation Developed for TS, TSS, and VSS

| Source | Sum of squares | Df | Mean square | F value | p-value prob > F |
|---|---|---|---|---|---|
| **TS** | | | | | |
| **Model** | 9.47E+09 | 13 | 7.29E+08 | 6.58 | 0.0148 |
| **A** | 1.46E+09 | 1 | 1.46E+09 | 13.21 | 0.0109 |
| **B** | 1.04E+09 | 1 | 1.04E+09 | 9.39 | 0.0221 |
| **AB** | 1.90E+09 | 1 | 1.90E+09 | 17.19 | 0.0060 |
| **BC** | 2.16E+09 | 1 | 2.16E+09 | 19.49 | 0.0045 |
| **$A^2B$** | 2.09E+09 | 1 | 2.09E+09 | 18.83 | 0.0049 |
| **$AB^2$** | 9.60E+08 | 1 | 9.60E+08 | 8.67 | 0.0258 |
| **$R^2$** | | | 93.45% | | |
| ***Insignificant terms; C, AC, $A^2$, $C^2$, ABC, $A^2C$** | | | | | |
| **TSS** | | | | | |
| **Model** | 2.18E+09 | 13 | 1.68E+08 | 28.22 | 0.0003 |
| **A** | 2.51E+08 | 1 | 2.51E+08 | 42.12 | 0.0006 |
| **B** | 6.62E+08 | 1 | 6.62E+08 | 111.23 | 0.0001 |
| **C** | 0.47E+08 | 1 | 0.47E+08 | 7.90 | 0.0307 |
| **AB** | 0.94E+08 | 1 | 0.94E+08 | 15.87 | 0.0073 |
| **AC** | 0.96E+08 | 1 | 0.96E+08 | 16.10 | 0.0070 |
| **BC** | 2.99E+08 | 1 | 2.99E+08 | 50.19 | 0.0004 |
| **$A^2$** | 2.83E+08 | 1 | 2.83E+08 | 47.59 | 0.0005 |
| **$C^2$** | 1.53E+08 | 1 | 1.53E+08 | 25.74 | 0.0023 |
| **ABC** | 2.45E+08 | 1 | 2.45E+08 | 41.19 | 0.0007 |
| **$A^2B$** | 5.50E+08 | 1 | 5.50E+08 | 92.32 | <0.0001 |
| **$A^2C$** | 0.47E+08 | 1 | 0.47E+08 | 7.97 | 0.0302 |
| **$AB^2$** | 2.01E+08 | 1 | 2.01E+08 | 33.67 | 0.0011 |
| **$R^2$** | | | 98.39% | | |
| **VSS** | | | | | |
| **Model** | 1.47E+09 | 11 | 1.33E+08 | 3.96 | 0.0305 |
| **B** | 5.41E+08 | 1 | 5.41E+08 | 16.06 | 0.0039 |
| **$A^2$** | 1.78E+08 | 1 | 1.78E+08 | 5.28 | 0.0506 |
| **$B^2$** | 1.59E+08 | 1 | 1.59E+08 | 4.72 | 0.0616 |
| **$AB^2$** | 1.72E+08 | 1 | 1.72E+08 | 5.12 | 0.0535 |
| **$R^2$** | | | 84.49% | | |
| ***Insignificant terms; A, B, C, AB, AC, BC, $C^2$, ABC, $A^2B$, $A^2C$** | | | | | |

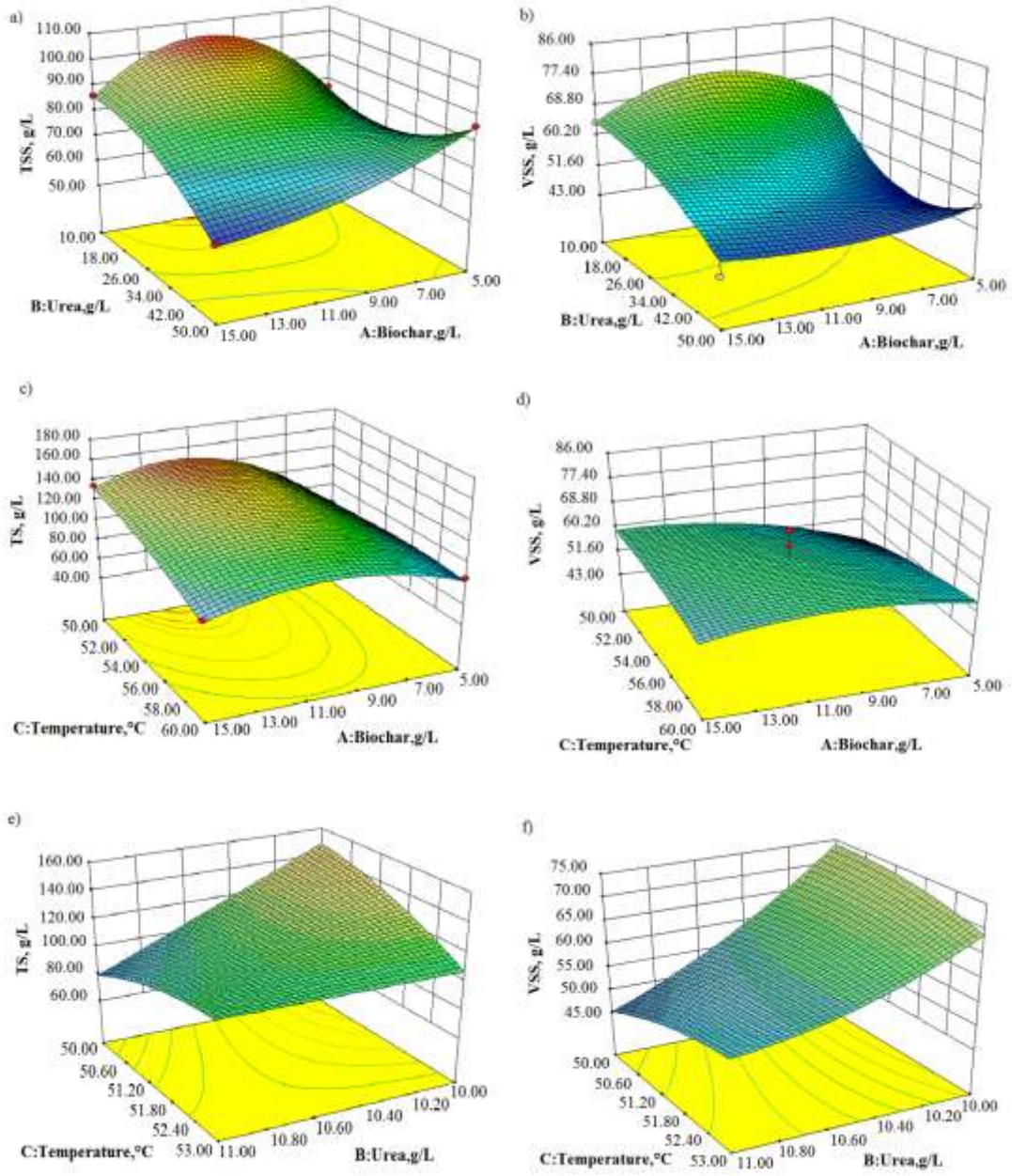


**Fig. 1.** Response surface 3-D plot indicating the effect of interaction between urea and bio-char on a) TS and b) VSS; temperature and bio-char on c) TS and d) VSS; and temperature and urea on e) TS and f) VSS

By comparing the F-values obtained from the studied factors (Table 2), it was concluded that the two-level interaction of urea and temperature (BC) had the greatest effect on total solids (F-value of 19.49), followed by bio-char and urea ($A^2B$ and AB) with F-values of 18.83 and 17.19, respectively. For total suspended solids, the highest F-value was urea (B) with 111.23, followed by the interaction of bio-char and urea ($A^2B$) with 92.32. Urea (B) was found to have the greatest effect on volatile suspended solids. Bio-char, urea, and temperature influenced the TS, TSS, and VSS and hence can be correlated with microbial growth in POMEBS aerobic sludge. Bio-char can act as a host for microbes; however, microbes can only utilize a certain amount of the carbon due to

the stable structure of bio-char. Bio-char has a very stable aromatic carbon structure that might not serve as an easily accessible carbon source for microbes (Kuzyakov *et al.* 2009). However, bio-char's macro-pores can serve as a habitat for microbes and also has the ability to retain nutrient (Fischer and Glaser 2012; Clough and Condron 2010). Microbes that colonize within the bio-char's pores utilize these nutrients for growth (Henriksen and Breland 1999). For thermophilic bacteria, the temperature for optimum growth was reported in the range of 50-60 °C (Baharuddin *et al.* 2010).

## Optimization and Validation Experiments

The experimental results and those predicted by the regression models are presented in Table 3. The results show that the predicted data calculated from the models and the experimental data are well fitted. The deviations between experimental and predicted values were 0.44%, 1.32%, and 0.61% for TS, TSS, and VSS, respectively. These numbers show that the statistical analysis is trustworthy to find the optimum conditions for POMEBS aerobic sludge.

**Table 3.** Verified Results of Model Equation

| Bio-char (g/L) | Urea (g/L) | Temperature (°C) | | TS (g/L) | TSS (g/L) | VSS (g/L) |
|---|---|---|---|---|---|---|
| 10.65 | 10.00 | 50.00 | Experimental | 147.99 | 105.21 | 73.40 |
| | | | Predicted | 148.64 | 103.82 | 73.85 |

## Scanning Electron Microscopy

Figure 2 shows the SEM pictures of POME anaerobic sludge and POMEBS aerobic sludge at 1000× and 10000× magnifications.

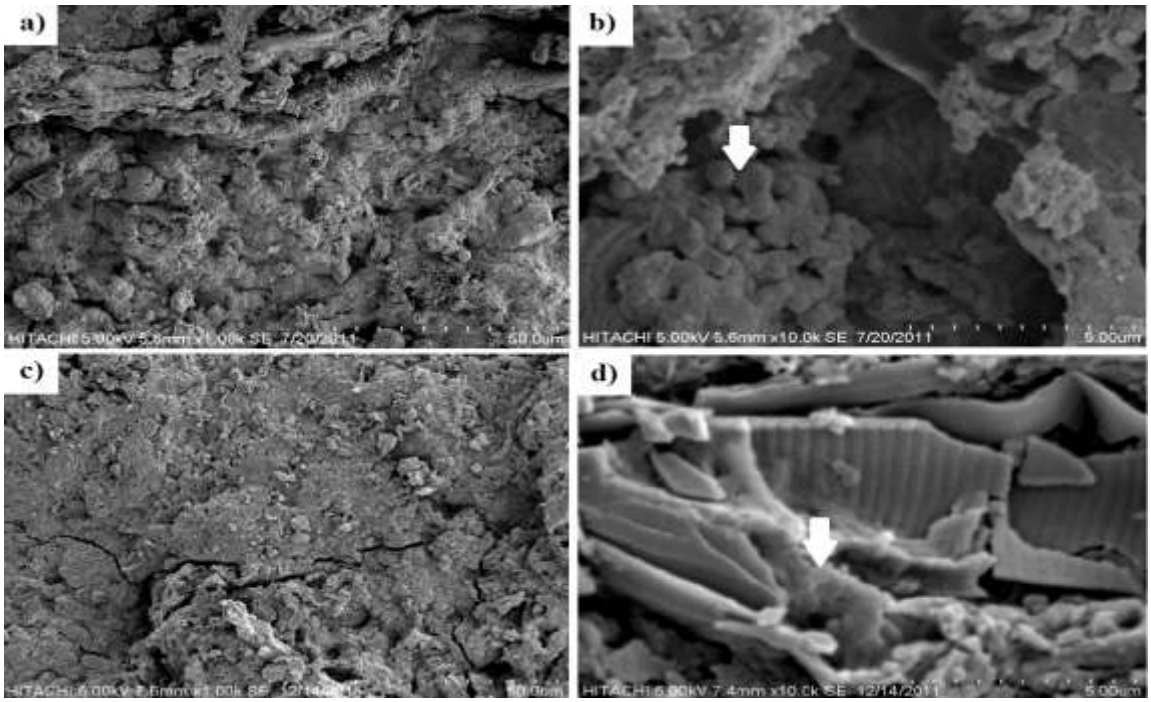


**Fig. 2.** SEM of (a) POME anaerobic sludge at 1000× magnification, (b) POME anaerobic sludge at 10000× magnification, (c) POMEBS aerobic sludge at 1000× magnification, and (d) POMEBS aerobic sludge at 10000× magnification

Figures 2 (a) and (b) show the structure of POME anaerobic sludge and coccus-like groups of bacteria (indicated by arrow) lying on the surface of POME anaerobic sludge. In Figs. 2 (c) and (d), the structure and coccus-like bacteria (indicated by arrow) were detected beneath the bio-char layer and fully covered by POME aerobic sludge. The presence of bacteria was also proven by the plate counting method. The bacteria count of POMEBS aerobic sludge ($3.7\times10^6$ CFU/mL) with the optimum conditions (10.65 g/L bio-char; 10 g/L urea; 50 °C temperature) was higher than POME anaerobic sludge ($2.5\times10^5$ CFU/mL).

## Denaturing Gradient Gel Electrophoresis (DGGE)

The DGGE profiles of POMEBS aerobic sludge in optimum conditions are shown in Fig. 3. The staining intensity of a band represents the relative abundance of that microbial species, which is shown in Table 4. The microorganism communities in POMEBS aerobic sludge were *Bacillus subtilis* strain TU2, *Bacillus* sp. MH-16, uncultured bacterium clone, uncultured *Firmicutes* bacterium, *Bacillus* sp. HS-V2, *Bacterium* FA_149, and *Bacillus subtilis* strain TBR2. Four of them were related to *Bacillus* species, which represents the most dominant species of bacteria in POMEBS aerobic sludge. All bands represent bacteria that are dominant in the thermophilic stage of composting in POMEBS aerobic sludge.

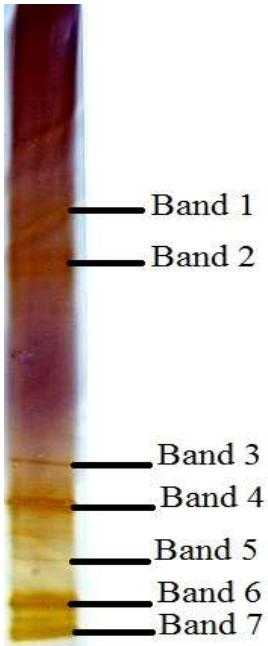


**Fig. 3.** DGGE band

*Bacillus subtilis* is a bacterium of the thermophilic phase and, because of its small size (0.5 to 3.0 µm), it allows rapid transfer of soluble substrates into the cell (Tuomela *et al.* 2000). In addition, *Bacillus* is a typical microorganism in the thermophilic stage of the composting process (Peters *et al.* 2000). In a previous study, uncultured bacterium and *Firmicutes* were also present during composting of OPEFB with partially treated palm oil mill effluent (POME) (Baharuddin *et al.* 2009); *Bacillus* and uncultured bacterium were also reported during the composting of oil palm fronds with POME anaerobic sludge (Ahmad *et al.* 2011). Therefore, the microbes that are present in POMEBS and the

previous composting process by Baharuddin *et al.* 2009 and Ahmad *et al.* 2011 are same, demonstrating that these microbes are beneficial for degradation.

**Table 4.** Phylogenetic of Affiliation of Excised DGGE Band for POMEBS Aerobic Sludge in Optimum Conditions

| Band No. | Closest relatives | Identity (%) | Accession no. |
|---|---|---|---|
| 1 | *Bacillus subtilis* strain TU2 16S ribosomal RNA gene, partial sequence | 91 | JX624786.1 |
| 2 | *Bacillus* sp. MH-16 16S ribosomal RNA gene, partial sequence | 91 | JQ068110.1 |
| 3 | Uncultured bacterium clone ncd1790a08c1 16S ribosomal RNA gene, partial sequence | 94 | JF155049.1 |
| 4 | Uncultured *Firmicutes* bacterium partial 16S rRNA gene, clone EJIR08_16 | 85 | HE573199.1 |
| 5 | *Bacillus* sp. HS-V2 16S ribosomal RNA gene, partial sequence | 99 | DQ988160.1 |
| 6 | Bacterium FA_149 16S ribosomal RNA gene, partial sequence | 75 | JQ765451.1 |
| 7 | *Bacillus subtilis* strainTBR2 16S ribosomal RNA gene, partial sequence | 74 | JF918976.1 |

## Fourier Transform Infrared Spectroscopy (FTIR)

Figure 4 shows infrared spectra comparison between POME anaerobic sludge and POMEBS aerobic sludge, which can be used to verify the presence of and changes in the organic groups.

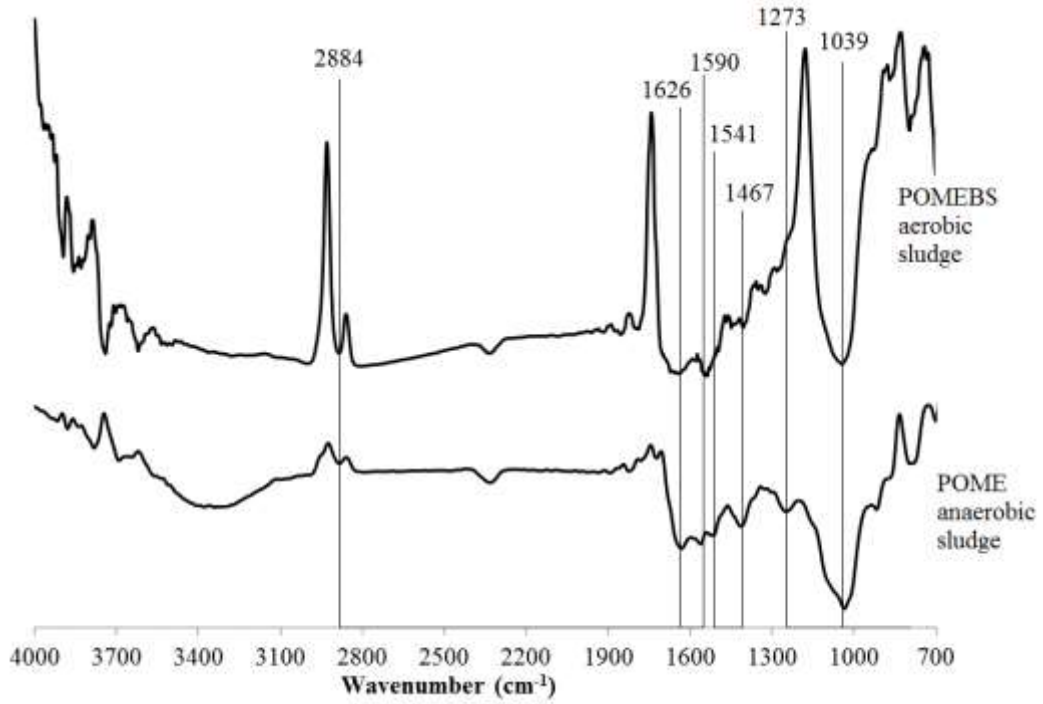


**Fig. 4.** FTIR analysis of POME anaerobic sludge and POMEBS aerobic sludge

The absorbance band at 2884 $cm^{-1}$ refers to hydrogen vibrations of the aliphatic methylene group. The rise in intensity is believed to be due to the addition of bio-char to the POMEBS aerobic sludge. Bio-char is an organic biomass with an aromatic structure that has a higher degree of stability compared to the original biomass (Kuzyakov *et al.* 2009).

The absorbance bands at 1626 and 1467 $cm^{-1}$ refer to the C=C group of alkene and $CH_2$ group of alkanes, respectively (Inyang *et al.* 2010). The increasing absorption band intensity of POMEBS aerobic sludge at 1590 $cm^{-1}$ may refer to the N-H vibration in the amine plane. Hence, the reduction in carbon chain indicates the presence of microbes due to a reduction of carbon and an increase of protein in the form of amines.

The increase in nitrogen content is also due to bio-char's ability to retain nitrogen within its pores (Clough and Condron 2010). Microbes that colonize within bio-char's pores utilize this nitrogen to live and grow (Henriksen and Breland 1999). The absorption band at 1273 $cm^{-1}$ was referred to as the $CO_2$ stretch of carboxylic acids groups. Since the available carbon was in an aromatic, stable form, less $CO_2$ was produced by microbes. The absorption band at 1039 $cm^{-1}$ was attributed to silica or clay mineral ingredients, such as Si-O-Si and SiO-H, which came from lignin that was available in POME anaerobic sludge (Wan Razali *et al.* 2012; Tuomela *et al.* 2000).

## Composting Profiles

Figure 5 shows the comparison between composting OPEFB with POMEBS aerobic sludge and POME anaerobic sludge regarding temperature, oxygen, and pH profiles. In the initial stage of the composting process, both temperature profiles increased rapidly to 70 to 71 °C and were maintained until day 3. The composting temperature of OPEFB with POMEBS aerobic sludge and POME anaerobic sludge dropped to 64 °C and 59 °C on day 4, respectively. An average temperature above 60 °C should be maintained for at least 4 days to kill pathogen microorganisms and to obtain a hygienized compost (Mohee *et al.* 2008). The temperature for OPEFB with POMEBS aerobic sludge then remained between 50 and 60 °C until day 22. After that, the temperature dropped to between 40 and 50 °C until day 33. At day 40, the temperature dropped to the environmental temperature (36 °C). On the other hand, the temperature of POME anaerobic sludge dropped to between 50 and 60 °C up to day 10. After that, the temperature decreased to between 40 and 50 °C until day 30. The higher temperature profile of OPEFB with POMEBS aerobic sludge indicates greater biological activity during the composting process. The temperature of compost is an easily measured indicator of biological activity because it changes in direct response to heat production (Som *et al.* 2009), and heat production during composting is almost completely caused by biological activity (Kutzner 2001).

The oxygen content for both composting processes reduced slightly, to 12%, on day 1. This is because of the rapid expansion of microbial populations due to the active consumption of readily degradable materials (Hock *et al.* 2009). After that, the oxygen level increased and remained between 15 to 19% until day 29 for both pH profiles. After day 30, the oxygen level was at 20% for both pH profiles.

The pH profiles for both composting processes showed an upsurge in the initial stage. This is due to the rapid metabolic degradation of organic acid. The moisture content was maintained around 65 to 70% throughout the composting process, due to the

design of the rotary drum, which was fully closed and insulated so that the water that vaporized returned to the compost materials.

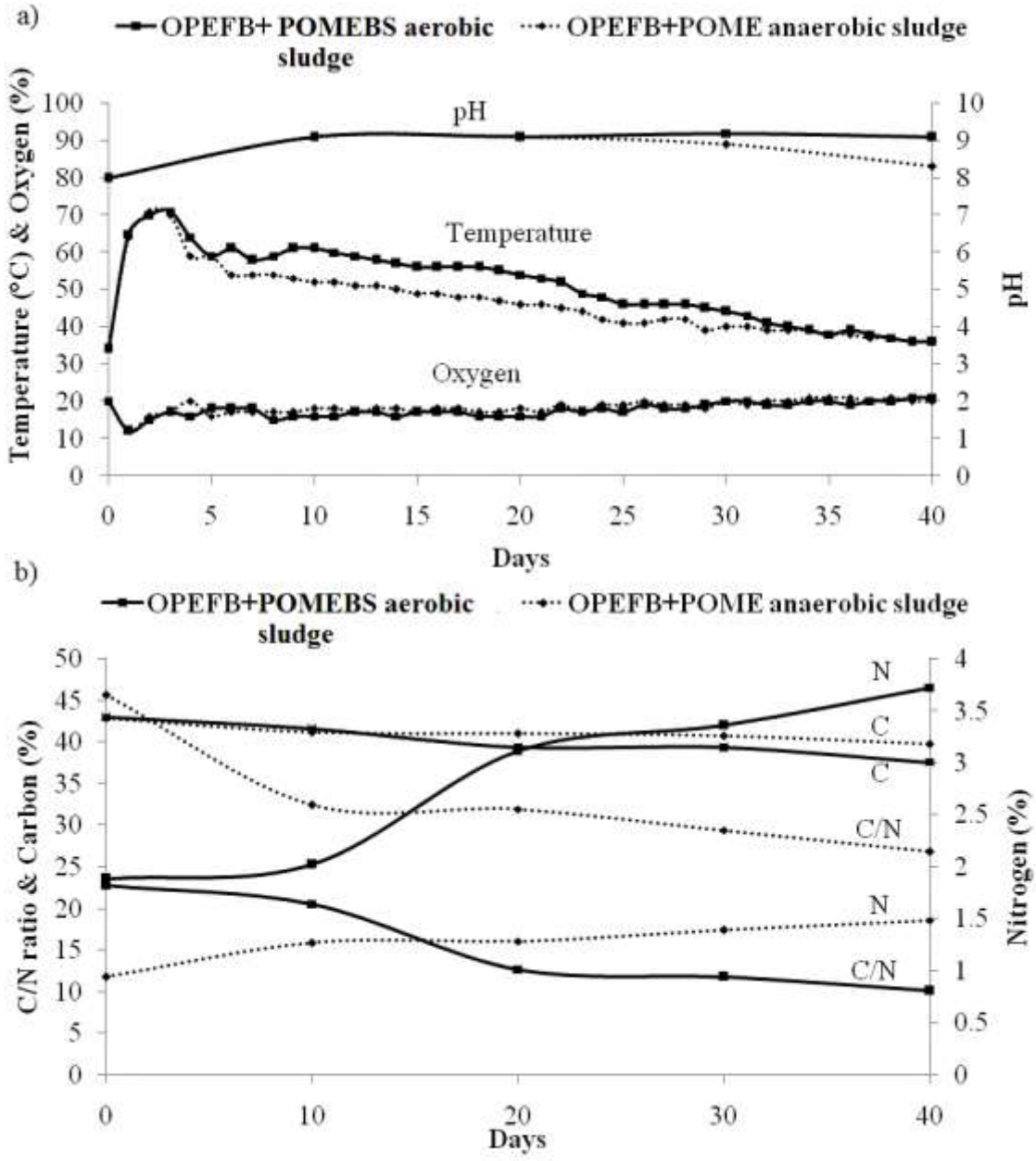


**Fig. 5.** Profile comparison between composting OPEFB with POMEBS aerobic sludge and OPEFB with POME anaerobic sludge for a) temperature, oxygen, and pH; b) carbon, nitrogen, and C/N ratio

## Thermogravimetric Analysis (TGA)

The TG and DTG thermograms in Fig. 6 show that the decomposition process occurred in two steps. The first step was the dehydration reaction in the temperature range of 50 to 120 °C, and the second step was the decomposition of hemicelluloses and cellulose in the temperature range of 220 to 400 °C (Table 5).

**Table 5.** Weight Losses (Percentage of Total Sample Weight) Corresponding to the Main Peaks Shown in the Thermograms in Fig. 8

| Samples | 220 to 315 °C | 315 to 400 °C | 200 to 500 °C |
|---|---|---|---|
| **EFB+POMEBS aerobic sludge** | | | |
| Day 1 | 17 | 30 | 52 |
| Day 40 | 11 | 15 | 39 |
| **EFB+POME anaerobic sludge** | | | |
| Day 1 | 16 | 34 | 55 |
| Day 40 | 17 | 23 | 50 |

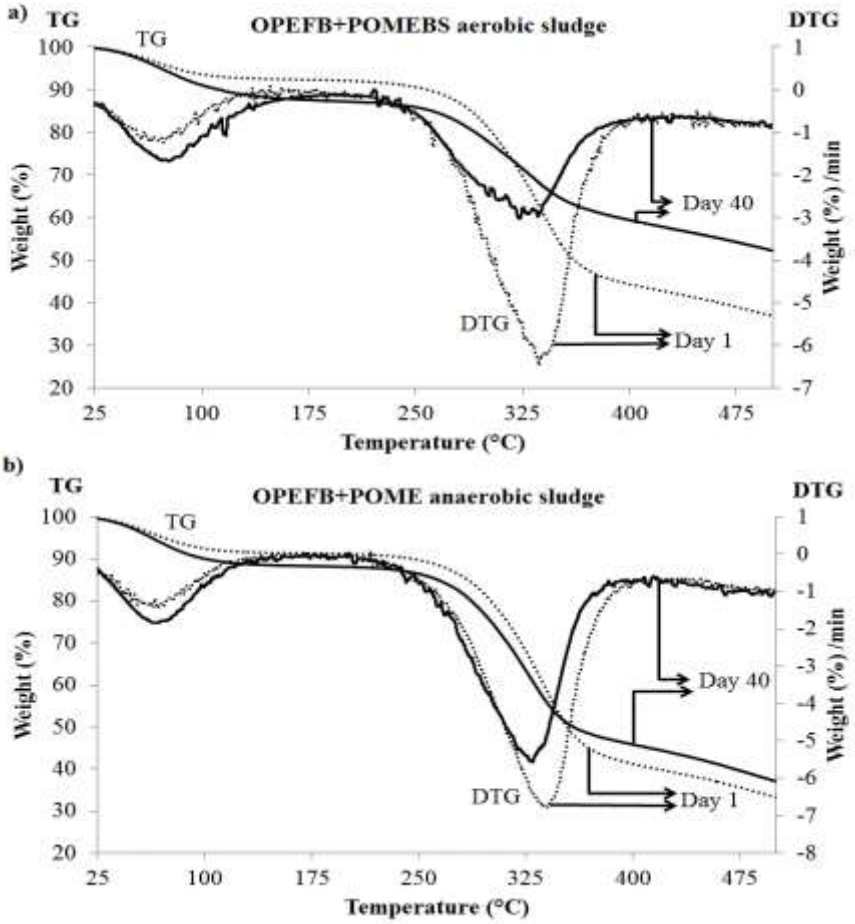


**Fig. 6.** TG and DTG data comparison of composting a) OPEFB with POMEBS aerobic sludge and b) OPEFB with POME anaerobic sludge

Figures 6 (a) and (b) show the comparison between composting OPEFB with POMEBS aerobic sludge and POME anaerobic sludge until day 40 of composting process. The day 1 sample of OPEFB composting with POMEBS aerobic sludge showed a 47% weight loss, which decreased remarkably to 26% on day 40. Composting of OPEFB with POME anaerobic sludge showed 50% weight loss for the sample on day 1, and the weight loss decreased to 40% by day 40. Despite using a small-scale system, composting OPEFB using POMEBS aerobic sludge achieved stabilized compost in 40 days, while composting OPEFB using POME anaerobic sludge required more time to achieve stability.

**Table 6.** Characteristics of OPEFB Bio-Char, POME Anaerobic Sludge, and POMEBS Aerobic Sludge at Optimum Condition (50 °C, 10.65 g/L Bio-Char, 10 g/L Urea), Pressed, Shredded OPEFB, Compost OPEFB + POMEBS Aerobic Sludge Day 40, Compost OPEFB + POME Anaerobic Sludge Day 40

| Parameter | OPEFB Bio-char | POME anaerobic sludge | POMEBS aerobic sludge | OPEFB | OPEFB+POMEBS aerobic sludge day 40 | OPEFB+POME anaerobic sludge day 40 |
|---|---|---|---|---|---|---|
| Carbon (%) | 57.4 | 31.3 | 37.3 | 44.1 | 37.6 | 39.7 |
| Nitrogen (%) | 1.9 | 4.5 | 3.1 | 0.6 | 3.7 | 1.5 |
| Phosphorus (%) | 0.4 | 1.5 | 2.2 | 0.1 | 0.8 | 0.3 |
| Potassium (%) | 4.3 | 2.6 | 3.4 | 1.4 | 6.2 | 3.4 |
| Magnesium (%) | 0.4 | 1.2 | 1.5 | 0.1 | 0.9 | 0.4 |
| Calcium (%) | 0.6 | 2.8 | 3.5 | 0.2 | 1.8 | 0.7 |
| C/N ratio | 30.2 | 7.0 | 12.0 | 73.5 | 10.1 | 26.8 |
| Zinc (mg/kg) | 59.5 | 105.1 | 159.3 | 22.4 | 115.5 | 47.3 |
| Manganese (mg/kg) | 65.9 | 299.3 | 326.2 | 26.4 | 283.5 | 96.8 |
| Ferrum (mg/mg) | 2160.1 | 9728.1 | 11480.0 | 41.2 | 6542.0 | 1968.0 |

### Comparison of Characteristics

Table 6 shows the characteristics of OPEFB bio-char, POME anaerobic sludge, and POMEBS aerobic sludge in optimum conditions (50 °C, 10.65 g/L bio-char, 10 g/L urea), pressed, shredded OPEFB, compost using POMEBS aerobic sludge, and POME anaerobic sludge at day 40.

The total nitrogen content decreased in POMEBS aerobic sludge in optimum conditions compared to POME anaerobic sludge. This result is due to the utilization of nitrogen by microbes to grow and proliferate (Henriksen and Breland 1999). Total carbon and phosphorus increased as a result of bio-char addition. The addition of bio-char in POMEBS aerobic sludge can also increase nutrient retention, hence improving soil fertility (Glaser *et al.* 2002). The N, P, and K values of the compost using POME anaerobic sludge were measured at 1.5, 0.3, and 3.4, while the N, P, and K values of the compost using POMEBS aerobic sludge stabilized at 3.7, 0.8, and 6.2, respectively.

## CONCLUSIONS

1. OPEFB compost with POMEBS aerobic sludge was matured and stabilized within 40 days with the highest N: P: K values (3.7, 0.8, and 6.2). POMEBS aerobic sludge also was able to speed up the degradation process of OPEFB, with nearly 50% weight reduction of organic matter after 40 days.
2. This indicates that POMEBS is able to achieve compost maturity and stability when easily accessible organic material, such as cellulose and hemicellulose from OPEFB material, is greatly consumed by microbes.

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