## Biochemical changes during oil palm (*Elaeis guineensis*) empty fruit bunches composting with decanter sludge and chicken manure

Wathida Kananam<sup>a</sup>, Thunwadee Tachapattaworakul Suksaroj<sup>a,b,\*</sup>, Chaisri Suksaroj<sup>b,c</sup>

<sup>a</sup> Faculty of Environmental Management, Prince of Songkla University, Hat Yai, Songkhla 90112 Thailand

<sup>c</sup> Environmental Engineering Program, Department of Civil Engineering, Faculty of Engineering, Prince of Songkla University, Hat Yai, Songkhla 90112 Thailand

\*Corresponding author, e-mail: thunwadee.t@psu.ac.th

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**ABSTRACT**: The aim of this study is to investigate the biochemical changes during oil palm (*Elaeis guineensis*) emptyfruit-bunches (EFB) composting when using decanter sludge or chicken manure as a nitrogen source. We found that the use of decanter sludge did not have an effect on any biochemical conditions of either aerobic or anaerobic EFB composting. The oil palm EFB compost with decanter sludge in an aerobic condition completed within 30 days whereas compost in the anaerobic condition failed to complete composting within 90 days. Adding red soil to the compost pile did not affect the microbial activity or enhance the composting time, but it reduced the odour generated from the pile. Bacteria played an important role in decomposing with a high organic degradation rate occurring within the first two weeks of composting. The composting product obtained met the compost nutrient standard.

KEYWORDS: palm oil, waste management, nitrogen source

## INTRODUCTION

One of the main solid waste products generated from a crude palm oil mill is oil palm (Elaeis guineensis) empty-fruit-bunches (EFB). For every 100 kg of oil palm fresh fruit bunches which are processed for oil production, 26–59 kg of oil palm EFB are wasted<sup>1</sup>. The dumping of EFB on land results in pollution of the surrounding area because these EFBs still contain oil which can be distributed through the local environment. Composting is a favoured alternative to manage this solid waste in Thailand. However the problem often encountered with oil palm EFB composting is its low degradation rate because oil palm EFB consists of hard biodegradable substances such as hemicelluloses and lignin. To increase the efficiency of oil palm EFB composting, co-composting substrates (usually microorganism activators and nitrogen sources) are added to the pile. The EFB mixed with nitrogen composts in less time than EFB without added nitrogen<sup>2</sup>. Animal manures, especially chicken manure, are often used as a nitrogen source for composting<sup>3</sup>. However, lower cost alternatives to chicken manure are gaining importance. In particular, decanter sludge is an attractive alternative nitrogen source due to its

high nitrogen content (about 2% by dry weight) and the fact that it is also a by-product of the palm oil production process and is in need of an appropriate management method as well.

In addition to nitrogen, other substrates such as microorganism seeds and soil can be added to the compost to enhance composting efficiency, decrease composting time, and increase the degradation rate. For municipal waste composting and it was found that the composting efficiency was the same when using a red soil instead of microorganism seed. Also, the Fe in red soil could be an electron accepter for microorganisms in anaerobic composting conditions<sup>4</sup>. The effects of decanter sludge on biochemical conditions, especially microbial activities during EFB composting, have not yet been reported. Hence the aim here is to study the biochemical effect of different nitrogen sources (chicken manure or decanter sludge from a palm oil mill) and red soil on oil palm EFB during composting.

 $<sup>^{\</sup>rm b}\,$  National Centre of Excellence for Environmental and Hazardous Waste Management (EHWM),

Satellite Centre at Prince of Songkla University, Hat Yai, Songkhla 90112 Thailand

## MATERIALS AND METHODS

## **Composting material**

Fresh oil palm EFB were cut to sizes of 2-5 cm using a grinder machine. Decanter sludge was collected from a palm oil factory. It was 3 days old when it was used for the experiments. Red soil was collected from Satun Province. The microorganism seeds used for the aerobic and anaerobic piles were the microbial activators LDD1 called "super LDD.1" (Land Development Department No. 1) and the microbial activator LDD2 called "super LDD.2". They were donated by the Land Development Department. LDD1 contained aerobic cellulose decomposer fungi (Corynascus sp., Scytalidium sp., Chaetomium sp. and Scopulariopsis sp.), and the bacteria (Bacillus sp.) and actinomycete (Streptomyces sp.)<sup>5</sup>. LDD2 contained facultative alcoholic yeast (Saccharomyces sp.), lactic acid bacteria (Lactobacillus sp.), and protein catabolism bacteria  $(Bacillus \text{ sp.})^6$ . The physicochemical properties of these materials are presented in Table 1.

## **Composting conditions**

The proportion of material mixed in each pile was set to obtain the initial carbon to nitrogen (C:N) ratio of 35–40:1. The mass of oil palm EFB, chicken manure, and decanter sludge used was 4.78, 3.89, and 4.62 kg of dry weight, respectively. In the piles mixed with red soil, the mass of red soil used was 3.17 kg for chicken manure and 5.16 kg for decanter sludge. Each condition was done in duplicate. The composting was performed in 20 gallon cylindrical tubs. The tub walls were perforated to permit oxygen flow only for the aerobic conditions. Super LDD.1 was added to the anaerobic piles and super LDD.2 was added to the anaerobic piles (100 g of LDD.1: 20 l of water: 1 ton of composting material, as recommended on product).

For the aerobic conditions, the piles were wetted with water and maintained at a moisture content of 50-70%. Due to the rapid increase of temperature within the aerobic piles during the first week of composting, the piles were turned every 3 days. After the first week, the piles were turned at weekly intervals. The anaerobic piles were wetted with excess water to keep the moisture over 80% and to set the pile without turning. All piles were left for 90 days. The temperature, electrical conductivity (EC) and pH of each pile were measured daily. Samples were collected from each pile every 3 days in the first week and then every 15 days to measure their organic carbon (OC), organic matter (OM), total nitrogen (total N), microbial community, and cellulase (CMCase) activities. The OC and OM were analysed according to method in Ref. 7. The total nitrogen of the samples was analysed using the Kjeldahl method. The microbial community in each pile was determined by the plate count technique. The method for cellulase activity measurement was that given in Ref. 8. The control experiment without seed inoculation was also conducted for both aerobic and anaerobic conditions. They were measured initially and at the end of composting (90 days). The odours of the piles were evaluated by sensation. Colour changes were also observed.

## Sampling method and sample analysis

For each pile, samples (100 g each) were collected at four different depths, mixed and then separated into quarters. The samples were analysed using the parameters as described in Table 2.

### **Statistical analysis**

The difference of the piles was analysed by SPSS using multivariate ANOVA (MANOVA).

## **RESULTS AND DISCUSSION**

# Physical characteristics of aerobic and anaerobic piles

During the first week of composting, all aerobic piles emitted foul odours, although the odours from the piles containing red soil were not as strong. This might be due to the fact that introduction of oxygen only by turning the pile was not sufficient during the composting initiation. Piles to which red soil was added contained Fe, which may be an alternative electron acceptor for oxygen<sup>4</sup>. As a result, malodorous gases such as ammonia were generated in lower quantities. Following the initial period, the odour gradually decreased until the 45th day, at which time it had a more earthy smell. At the end of composting (90th day), all piles had lost their odour and the composting materials were completely converted to humus.

For the anaerobic piles, the foul odour persisted for the full 90 day composting period. These odours include a wide range of compounds which can be formed anaerobically. The most notorious of these are hydrogen sulphide, dimethyl sulphide, volatile fatty acids, amines, and methyl mercaptan<sup>9</sup>. Although the anaerobic composting was performed in a closed system, they were not sealed and so fugitive emission could occur.

### Change of temperature in the piles

The temperature of the pile is an important parameter used to indicate the microorganisms' activities. The

Parameters	EFB	chicken manure	decanter cake	red soil
MC (%)	$20.3 \pm 1.3$	$9.35\pm0.12$	$57.3\pm2.0$	$7.47\pm0.29$
pН	$8.19\pm0.06$	$8.00\pm0.09$	$7.61\pm0.17$	$7.55\pm0.08$
EC (dS/m)	$2.93\pm0.11$	$9.65\pm0.08$	$2.78\pm0.18$	$0.11\pm0.01$
OC (%) <sup>a</sup>	$52.36 \pm 0.26$	$14.7\pm0.8$	$45.0 \pm 0.5$	$0.84\pm0.07$
OM (%) <sup>a</sup>	$90.3 \pm 2.0$	$25.4 \pm 1.3$	$77.6\pm0.8$	$1.45\pm0.12$
N (%) <sup>a</sup>	$0.56\pm0.06$	$1.51 \pm 0.04$	$2.18\pm0.05$	$0.12\pm0.02$
C/N ratio <sup>a</sup>	$94\pm9$	$9.74\pm0.26$	$20.7\pm0.6$	$7.1 \pm 1.1$
$P_2O_5^a$	$0.95\pm0.10$	$2.90\pm0.15$	$1.40\pm0.07$	$1.07\pm0.04$
$K_2O(\%)^a$	$1.73\pm0.04$	$2.46\pm0.09$	$2.55\pm0.14$	$0.75 \ \pm 0.09$

**Table 1** The physicochemical properties of raw materials (mean  $\pm$  SD).

<sup>a</sup> In this and subsequent tables these quantities are total dry basis; MC: moisture content; OM: organic matter

Table 2 Physicochemical properties of compost under anaerobic conditions (mean  $\pm$  SD).

Parameters	Control		An 1		An 2		An 3		An 4	
	0 day	90 day	0 day	90 day	0 day	90 day	0 day	90 day	0 day	90 day
pH EC (dS/m) OC (%) OM (%) N (%) C:N ratio P <sub>2</sub> O <sub>5</sub> (%) K <sub>2</sub> O (%)	$\begin{array}{c} 8.07 \pm 0.04 \\ 2.72 \pm 0.06 \\ 55.4 \pm 1.4 \\ 95.5 \pm 2.4 \\ 0.56 \pm 0.02 \\ 98.4 \pm 1.4 \\ \text{NM} \\ \text{NM} \end{array}$	$\begin{array}{c} 8.15 \pm 0.07 \\ 2.35 \pm 0.04 \\ 49.1 \pm 1.2 \\ 85.0 \pm 2.1 \\ 0.79 \pm 0.02 \\ 62.2 \pm 0.9 \\ \text{NM} \\ \text{NM} \end{array}$	$\begin{array}{c} 7.49 \pm 0.12 \\ 1.16 \pm 0.18 \\ 42.3 \pm 2.1 \\ 72.9 \pm 3.5 \\ 1.09 \pm 0.05 \\ 38.9 \pm 0.8 \\ 1.88 \pm 0.29 \\ 1.62 \pm 0.14 \end{array}$	$\begin{array}{c} 7.51\pm 0.15\\ 1.30\pm 0.16\\ 38.2\pm 1.5\\ 65.8\pm 2.5\\ 1.50\pm 0.11\\ 25.6\pm 1.6\\ 2.07\pm 0.17\\ 1.85\pm 0.08 \end{array}$	$\begin{array}{c} 7.44 \pm 0.08 \\ 0.90 \pm 0.05 \\ 33.0 \pm 1.8 \\ 56.9 \pm 3.1 \\ 0.83 \pm 0.02 \\ 40.0 \pm 2.9 \\ 1.60 \pm 0.32 \\ 1.69 \pm 0.13 \end{array}$	$\begin{array}{c} 8.12 \pm 0.05 \\ 1.04 \pm 0.12 \\ 29.0 \pm 1.2 \\ 50.0 \pm 2.1 \\ 1.07 \pm 0.15 \\ 28 \pm 4 \\ 1.69 \pm 0.08 \\ 1.61 \pm 0.09 \end{array}$	$\begin{array}{c} 7.23 \pm 0.14 \\ 0.94 \pm 0.10 \\ 57.59 \pm 0.15 \\ 99.30 \pm 0.27 \\ 1.65 \pm 0.06 \\ 35.0 \pm 1.3 \\ 1.01 \pm 0.09 \\ 2.25 \pm 0.15 \end{array}$	$\begin{array}{c} 7.77 \pm 0.11 \\ 1.24 \pm 0.14 \\ 52.1 \pm 0.8 \\ 89.8 \pm 1.4 \\ 2.59 \pm 0.22 \\ 20.2 \pm 1.4 \\ 1.23 \pm 0.18 \\ 2.75 \pm 0.23 \end{array}$	$\begin{array}{c} 7.35 \pm 0.09 \\ 0.68 \pm 0.04 \\ 38.3 \pm 1.2 \\ 66.0 \pm 2.0 \\ 0.99 \pm 0.02 \\ 38.5 \pm 0.7 \\ 1.28 \pm 0.03 \\ 1.39 \pm 0.12 \end{array}$	$\begin{array}{c} 7.87 \pm 0.10 \\ 1.05 \pm 0.06 \\ 34.9 \pm 1.9 \\ 60.2 \pm 3.2 \\ 1.58 \pm 0.27 \\ 22.4 \pm 2.9 \\ 1.36 \pm 0.04 \\ 1.51 \pm 0.07 \end{array}$

NM: no measurement

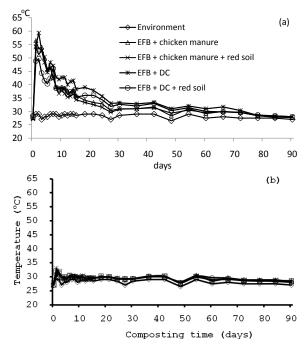
initial temperature of all piles was approximately 28 °C. The temperature of all piles rapidly increased to maximum after 2 days. This was 49-59 °C for aerobic piles and 31-34 °C for anaerobic piles. For the aerobic condition, the highest temperature was observed in a compost pile consisting of oil palm EBF mixed with decanter sludge (Ae 3) (59 °C on the second day) which was slightly higher than that which had chicken manure (Ae 1) (57 °C 24 h after initiation). The highest temperature observed in the aerobic piles was within the range of temperatures that could destroy pathogenic organisms and undesirable weed seeds  $(45-65 \circ C)^{10}$ . After that, the temperature in the aerobic piles decreased continuously and the temperatures were lower than 30 °C within 1 month. This phenomenon could be explained by the fact that the aerobic piles were more frequently turned in the initial phase and so there was sufficient oxygen for aerobic biological reactions which are highly exothermic. After that, the turning time interval was changed to one week to prevent the nitrogen loss from ammonia vaporization during prolonged high temperatures. This could reduce the biological reaction rate due to the lower oxygen introduced and cause the decrease in temperature. Also, it is possible to bring about a temperature drop by decreasing the reaction rate due to the limit of substrate in the pile and watering.

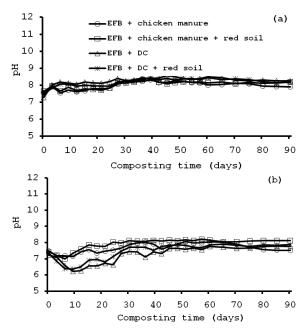
Temperatures in anaerobic piles increased slightly

from 27–27.2 °C in the first few days of composting to 31–33 °C and then rapidly decreased to 30 °C where the temperature remained for the rest of the composting period (Fig. 1). The smaller temperature variation in the anaerobic composting is due to a decreased degradation rate in the anaerobic condition<sup>11</sup>. The degradation of anaerobic piles was very slow and ineffective at destroying pathogenic organisms irrespective of the use of chicken manure or decanter sludge and whether or not red soil was applied to the pile. This result could be explained and confirmed by changes in microorganism quantities, cellulase activity, and C:N ratio monitoring.

### Change of pH in the piles

The pH of both aerobic and anaerobic piles changed slightly during composting (pH 7.50–8.60) (Fig. 2). This range was appropriate for organic matter degradation<sup>12</sup>. However, the change in pH of the piles was independent of the use of chicken manure or decanter sludge and also independent of whether or not red soil was used. The pH in all piles increased slightly in the first 45 days of composting and gradually decreased after that. At the end of composting, the pH of the aerobic piles was in the range of 7.90–8.25 while the pH of anaerobic piles was in the range of 7.51–8.12. The pH change of the piles was due to microbial activity. In the aerobic condition, organic N is transformed





**Fig. 1** Temperature change in (a) aerobic and (b) anaerobic composting systems.

**Fig. 2** pH change in (a) aerobic and (b) anaerobic composting systems.

into NH<sub>3</sub> or NH<sub>4</sub><sup>+</sup> in ammonification, increasing the pH of the pile<sup>13, 14</sup>. If the oxygen content is sufficient, ammonia oxidizing bacteria and nitrifying bacteria will transform NH<sub>3</sub> into NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>, in that order. This process is called nitrification and releases H<sup>+</sup>, reducing the pH of the environment<sup>15</sup>. When the piles contain insufficient levels of oxygen, NH<sub>3</sub>-decay reactions occur and produce different nitrogen compounds including amines, causing the emission of a foul odour from the pile<sup>9</sup>.

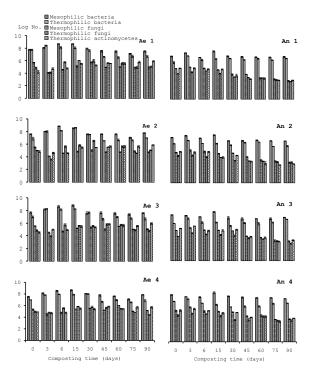
### Microbial community

Fig. 3 presents the microbial community change in both aerobic and anaerobic piles. This figure indicates that bacteria are the dominant group in all piles (mesophilic and thermophilic bacteria, respectively) due to the important role of decomposer that bacteria play in compost piles<sup>16</sup>. In most of the aerobic piles of this study, the bacteria quantity increased slightly until the 15th day of composting and then it gradually decreased and remained constant until the end of composting. While a small multiplication of fungi and actinomycetes was found during the latter composting (Fig. 3), these microorganisms could have originated from all compost substrates and seed. But the selected microorganisms inoculated could enhance the effective compost microorganism number, especially the bacteria, because it was the main group that existed in seed and required less reproduction time than other groups. As a result, the main microorganism group in the compost was bacteria.

For anaerobic conditions, the microbial community was not affected by the type of N source and was independent of whether or not red soil was added. The quantity of microorganisms counted in these piles was no different from that counted for aerobic piles. There was little change in the numbers of bacteria whereas the fungi and actinomycetes quantities decreased appreciably. The reason is that most fungi need oxygen for growth and activity and so anaerobic conditions were not suitable for their survival.

### Cellulase (CMC-ase) production

Changes in cellulase activity of the piles were also monitored to evaluate the microbial activity (Fig. 4). An obvious increase in cellulase activity occurred in the aerobic piles during the first two weeks of composting (the highest cellulase activity measured in aerobic piles was 0.42 unit/g) while the cellulase activity of anaerobic piles also increased during the first two weeks, but by smaller amounts than in the aerobic piles (the highest cellulase activity measured in anaerobic piles was 0.19 unit/g). This is due to the humid environment of the anaerobic conditions which is not suitable for the growth of cellulase-producing organisms<sup>17</sup>. The result of statistical analysis con-

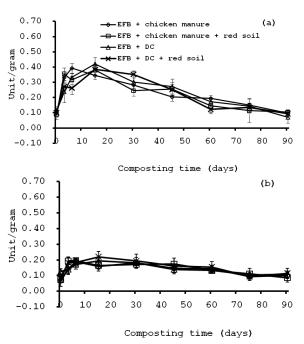


**Fig. 3** Microbial changes in aerobic (Ae) and anaerobic condition (An). 1: EFB + chicken manure; 2: EFB + chicken manure + red soil; 3: EFB + decanter cake; 4: EFB + decanter cake + red soil.

firmed that bacteria and fungi quantities are significant treatment factors while the actinomycete has no significant effect on the compost ( $\alpha = 0.05$ ). This is true regardless of whether the piles contain chicken manure or decanter sludge, and whether or not they contain red soil. This means that decanter sludge and red soil do not significantly affect microbial activities for organic matter degradation.

## **Compost completion**

The organic matter and organic carbon contained in all piles gradually decreased with composting time. The total degraded organic matter and organic carbon in aerobic conditions were higher than the average degraded organic matter and organic carbon in anaerobic conditions. The organic matter content could be used to indicate when composting is complete. The criterion of organic matter percentage in compost standards varies considerably among different countries. The organic matter content in compost should not be less than 20–30%. However, if the organic matter content of composting material is higher than 60%, the composting is considered to be incomplete. In this study, the organic matter content of compost



**Fig. 4** Change of cellulase activity in (a) aerobic composting systems and (b) anaerobic composting systems.

product samples obtained from the aerobic piles was in the range of 32-57%, as presented in Table 3. For anaerobic conditions, all compost still contained organic matter at concentrations higher than 60%, except pile An 2 (EFB + chicken manure + red soil) which contained less than 60% since initiation.

A C:N ratio lower than 20:1 is the criterion used in this study to determine the completion of composting<sup>18</sup>. The C:N ratios of all compost piles decreased with composting time. At the end of composting, the C:N ratio of all piles in the aerobic condition was lower than 20:1 (Table 3) whereas all anaerobic piles still had C:N ratios higher than 20:1 (Table 2). For the aerobic piles, the pile Ae 3 (EFB + decanter sludge) completed on the 30th day and the completion of pile Ae 4 (EFB + decanter sludge + red soil) occurred on the 60th day which is faster than the piles composted with chicken manure (Fig. 5). However, the result of MANOVA shows that the EFB compost with only decanter sludge yielded faster composting times than compost with decanter sludge and red soil ( $\alpha = 0.05$ ).

#### Nutrients of compost product

The important nutrients (nitrogen-phosphoruspotassium, N-P-K) met the compost standards of the Department of Agriculture of Thailand (N-P-K higher than 1.0-0.5-0.5% by dry weight). The compost

Ae 4

 $1.03 \pm 0.14$ 

 $38.6\pm3.8$ 

 $1.27 \pm 0.10$ 

 $1.39 \pm 0.12$ 

90 day

 $8.18\pm0.05$ 

 $1.48 \pm 0.18$  $21.1 \pm 0.6$ 

 $36.4 \pm 1.0$ 

 $2.00 \pm 0.06$ 

 $10.57\pm0.11$ 

 $1.37 \pm 0.03$  $1.57 \pm 0.05$  $1.51 \pm 0.07$ 

<b>Table 3</b> Physicochemical properties of compost under aerobic conditions (mean $\pm$ SD).										
Parameters	s Control		Ae 1		Ae 2		Ae 3		Ae	
	0 day	90 day	0 day	90 day	0 day	90 day	0 day	90 day	0 day	
pH	0.00 0.01		$7.53 \pm 0.09$		$7.64 \pm 0.10$		$7.27 \pm 0.12$	0.20 22 0.00		
EC (dS/m) OC (%)	$2.79 \pm 0.06$ $54.1 \pm 1.5$	$2.00 \pm 0.06$ $42.2 \pm 2.1$	$2.37 \pm 0.03$ $40.7 \pm 2.0$	$1.36 \pm 0.11$ $23.2 \pm 0.4$	$2.25 \pm 0.03$ $30.9 \pm 2.3$	$1.35 \pm 0.08 \\ 18.5 \pm 0.5$	$1.96 \pm 0.05 \\ 57.5 \pm 0.3$	$1.62 \pm 0.15$ $33.1 \pm 0.4$	$1.91 \pm 0.16$ $39.4 \pm 1.4$	
OM (%)	$93.3 \pm 2.6$	$77.6 \pm 3.7$	$70.1 \pm 3.5$	$40.0 \pm 0.6$	$53.3 \pm 3.9$	$32.0 \pm 0.9$	$99.1 \pm 0.5$	$57.1 \pm 0.7$	$67.9 \pm 2.4$	

 $50.9 \pm 2.9$  $53.3 \pm 3.9$  $0.78 \pm 0.05$ 

 $39.8 \pm 0.8$ 

 $1.70 \pm 0.03$ 

 $1.69 \pm 0.13$ 

 $1.60 \pm 0.13$ 

 $14.6\pm1.3$ 

 $\begin{array}{c} 2.57 \pm 0.14 \\ 1.85 \pm 0.08 \end{array}$ 

 $70.1 \pm 3.5$  $1.08 \pm 0.14$ 

 $38.1 \pm 3.6$ 

 $2.16 \pm 0.16$ 

 $1.62 \pm 0.14$ 

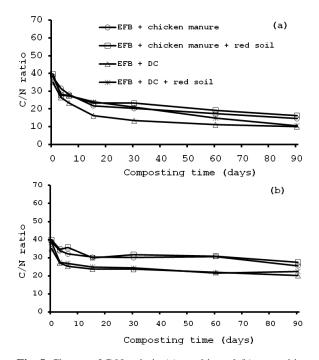


Fig. 5 Change of C:N ratio in (a) aerobic and (b) anaerobic composting systems.

that used decanter sludge had a higher total nitrogen content than that using chicken manure. However, the opposite trend was observed for phosphorus  $(P_2O_5)$ values, as the compost from piles using decanter sludge had lower phosphorus values than the piles with chicken manure ( $\alpha = 0.05$ ). This is caused by the lower phosphorus content in decanter sludge. The phosphorus content of the completed compost samples were slightly greater than the P content of samples from the initial composting period in all piles and they were in the range of 1.4-2.6% by dry weight for the aerobic conditions and 1.2-2.1% by dry weight for the anaerobic conditions at the end of composting. For potassium values measured in terms of percentage of K<sub>2</sub>O by dry weight, the compost obtained from aerobic and anaerobic piles contained total K<sub>2</sub>O of 1.5-2.8% and 1.4-1.6%, respectively

(Tables 2 and 3).

 $1.14 \pm 0.02$ 

 $16.3 \pm 0.6$ 

 $2.18 \pm 0.15$ 

 $1.61 \pm 0.09$ 

 $\begin{array}{c} 99.1 \pm 0.5 \\ 1.64 \pm 0.11 \end{array}$ 

 $35.1 \pm 2.3$ 

 $1.29 \pm 0.07$ 

 $2.25 \pm 0.15$ 

For the control pile without adding microorganism seeds, the analysis of compost products from both conditions at 90 days indicated that their composting was not complete. These compost products still had C:N ratios higher than 20:1 and the organic matter was still higher than 60% (Tables 2 and 3). This result confirmed that the addition of seed organism is a factors that can decrease the EFB compost time.

 $57.1 \pm 0.7$  $3.30 \pm 0.24$ 

 $10.1\pm0.8$ 

 $1.59 \pm 0.13$ 

 $2.75 \pm 0.23$ 

From the results obtained, we found that decanter sludge could be used as an effective nitrogen source for EFB composting instead of chicken manure in the proportion of 1:1 (EFB:decanter sludge). Although the composting under anaerobic conditions was unable to complete within 90 days, the organic carbon component of composting materials continued to decrease after that time. This means that the EFB composting under anaerobic conditions could be accomplished but takes more time than aerobic composting. When the EFB is heavy, it is more beneficial if EFB is composted under anaerobic conditions because it does not require turning. Although EFB composting under aerobic conditions is more effective, it requires a turning machine or intensive labour.

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N(%)

C:N ratio

P<sub>2</sub>O<sub>5</sub> (%) K<sub>2</sub>O(%)

 $93.3 \pm 2.6$  $0.57 \pm 0.04$ 

 $95\pm 6$ 

NM

NM

 $77.6 \pm 3.7$  $0.86 \pm 0.06$ 

 $45.6 \pm 3.7$ 

NM

NM

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