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Original Article

Effect of inoculum size on production of compost and enzymes from palm oil mill biogas sludge mixed with shredded palm empty fruit bunches and decanter cake

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Abstract

The effect of inoculum size on production of compost and enzymes from palm oil mill biogas sludge (POMS) mixed with shredded palm empty fruit bunches (PEFB) and decanter cake (DC) was studied using the mixed culture LDD1 as an inoculum. Three piles of 50 kg mixture (POMS:PEFB:DC = 2:1:1) with different inoculum sizes of 0.0075% (treatment A), 0.015% (treatment B), and 0.030% (treatment C) were set up. The physicochemical parameters were measured during the composting. All the compost appeared dark brown in color, crumbly, attained an ambient temperature and had the C/N ratio of 11:1 after 40 days fermentation, indicating the maturity of the compost. The optimal inoculum size was found to be 0.030% (w/w). For enzyme production, the highest carboxymethylcellulase (CMCase) activity was 3.23 Unit/g substrates at 12 days incubation whereas the highest xylanase activity was 3.11 Unit/g substrates at 6 days incubation. At the end of 60 days fermentation, the compost (treatment C) had a TN-P_2O_5-K_2O of 3.10-1.29-2.01% (dry basis). Therefore, the compost quality complied with the national compost standard set by the Ministry of Agriculture, Thailand.

Keywords: palm oil mill biogas sludge, composting, palm oil mill wastes, empty fruit bunch

1. Introduction

Palm oil production is a major agro-industry in Southern Thailand. Palm oil mills generate significant quantities of wastes such as empty fruit bunches (EFB) (20-28%), decanter cake (DC) (4%), fibers (11-13%), ash from boilers (4%) and palm oil mill effluent (POME) (0.5-1.2 m^3/t fruit fresh bunches (Prasertsan and Prasertsan, 1996). EFB was not used as a source of fuel (for boiler to generate steam at the palm oil mills) due to its high moisture content (60-70%) as well as high potassium content (6.5%) and air pollution problem (Singh *et al*., 2010). EFB is mainly used as mulch in the oil palm plantations to control weeds, prevent erosion and maintain soil moisture. However, due to escalating labor, transportation, and distribution costs of EFB in the field, its utilization as mulch is becoming more expensive (Alam *et al*., 2009). Decanter cake is known to be rich in N, P_2O_5, K_2O, CaO, and MgO (Haron *et al.,* 2008). In addition, its high moisture content can further accelerate the rate of compost formation (Yahya *et al*., 2010). POME is composed of high organic content mainly cellulosic materials with oil and fatty

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acid and able to support bacterial growth to reduce its pollution strength. Anaerobic processes are mostly employed in treating POME and recently the purpose has been shift for biogas production to generate electricity. Wastewater treatment or biogas production from POME generate huge amount of sludge every year. The palm oil mill biogas sludge (POMS) from anaerobic reactors contains high moisture content (85%), low carbon content (33% dry weight) and high nutrient value (3.6% N, 0.9% P, and 2.1% K dry weight) (Yaser *et al*., 2007). POMS is usually discharged from biogas reactor to sand bed and dried, then used as fertilizer or soil conditioner. However, this process becomes a problem during rainy season due to slow rate of drying. The problems are sludge flooding, insects and bad odor (Zahrim *et al*., 2007).

An alternative approach was to utilize POMS, shredded EFB and decanter cake, as raw materials for making compost. Mixing POMS with EFB and DC can improve the efficiency of the composting process by providing additional carbon, retaining nutrient, and reducing odor (Bhamimarri *et al*., 1996; Zahrim *et al*., 2007). In addition, composting of the non-shredded EFB with POME could simultaneously treat the solid and liquid waste from palm oil mill. With the aim to reduce the composting yard area, production of semi-compost in industrial scale windrow was studied and the nutrient in compost is acceptable for soil conditioner (Zahrim and Asis, 2010). Utilization of these wastes, instead of left them unused, could mitigate environmental problem in nearby communities and palm oil mills (Yahya *et al*., 2010).

Composting is basically a biodegradation process in which organic wastes are transformed and stabilized by the metabolic activities of succession of mixed microbial population (Baharuddin *et al*., 2009). The decomposition of organic wastes during the composting process is carried out by succession of microbial communities (Hiroaki *et al*., 2006) processing various enzymes. Cellulase is an enzymes system that breaks down cellulose, the main component of the cell walls of plant biomass. This enzymatic system is responsible for the hydrolysis of cellulose which is the most important step in the economical production of bioethanol, single cell proteins and other chemicals (Alam *et al*., 2009). The enzyme endo-1,4-β-xylanase, β-xylosidase, α-glucuronidase, β-L-arabinofuranosidase and acetylxylan esterase including hemicellulose were reported to act on the different hetero-polymers available in nature. In glucomannan degradation, β-mannanase and β-mannosidase cleave the polymer backbone. Like cellulose, hemicellulose is also an important source of fermentable sugars for biorefining applications.

In previous studies, the mixed culture LDD1 was used as an inoculum for production of compost and it contained cellulase and xylanase producing microorganisms. The optimum mixture ratio of POMS:PEFB:DC was reported to be 2:1:1 after 60 days incubation (Nutongkaew *et al*., 2011). This study aims to further investigate on the effect of inoculum size of the LDD1 on the production of compost and lignocellulosic enzymes.

2. Methodology and Experimental Design

2.1 Raw materials and seed inoculum

Palm oil mill biogas sludge was obtained after discharging the biogas effluent containing sludge onto a sand bed and sun-dried. Biogas effluent (BE) was obtained directly from the biogas reactor in the same palm oil mill. Wastes generated from the palm oil milling process included decanter cake (DC) which was the solid residue discharged from the decanter (3-phase separator). Palm oil empty fruit bunches (PEFB) was discharged from a rotary drum thresher after separation of the palm fruits. PEFB was shredded (by using an accelerated shredder machine) to facilitate the mixing with other solid wastes and enhance the composting rate. Palm oil fuel ash (POFA) was obtained from a boiler whereby palm pressed fiber was used as a source of fuel for steam production. All of these wastes were collected from palm oil mills in Southern Thailand. The seed inoculum used was the microbial activators LDD1 called 'super LDD1' provided by the Land Development Department, Ministry of Agriculture, Thailand. The super LDD1 are the mixed cultures of aerobic cellulose decomposer fungi (*Corynascus* sp., *Scytalidium* sp., *Chaetomium* sp., *Scopulariopsis* sp., *Helicomyces* sp., and *Trichoderma* sp.), the bacteria (*Bacillus* sp.) and Actinomycete (*Streptomyces* sp.) (Leaungvutiviroj *et al*., 2007).

2.2 Experimental setup

An experimental composting system with a weight of 50 kg each was set up. The mixture was consisted of POMS, PEFB, and DC at a ratio of 2:1:1 (by weight) (Nutongkaew *et al*., 2011). Three treatments, each in triplicate, were set up. Treatment A, B, and C were inoculated with 3.75, 7.5, and 15 g LDD1/50 kg mixed material, corresponding to the inoculums size of 0.0075, 0.015, and 0.030% (w/w), respectively. BE was added to adjust the moisture content to 60-70% and POFA was added to adjust the pH to 7-7.5. After mixing well, each treatment materials were put into a reactor (0.60 m W × 1.0 m L × 0.60 m H) (Figure 1). During 60 days incubation period, the BE was added to keep the final moisture content of the compost within the range of 60-70%

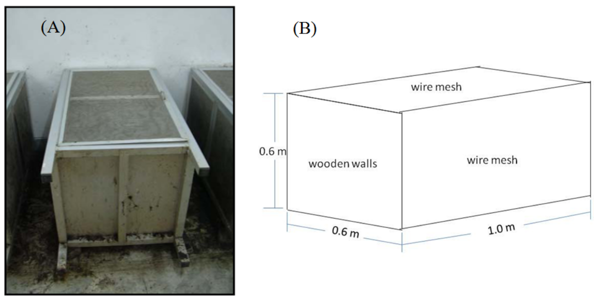

Figure 1. Perforated rectangular reactor for compost production: Photo image (A), Schematic diagram (B).

and turned each pile every 10 days for aeration. The addition of BE was stopped 1-2 weeks prior to harvesting in order to avoid the final product from being too wet.

2.3 Sampling

Temperature was measured at the core of the reactor every day. Samples were collected at different locations (the center of the four sides of each reactor/pile). The mixture was manually mixed and divided into two parts. One part was stored at 4°C while the other was determined for the activity of carboxymethylcellulase (CMCase) and xylanase. The pH and moisture content were determined every five days. The organic matter (OM), total organic carbon (TOC), and total nitrogen (TN) were determined every 10 days for 60 days cultivation and phosphorus was determined every 20 days, while potassium was determined at the end of fermentation. All experiments were conducted in triplicates.

2.4 Analytical methods

Thermometer was used for temperature measurement. The compost pH was determined by adding 10 g sample to 100 ml distilled water, mixed well on a rotary shaker for 30 min before measured by using pH meter. For moisture content, the mixture samples were oven-dried at 105°C for 24 hrs (AOAC, 1990). Oven-dried sample were finely ground to represent the whole sample. The organic matter (OM) was determined as volatile solid (AOAC, 1990). Ash content was determined by burning sample at 600°C for 3 hrs (AOAC, 1990). Total organic carbon (TOC) was determined by using the following formula (Hoyos, 2002):

$$TOC\ (\%) = Organic\ matter\ (\%)/1.8 \quad (1)$$

Total nitrogen (TN) was determined using the Kjeldahl method (AOAC, 1990) with C to N ratio determined as TOC/TN. Phosphorus (P) and potassium (K) was determined using $HNO_3/HClO_4$ digestion (AOAC, 1990).

The enzymes were extracted from the compost by adding 10 g sample to 100 ml distilled water, mixed with a rotary shaker for 1 hr at 200 rpm and 20°C. The extract was centrifuged at 8,000 rpm (5449 *x g*) for 10 min and the filtrate was determined for enzymes activity. CMCase activity was assayed according to method of Mandels and Weber (1969) and xylanase activity was assayed according to method of Tang *et al*. (1987). After 10 min incubation at 50°C, the liberated reducing sugar was determined by the Somogyi-Nelson Method (Nelson, 1944). One unit is defined as the amount of enzyme required to liberate 1 µmol of reducing sugar per minute under the assay conditions. Enzymes yield was expressed as Unit/g of dry substrate.

2.5 Statistical analysis

The data presented were analyzed using SPSS (SPSS Inc., version 15.0). One-way analysis of variance (ANOVA) was carried out to compare the means of different treatment where significant *F* value was obtained. Differences between individual means were tested using Duncan's Multiple Rang Test (DMRT) at 0.05 significant levels. The data was performed in triplicate.

3. Results and Discussion

3.1 Characteristics and chemical composition of palm oil mill wastes

The characteristics and chemical composition of palm oil mill wastes are shown in Table 1. Palm empty fruit bunches (PEFB) exhibited the highest total organic carbon (52.83%) while the palm oil mill biogas sludge (POMS) had the highest nitrogen content (3.6%). Palm oil fuel ash (POFA) contained only mineral; 2.17 % P and 1.93 % K. The C/N ratio of palm

Table 1. Characteristics and chemical composition of palm oil mill waste materials.

Composition	Palm oil mill waste materials				
	Biogas sludge	Empty fruit bunches	Decanter cake	Ash	Biogas effluent
pH	8.41	6.35	4.81	10.00	7.58
Moisture (%)	22.23±0.72[c]	45.51±0.94[b]	78.42±0.12[a]	ND	ND
Total organic carbon (%)	36.06±0.03[c]	52.83±0.05[a]	47.73±0.11[b]	-	ND
Oil and grease (%)	1.15±0.03[b]	1.85±0.10[a]	0.92±0.12[c]	0.65±0.06[d]	ND
Nitrogen (%)	3.6±0.30[a]	0.9±0.10[c]	2.37±0.10[b]	0.08±0.02[c]	1,880 mg/L
Phosphorus (%)	1.58±0.09[b]	0.37±0.03[c]	0.33±0.03[c]	2.17±0.01[a]	15.9 mg/L
Potassium (%)	1.72±0.10[b]	1.73±0.04[b]	0.85±0.10[c]	1.93±0.11[a]	2.00±0.9[a]
Ash (%)	35.09±0.06[b]	4.90±0.08[d]	14.08±0.21[c]	86.73±0.75[a]	ND
COD (mg/l)	ND	ND	ND	ND	2,270
C/N ratio	10.12	58.70	20.14	-	1.21

ND: Not detect, percentage (%) was based on dry weight; Different letters in the same row indicate significant differences at p<0.05 according to Duncan's multiple rang test.

oil mill biogas sludge, palm empty fruit bunches, decanter cake (DC) and biogas effluent (BE) were 10.12, 58.70, 20.14, and 1.21, respectively. The high C (52.83%) and low N (0.9%) of palm empty fruit bunches, therefore, should be mixed with the low C and high N of the palm oil mill biogas sludge (36.06 % C and 2.37 % N) and decanter cake (47.73% C and 2.37 % N). Unlike the other three wastes, biogas effluent was low in both C and N as they were assimilated during biogas production. Hence, it was only used as a source of water to adjust the moisture content to the desired value.

3.2 Physicochemical and biochemical changes during composting

3.2.1 Temperature profile

The temperature of the pile is an important parameter used to indicate the degree of microbial activities and the composting progress. Using a variable temperature profile is required to promote contaminant degradation and microbial activity (Antizar-Ladislao *et al*., 2007; Yu *et al*., 2011). In this study, the initial temperature of all piles was approximately 28°C. During the composting period, the ambient temperature was observed around 28 to 32°C. The temperature of composting piles increased sharply to 45°C after 24 hrs of treatment reflecting active microbial decomposition in the composting piles (Figure 2A). From 2 to 20 days incubation, the temperature was around 33-45 °C. Based on temperature profile during composting, two stages in the treatment process can be observed; thermophilic (from day 2 to day 20) and mesophilic (from day 21 to day 40). High temperature accelerated the breakdown of protein, fats and complex carbohydrates such as cellulose and hemicelluloses (Olfa *et al*., 2008). Most studies reported that the optimum temperature range of effective decomposition was 50-70°C (Wong *et al*., 2001; Baharuddin *et al*., 2009), but temperature of 45-55°C must be maintained for maximum biodegradation (Stentiford *et al*., 1996; Baharuddin *et al*., 2009). Thus, the temperature profile obtained in this study met the sanitary requirement without external exertion of heat energy to the composting pile. The rapid increase in temperature might be due to the turning process. Moreover, large amounts of readily digestible components in the POMS were available for microbial utilization. The compost indicates a good degree of stability when temperature during composting approaches the ambient level (Satisha and Devaranjan, 2007; Raj and Antil, 2011). At the end of treatment, the average temperature inside the pile was 30°C. It was also suggested that the capacity of the composting pile could preserve heat by limiting heat loss to the surrounding.

3.2.2 Moisture content and pH value

The moisture content was the critical factor that determined the decomposition rate in composting. According to Luo *et al*. (2008), microbial dependability of water to support growth could affect biodegradation of organic matters. In this study, BE was added to the composting pile to maintain the optimum moisture content (60-70%) throughout the treatment. The changes of moisture content during composting of the three piles using three inoculum sizes are shown in Figure 2B. The moisture content of the matured compost (44.94%) was 60% of the initial raw materials (75%). High temperature and frequent turning of the composting piles contributed to water loss. Therefore, the addition of BE was essential to sustain the microbial activity under the optimum moisture content.

The initial pH of composting pile was 8.14 and this was probably due to the high pH values of POFA (pH 10.00) and BE (pH 7.58) (Figure 2C). The pH fell within the range of

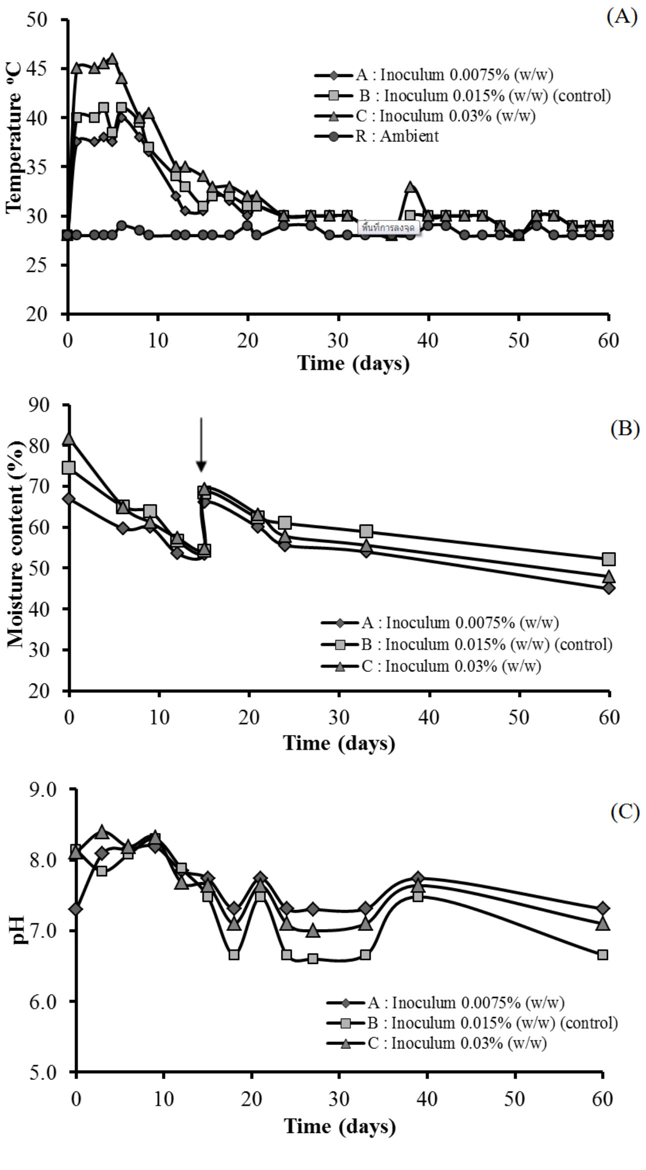

Figure 2. Profile of compost temperature (A), moisture content (B) and pH (C) during co-composting of palm oil mill biogas sludge mixed with palm oil mill wastes and biogas effluent (ratio 2:1:1) using the Super LDD1 as an inoculums.

6.6 to 7.3 and was almost constant after day 12 of composting period. The slight decline of pH after day 24 (pH<7.5) was probably due to the volatilization of ammonium and released of hydrogen ions from the nitrification process. In maturity phase, the pH was almost neutral and stabilized which was probably due to buffering nature of humic substances (Satijah and Devarajan, 2007; Hock *et al*. 2009).

3.2.3 C/N ratio

The C/N ratio is widely used as an indicator of compost maturity (Bernal *et al*., 1998; Raj and Antil, 2011). However, some researchers reported that the C/N ratio was not a good indicator of mature compost because it has large variability in raw materials and often gives a misleading indication of maturity and it may not reflect a materials which is sufficiently decomposed (Sellami *et al*., 2008). In this study, the nitrogen content (Figure 3A) increased steadily whereas carbon content (Figure 3B) was reduced gradually throughout the composting process. This phenomenon may be attributed from the microbial activity on cellulosic substrate and nitrogen, resulted in the increase of the microbial protein and humic substances (Thambirajah *et al*., 1995; Baharuddin *et al*., 2009). On the other hand, the biologically degradable organic matter is converted to CO_2 and H_2O and is removed from the compost (Vuorinen and Saharinen, 1997; Baharuddin *et al*., 2009). Despite the high C/N ratio of EFB, the addition of POMS, DC and BE reduced the initial C/N ratio of composting material from14 to about 10 (Figure 3C).

3.2.4 CMCase and Xylanase activity

The decomposition of organic materials was performed by microorganism. The enzymatic process is specific to materials to be composted (Baharuddin *et al*., 2009). Three treatments contained a high proportion of lignocellulosic materials which could be decomposed by biological processes. This is accomplished through a complex reaction of various enzymes such as carboxymethylcellulase (CMCase), xylanase and lignin peroxidase. Therefore, enzyme activity profiles throughout the process reflect microbial activity and compost stability. In this study, the LDD1 was used as an inoculum for composting. The enzyme activity increased rapidly on the first day of fermentation with simultaneously increase of temperature. There were two peaks for the CMCase activity at day 12 and 27 and day 6 and 27 for xylanase activity (Figure 4). The optimal inoculums size was 0.030% LDD1 (w/w) (treatment C), giving the maximum CMCase activity of 3.23 Unit/g substrate at 12 day incubation and xylanase activity of 3.11 Unit/g substrate at 6 day incubation. The maximum CMCase activity from this study was about one fourth of that (13.6 Unit/g) reported by Baharuddin (2009) who determined the CMCase activity at 30 days of the co-composting of palm empty fruit bunches and partially treated palm oil mill effluent. Furthermore, the difference was also due to partly the difference in inoculum source, fermentation time and

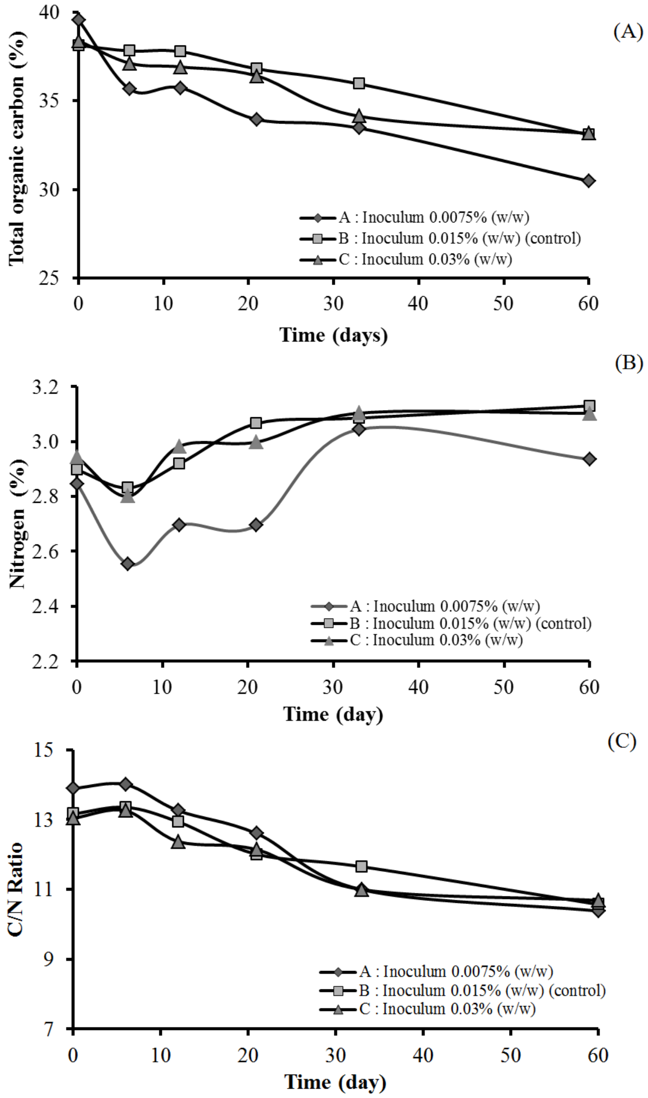

Figure 3. Profiles of total organic carbon (A), nitrogen concentration (B) and C/N ratio (C) throughout composting process by co-composting of palm oil mill biogas sludge mixed with palm oil mill wastes and biogas effluent.

enzyme activity determination, for example the type of CMC used, incubation temperature, and time.

3.2.5 Nutrients and chemical composition

Nitrogen, phosphorous, and potassium are the nutrients which are utilized in greatest quantities by plants. The chemical compositions of the matured composts are displayed in Table 2. The mean values for nitrogen, phosphorus and potassium of the compost using 0.03% inoculums (treatment C) were greater than those of the compost using 0.075% and 0.015% inoculums (treatment A and B). On the other hands, the organic matter, organic carbon and pH of the treatment A and B were higher than those of the treatment C. In this study, the nutrients content of the matured compost

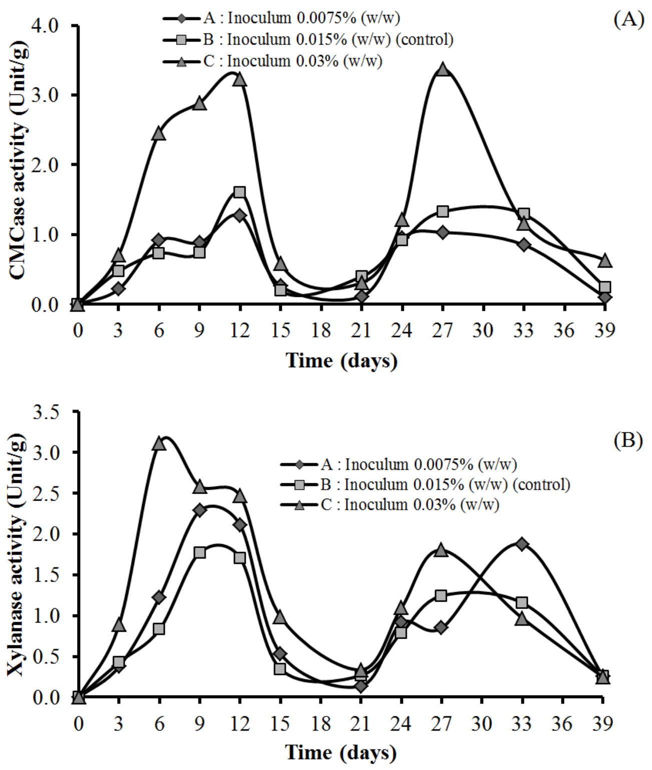

Figure 4. Profiles of CMCase (A) and xylanase (B) activity throughout composting process by co-composting of palm oil mill biogas sludge mixed with palm oil mill wastes and biogas effluent.

(treatment C) were 3.1% TN, 1.3% P, and 2.0% K. Hence, it showed significantly higher ($p<0.05$) TN, P, and K, when compared with other treatments. The nitrogen and nutrients level of the matured compost (treatment C) was comparable to the compost using PEFB (2.2% TN, 1.5% P, 2.8% K) and partially treated POME (Baharduddin *et al.*, 2009). The final compost product in this study was suitable for plant nutrients, safe and no detrimental effect.

4. Conclusion

The optimal inoculum size for making compost was 15 gLDD1 per 50 kg batch, which was equivalent to 0.03% (w/w). During composting, the highest enzymes activities were 3.11 Unit/g at 6 days incubation for xylanase and 3.23 Unit/g at 12 days incubation for CMCase. At the end of fermentation 40 days, the C/N ratio was 11:1. In addition, the nutrients TN-P_2O_5-K_2O of the compost were 3.10-1.29-2.01% dry weight.

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Table 2. Characteristics and chemical composition of matured compost.

Element of compost standard	Treatment A 0.0075% (w/w)	Treatment B 0.015% (w/w)	Treatment C 0.030% (w/w)
C:N ratio (<20:1)	10.38	10.54	10.68
TN % (>0.5%)	2.94 ± 0.01^{b}	3.13 ± 0.03^{a}	3.10 ± 0.03^{a}
P_2O_5 % (>0.5%)	0.88 ± 0.01^{c}	1.15 ± 0.06^{b}	1.29 ± 0.01^{a}
K_2O % (>1%)	1.63 ± 0.10^{c}	1.84 ± 0.04^{b}	2.01 ± 0.10^{a}
Moisture content % (<30%)	44.95 ± 1.01^{b}	52.11 ± 1.25^{a}	47.95 ± 4.81^{ab}
Organic matter % (>25%)	61.32 ± 0.33^{a}	60.20 ± 0.20^{b}	59.67 ± 0.01^{c}
pH (6.0-8.0)	7.31	6.66	7.10

Percentage (%) was based on dry weight; Different letters in the same row indicate significant differences at $p<0.05$ according to Duncan's multiple rang test.

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