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EFFECT OF PRE-TREATMENT AND INOCULANT DURING COMPOSTING OF PALM OIL EMPTY FRUIT BUNCHES

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In this work, untreated empty fruit bunch (EFB) or microwave-assisted NaOH pretreated EFB with palm oil mill effluent (POME) were composted under mesophilic conditions either in the presence or absence of *Bacillus amyloliquefaciens D203* for sixty days. During pretreatment conditions, the EFB was mixed with 1% (w/w) sodium hydroxide and then exposed to microwave irradiation. The composting process was evaluated based on the evolution of pH, electrical conductivity, moisture content, organic matter loss, zeta potential and phytotoxicity. The strain *Bacillus amyloliquefaciens D203* is not suitable for EFB-POME composting due to lower organic matter loss. The microwave-assisted NaOH pretreatment contributed to ~15% more organic matter loss than was found in the untreated sample while its germination index was >50%.

Keywords: Composting, Empty fruit bunch, Palm oil mill effluent, Microwave pretreatment, Zeta potential, Phytotoxicity

INTRODUCTION

The palm oil industry produces millions of tonnes of wastes yearly; these include empty fruit bunch (EFB) and palm oil mill effluent (POME). Fresh POME is a highly viscous liquid, brownish in colour which is discharged at a temperature of 80–90 °C. It is extremely poisonous with very low pH between 3.5 and 4.2, high chemical and biological oxygen demand (COD: 16–100 g/L, BOD5, 30 °C: 10–44 g/L), high suspended solids (SS: 5–54 g/L), and high salt content (Alhaji et al., 2016). Annually, there are 90 million tonnes of renewable biomass accumulated and EFB accounts for approximately 9% of this (Bari et al., 2010). Composting, an aerobic biological treatment, could simultaneously manage the EFB and POME (Bukhari et al., 2014, Zahrim et al., 2015) and has the potential to be used as a soil conditioner.

EFB is an abundant lignocellulosic biomass with a worldwide annual production. Like other types of lignocellulosic biomass, EFB is mainly composed of lignin, cellulose and hemicelluloses, as well as other minor elements. For the usual lignocellulose complex found in EFB, cellulose maintains the crystalline fibrous structure and it appears to be at the core of the complex. Hemicellulose is located both between the micro- and macrofibrils of cellulose. Meanwhile, lignin, bound in the interfibrous area, provides the structural matrix in which cellulose and hemicellulose is embedded (Harmsen, Huijgen, Lopez and Bakker, 2010). The recalcitrant structure of EFB causes longer composting time to be needed and hence, the composting area required will be larger. In general, the conventional composting process takes 60 – 90 days (Zahrim and Asis, 2010, Zahrim et al., 2015).

Suitable pretreatment has been shown to assist in reducing the time and space required for composting. From previous literature, the combination of microwave and alkali pretreatment was able to remove more lignin with a shorter pretreatment time compared to other pretreatment methods. Binod et al. (2012) compared various types of microwave pretreatment such as microwave-acid, microwave-alkali and combined microwave-acid-alkali, using sugarcane bagasse as the lignocellulosic waste. It was deduced that the maximum lignin removal was attained for microwave-NaOH pretreatment which achieved about 96% removal (Binod et al., 2012). The microwave-assisted sulphuric acid pretreatment was not able to remove lignin at a very high rate with maximum lignin removal of only 34.5% (Jung et al., 2013). The lignin molecular architecture, where different non-phenolic phenylpropanoid units form a complex three-dimensional network linked by a variety of ether and carbon–carbon bonds (Ruiz-Duenas and Martinez, 2009), make the EFB resistant to microbial attack. Besides that, addition of the right combination of microorganisms can speed up the composting process by aiding biodegradation of the EFB structure. It was reported there are 27 strains of indigenous microbes from POME anaerobic sludge alone which were found to exhibit cellulolytic and hemicellulolytic activity, which could enhance biodegradation of EFB and shorten the composting process to as little as 40 days (Zainudin et al., 2013). Other than that, inoculation also improves carbon metabolism of microorganisms, which is indicated by the more stable profile of low molecular weight organic acids (LMWOAs) which are consumed by microorganisms as a nutrient source (Lim et al., 2015)

Based on our knowledge and an extensive review of the literature, there are no studies dealing with microwave pretreatment of combination of EFB and POME for the composting process. As a result, microwave-assisted NaOH pretreatment prior to composting was carried out in this study. Additionally, inoculation of *Bacillus amyloliquefaciens D203* was carried out to study the performance of the pretreated compost.

METHODS

Composting Materials

EFB and POME were collected from Merotai Composting Plant, Tawau, Sabah palm oil mill. The EFB was cleaned and dried before they were stored at room temperature. The characteristics of the EFB and POME are presented in **Table 1** and **Table 2** respectively.

Table 1. Physio-chemical Properties of EFB of Palm Oil Industry (Kavitha et al., 2013)

Parameters	EFB
pH	7.20
Electrical Conductivity (dS m^{-1})	2.70
Organic Carbon (%)	45.10
Total Nitrogen (%)	0.55
C/N ratio	82.00
Total Phosphorus (%)	0.02
Total Potassium (%)	1.28
Total Iron (mg kg^{-1})	210.00
Total Zinc (mg kg^{-1})	71.00
Total Copper (mg kg^{-1})	26.00
Total Manganese	88.00
Cellulose (%)	33.00
Hemicellulose (%)	30.00
Lignin (%)	34.00

Table 2. Physio-chemical Properties of Palm Oil Mill Effluent (POME) (Kavitha et al., 2013)

Parameters	POME
Colour	Yellow
pH	4.70
Electrical Conductivity	25.20
BOD (mg L^{-1})	25,000
COD (mg L^{-1})	50,000
TDS	22,000
TSS	17,000
Nitrate (mg L^{-1})	35.00
Total Nitrogen (mg L^{-1})	741.00
Total Phosphorus (mg L^{-1})	176.00
Total Potassium (mg L^{-1})	2,277
Total Iron (mg L^{-1})	46.50
Total Zinc (mg L^{-1})	2.30
Total Manganese (mg L^{-1})	615.00
Total Copper (mg L^{-1})	0.89
Boron (mg L^{-1})	7.60
Calcium (mg L^{-1})	439.00

Co – Composting Trials and Physiochemical Analysis

The co-composting trials were run in the Chemical Environmental Engineering Laboratory, Universiti Malaysia Sabah. Approximately 40 g of EFB was mixed with 72.40 g of POME for every different set of compost. The mixtures were prepared using the following conditions with Set 1 as control:

Set 1: Untreated EFB + POME (control)
Set 2: Untreated EFB + POME + Inoculants
Set 3: Pre-treated EFB + POME
Set 4: Pre-treated EFB + POME + Inoculants

For Set 3 and 4, the EFB was soaked in 1% w/w NaOH at a solid-liquid ratio of 1:10 (Binod et al., 2012). Microwave pretreatment was carried out in a domestic microwave (Model: ELBA, EMO-A2072(SV)) at a power of approximately 487 W for an exposure time of 4 minutes (Binod et al., 2012). The output power was determined by measuring the temperature difference and then using the formula below (Gallawa, 2013):

$$\text{Output power} = (T_2 - T_1) \times 70 \qquad (1)$$

where T_1 is the initial temperature and T_2 is the final temperature.

For Set 2 and 4, inoculants were added to POME before composting. Each mixture was carefully homogenized; moisture was adjusted to 70% (optimum value for composting). The duration of composting was 60 days. The composting experiment was performed in three replicates in vertical plastic bottles (1.5 L) with 1/4 upper part cut off and left uncovered. The addition of POME was carried out on the 0, 25th, 44th and 57th days, when the moisture content of the mixture fell below 60% and at the same time the mixture was being turned.

The temperature was measured at 5 day intervals. The pH and conductivity of every set of compost were determined on the aqueous extract of the compost using a pH meter (Hanna Instrument (Model:HI 9811-5)) by adding 20 g of the sample to 100 ml of distilled water, mixing with magnetic stirrer for 20 minutes, allowing the mixture to stand for 24 hrs and then filtering (Zahrim et al., 2007). Moisture content was determined by drying the sample at 105°C for 24 h (Zahrim et al., 2007). Total organic carbon was calculated after calcination in a furnace at 550°C for 4 h. Organic matter loss was determined by the equation below (Paredes et al., 2000), (Zahrim et al., 2007):

$$OM\ loss\ (\%) = 100 - 100\frac{[Ash_i(100 - Ash_f)]}{[Ash_f(100 - Ash_i)]} \qquad (2)$$

Where Ash_i is the initial level of ash and Ash_f is the final level.

Zeta Potential Analysis

The aqueous extract of the compost was used as the sample for zeta potential analysis. Undiluted samples were used and tested using a Malvern-Zetasizer Nano Series model ZS.

Phytotoxicity Test

To determine the germination index (GI), cabbage seeds were used and soaked in distilled water for 48 hours with the distilled water being changed every 24 hours.10 cabbage seeds were tested in 5 ml of water-soluble extracts of compost (from 20 g of sample into 100 ml of distilled water) in petri dishes on a piece of filter paper in a dark cupboard at room temperature for 3 days. Another 10 cabbage seeds were tested in 5 ml distilled water on just a piece of filter paper as the control. Two replicates were made. The number of germinated seeds was counted and the growth of roots was measured using the grid intersection method (Rowell, 1994) after 3 days. The percentage of relative seed germination (RSG), relative root growth (RRG) and germination index (GI) were calculated according to the following formulae (Miaomiao et al., 2009):

$$RSG\ (\%) = \frac{number\ of\ seeds\ germinated\ in\ sampple\ extract}{number\ of\ seeds\ germinated\ in\ control} x100 \quad (3)$$

$$RRG\ (\%) = \frac{root\ length\ in\ sample\ extract}{root\ length\ in\ control} x100 \quad (4)$$

$$GI\ (\%) = \frac{RSG}{RRG} x100 \quad (5)$$

Statistical Analysis

The average value and standard deviation of the data were calculated using Microsoft Excel. The standard error was computed and errors bars were determined for the data.

RESULTS AND DISCUSSION

pH

In this study, the composting process mainly undergoes mesophilic composting at a temperature that varies from 25.9˚C to 29.2˚C. The changes in pH of different sets of compost are shown in **Figure 1**. The initial pH values for control, untreated EFB + POME + inoculants, pretreated EFB + POME and pretreated EFB + POME + inoculants were 6.4, 6.5, 6.9 and 6.7 respectively. The pH changes for control and untreated EFB + POME + inoculants exhibited a different pattern from that of pretreated EFB + POME and pretreated EFB + POME + inoculants for the first 20 composting days; for the remaining composting days, similar patterns of pH change were presented by all 4 sets.

For the first 20 days, the pH for Sets 1 and 2 increased from 0 – 10 days followed by a decrease until 20 days; pH for set 3 and 4 decreased from 0 – 10 days followed by an increase until 20 days. Generally, the pH was maintained in the range of 6.4 – 9.5. The pH change is due to the microbial activity (Kananam et al., 2011).

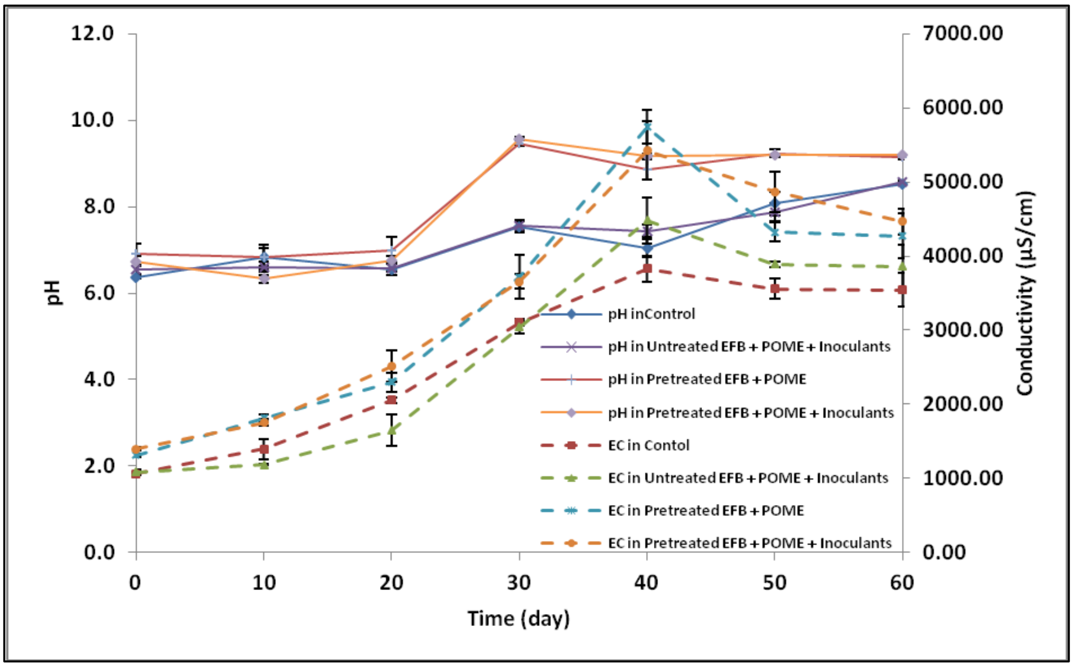

Fig. 1: pH and electrical conductivity versus time during composting process for untreated and pretreated EFB under different conditions. EFB denotes Empty Fruit Bunch, POME: Palm Oil Mill Effluent

At the beginning, a slight decrease in pHnoted for pretreated EFB + POME and epretreated EFB + POME + inoculants can be explained by the production of organic acids. These result from dissolved CO_2 in the medium and by-products from the degradation of easily degradable compounds such as polysaccharides and fats (Yang et al., 2016). On the other hand, the general increase for all sets later can be explained by production of ammonia from the degradation of amines such as proteins and nitrogenous bases which releases bases existing in the organic waste (Ouatmane et al., 2000). According to Arrhenius theory, bases will dissociate in aqeous solution to produce hydroxide. This dissociation of bases in aqueous solution is summarised below.

$$NaOH\ (aq) \rightarrow Na^{+}\ (aq) + OH^{-}\ (aq) \quad (6)$$

(Lister and Renshaw, 1991)

$$NH_4OH\ (aq) \rightarrow NH_4^{+}\ (aq) + OH^{-}\ (aq) \quad (7)$$

(Mittal, 2002)

The increase in pH is generally thought to be the result of volatilization and microbial decomposition of the organic acids and subsequent release of ammonia through mineralization of organic nitrogen sources (Pan and Sen, 2013).

Microwave irradiation was able to cause the deposition metallic looking silica spheres (Tuval and Gedanken, 2007). From previous studies, there were many silica bodies found on the surface of EFB strands (Baharuddin et al., 2009, Zainudin et al., 2014). Similarly in this study, the inorganic metals, sodium ions from sodium hydroxide might attach to the silica bodies on the lignocellulosic surface to form sodium silicate. The bonds in sodium silicate can be strengthened through application of microwave radiation (Jina et al., 2009). Sodium ions can be attached on fibre through Donnan equilibrium (Stenius, 2011). In addition, the difference in osmotic pressure and electrical potential between the lignocellulosic surface with more negative charge than the bulk solution can result in the movement of Na^{+} to the fibre. The selectively permeable lignocellulosic membrane only allows passage of certain charged ions, such as Na^{+}. Na^{+} attaches to the fibre in an attempt to balance the large negative charge on the fibre (Philipse and Vrij, 2011). During composting, silica and sodium might gradually detached from the EFB structure. Generally, the control and untreated EFB + POME + inoculants exhibited lower pH due to the sole presence of ammonia ions. The elevated pH of pretreated EFB + POME and pretreated EFB + POME + inoculants might be due to the combined presence of sodium ions and ammonia produced during composting.

Electrical Conductivity

The variation of conductivity reveals the extent of mineralization of the organic substrate and the release of the ionic loads into the medium (El Fels et al., 2014). The electrical conductivity changes for the control, untreated EFB + POME + inoculants, pretreated EFB + POME and pretreated EFB + POME + inoculants, presented in **Figure 1**. The electrical conductivity change for every set of compost exhibited a similar pattern by which electrical conductivity increased initially from 0 day until a maximum value

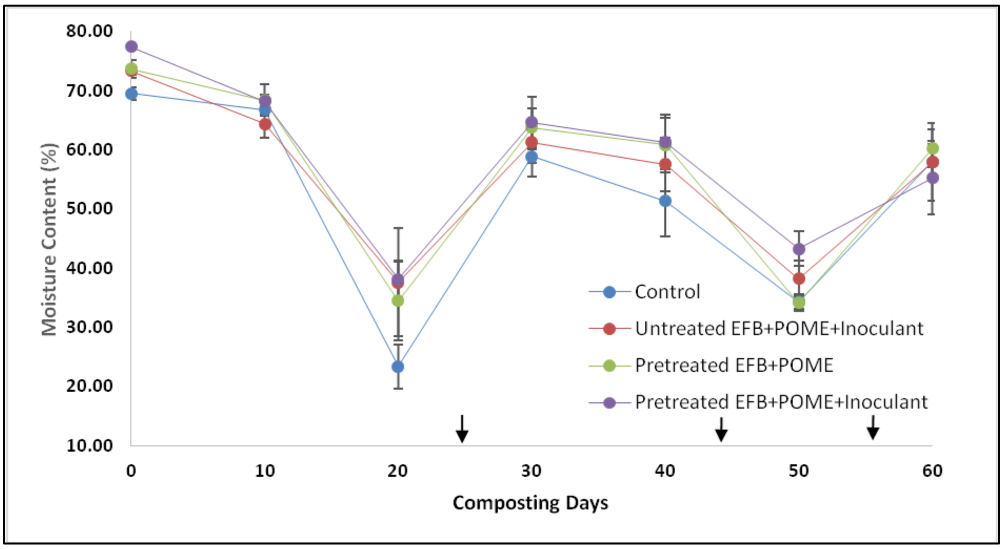

Fig. 2: Moisture Content versus time during composting process for untreated and pretreated EFB under different conditions. EFB denotes Empty Fruit Bunch, POME: Palm Oil Mill Effluent. Arrows indicate the addition of POME on the 25th, 44th, and 57th day.

of 3830, 4483, 5740 and 5423 μS/cm respectively on day 40, followed by a decrease until the end of the composting process. This increase of electrical conductivity might be caused by the release of mineral salts and ammonium ions from the decomposition of organic matter (Yang et al., 2016). The volatilization of ammonia and precipitation of mineral salts resulted in the decrease of electrical conductivity while the composting process continued (Gao et al., 2010).

For mature and safe compost, the proper value of electrical conductivity should be less than 4000 μS/cm as a value exceeding this value would have an adverse effect on plant growth, resulting in low germination rate and withering of plants (Lin, 2008). The final electrical conductivity values of control and untreated EFB + POME + inoculants were 3540 and 3863 μS/cm respectively, i.e. less than 4000 μS/cm, indicating that they are safe for plants; final electrical conductivity values of pretreated EFB + POME and pretreated EFB + POME + inoculants were 4270 and 4463 μS/cm respectively, which were slightly higher than 4000 μS/cm, indicating that the compost is insufficiently stable favourable for plant growth. The higher electrical conductivity for pretreated EFB + POME and pretreated EFB + POME + inoculants was due to the combined presence of Na^+ ions and silica; Control and untreated EFB + POME + inoculants exhibited lower electrical conductivity due to the sole presence of silica. High electrical conductivity is reported to be unfavourable for plant growth. Thus, compost production via pretreated EFB + POME and pretreated EFB + POME + inoculants with high electrical conductivity needs to be mixed with other compost with lower electrical conductivity to make it usable (Vakili et al., 2012).

Moisture Content

The evolution of the moisture content of the different sets of compost is shown in **Figure 3**. Moisture content is a critical factor to optimize the composting system because the microbial dependence on water to support growth could affect the biodegradation of organic matter (Hock et al., 2009). The main mechanism of water removal in this composting process was the evaporation of water d microbial heat generation as well as natural aeration, which dries the compost material continuously. The continuous decrease in the moisture content during composting is an indication of organic matter decomposition (Kulcu and Yaldiz, 2004).

In this study, POME was added to maintain the optimum condition between 50 and 70% (Ahmad et al., 2011). The initial moisture content for the control (Set 1), untreated EFB with POME and inoculant (Set 2), pretreated EFB with POME (Set 3), and pretreated EFB with POME and inoculant (Set 4) are 69.6%, 73.4%, 73.68% and 77.4% respectively. Each set of compost exhibited the same pattern throughout the composting process; that is, a decrease in moisture content prior to addition of POME. The final moisture content for the different compost sets were 58.0 % (Set 1), 57.9 % (Set 2), 60.2 % (Set 3) and 55.3 % (Set 4).

Total Organic Content (TOC)

The total organic carbon of the compost is measured to assess the rate of decomposition in the composting process. From **Figure 4**, it can be seen that the total organic carbon percentage of each set decreases slightly through composting.

The highest organic matter loss was exhibited by the pretreated EFB with POME which gave 63.76% organic matter loss. This was followed by the control (untreated EFB), pretreated EFB with POME plus inoculant and then untreated EFB with POME plus inoculant with organic matter loss of 55.44%, 36.81%, and 32.43%, respectively. Highest OM loss for

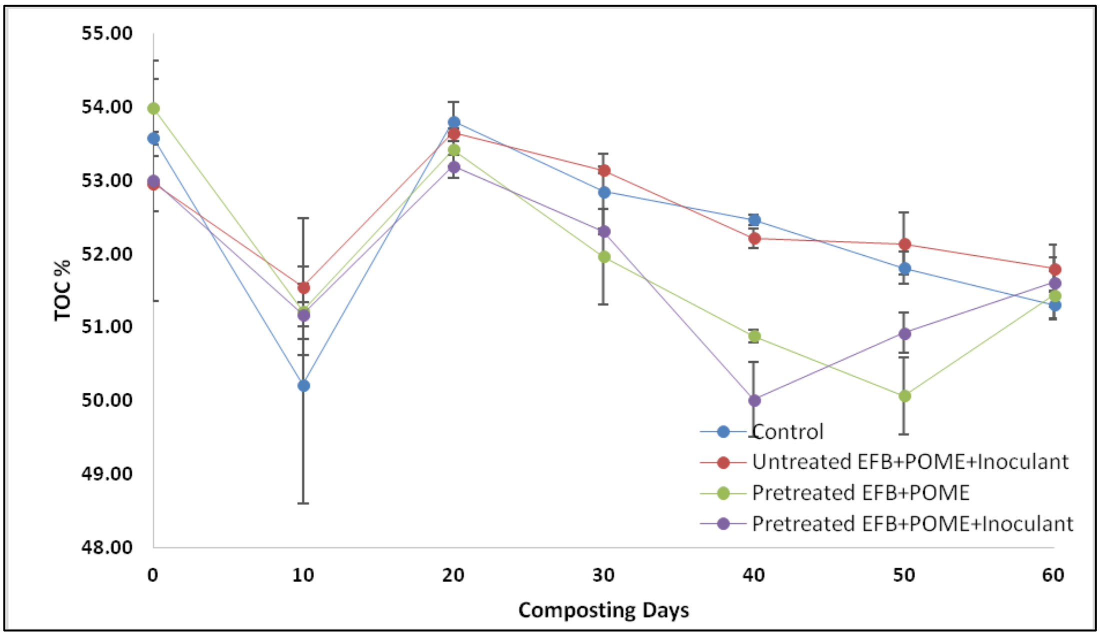

Fig. 3: Percentage of total organic carbon versus time during composting process for untreated and pretreated EFB under different conditions. EFB denotes Empty Fruit Bunch, POME: Palm Oil Mill Effluent.

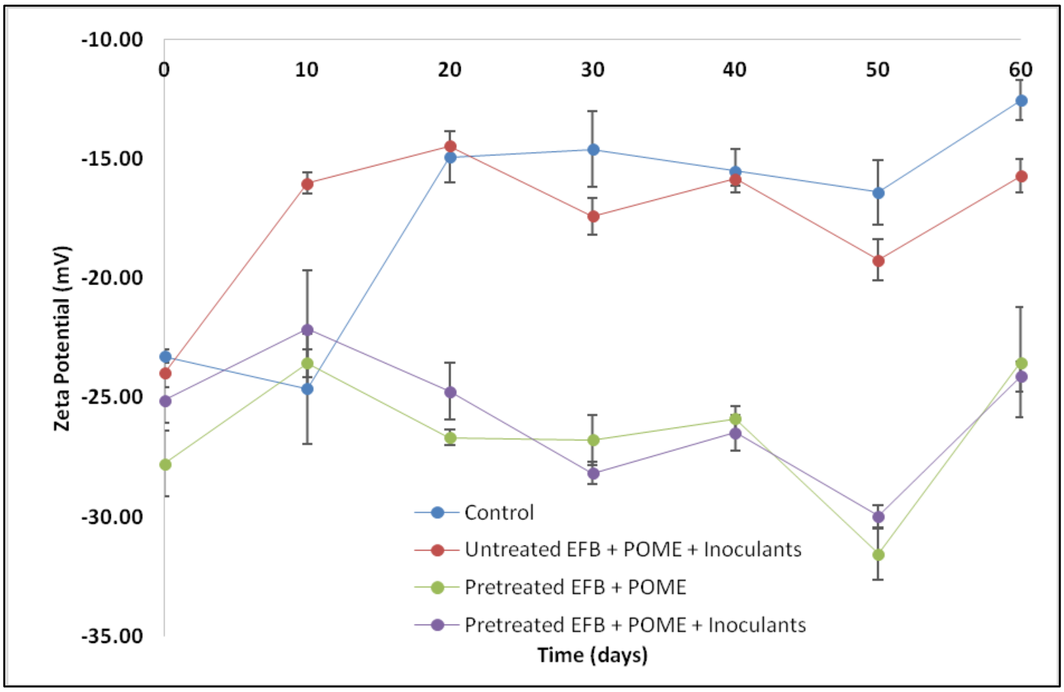

Fig. 4: Zeta Potential versus time during composting process for untreated and pretreated EFB under different conditions. EFB denotes Empty Fruit Bunch, POME: Palm Oil Mill Effluent

pretreated EFB is due to modifications in hemicellulose structure or the disruption of some linkages between hemicellulose, cellulose and lignin during the pretreatment (Diaz et al., 2015); this induces the indigenous microorganisms to utilize more organic matter. However, inoculation of *Bacillus amyloliquefaciens D203* inhibiting the utilization of OM might be due to the antifungal properties of *Bacillus amyloliquefaciens D203;* this may have inhibited the growth of mesophilic fungi in the compost hence reducing the biodegradation rate (Alvarez et al., 2012, Ji et al., 2013).

The OM loss in this study (32%–63%) was comparable to the results reported by (Zhao et al., 2016), which had a maximum OM loss of 34%-54%. On the otherand, the OM loss in composting of some agricultural waste was also reported to be in the range of 42%-58% (Kulcu and Yaldiz, 2004), which lies within the range found in this study. Moreover, (Petric and Mustafic, 2015) reported values of 37%-50% in composting of wheat straw with poultry manure.

Zeta Potential

The surface charge was measures using a zetasizer for compost (**Figure 8).** The zeta potential for Sets 1 and 2 show a lower initial and increase with composting time. However, the NaOH – microwave pretreatment might have led the particle surface to be dominated by the presence of carboxyl (-COOH), carbocylate (-COOH) and alcoholic groups (-OH) (Sun et al., 2002, Bellmann et al., 2004); this could cause the decrease of zeta potential value as the compost tends to become more hydrophilic (Zahrim et al., 2014, Bellmann et al., 2004, Zahrim et al., 2016). In addition, silica bodies might be removed during pre-treatment. In Set 1 and 2, the concentration of silica in the solution increases and cause the deposition of silica onto the EFB surface whereas in Set 3 and 4, Na^+ is absorbed on the EFB surface.

Phytotoxicity

The germination test has usually been used to evaluate the compost maturity and phytotoxicity of biowastes (Miaomiao et al., 2009). This index had been proven to be a more sensitive parameter to illuminate both low toxicity affecting root growth and high toxicity affecting germination (Zucconi et al., 1981). **Figure 6** presents results of seed germination inhibition and root growth for the cabbage seeds, taken every 10 days during composting. The germination indices (GI) obtained for each set of compost demonstrated a trend of decreasing phytototxicity with composting time.

After the first month of composting, there is a significant increase in the germination index, especially on the 30th day for all sets of compost; after the first month of composting this index (GI) underwent a slight decline. As composting proceeded, GI increased and reached ~ 60 (for both pretreated compost samples i.e. Sets 3 & 4) and > 80 (for both untreated compost samples i.e. Sets 1 & 2) at the termination of composting. (Zucconi et al., 1981) reported that GI's above 80% indicated the disappearance of phytotoxicity in compost, while GI > 50% indicated no detriment to olive plant growth (Tam and Tiquia, 1994, Zucconi et al., 1981).

In summary, high temperature microwave pretreatment causes the formation of complex refractory products and inhibitory digestion compounds that

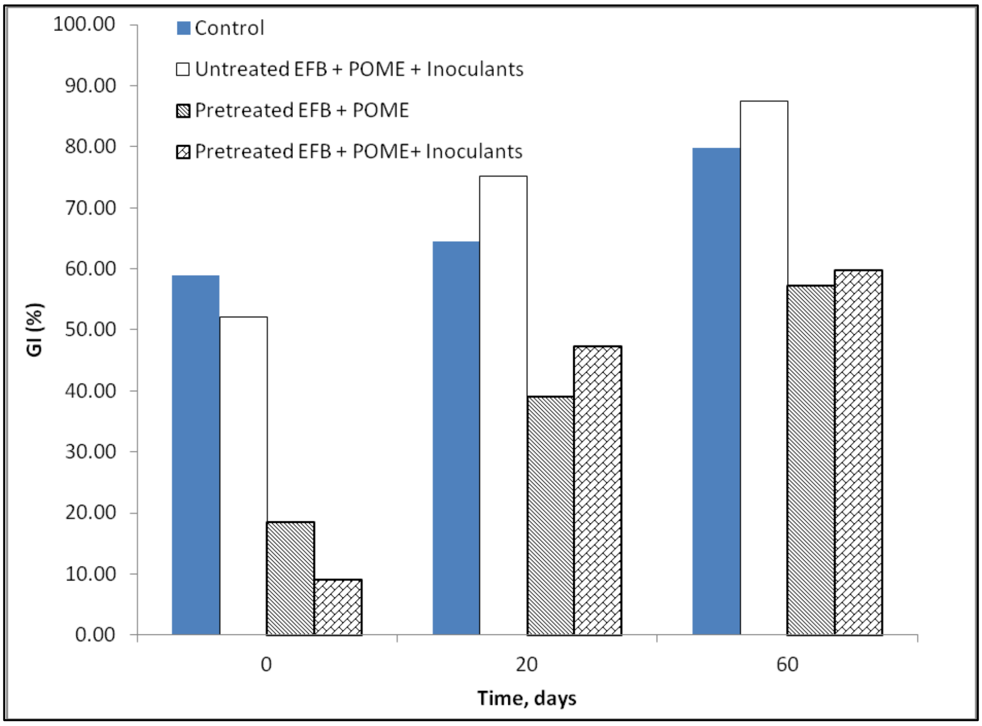

Fig. 6: GI versus time during composting process for untreated and pretreated EFB under different conditions. EFB denotes Empty Fruit Bunch, POME: Palm Oil Mill Effluent

affect the pretreated EFB composts. These show smaller GI values as microwave pretreatment of biomass did not improve the digestion of waste when compared with untreated EFB compost (Shahriari et al., 2013). It was reported that the present of furan compounds produced from hydrothermal pretreatment inhibited the microbial activities of composting microorganism, thus delaying the start of degradation of organic matter in pretreated EFB composting (Nakasaki et al., 2015). For compost without inoculation, the compost was characterized by higher phytotoxicity, synonymous with results which show that inoculation composts have the lower phytotoxicity (Piotrowska-Cyplik et al., 2013). Interestingly, inoculant is found to increase the GI for pretreated compost, which is supported by other research (Nakasaki et al., 2015). Phytotoxicity or poor plant response can result from several factors such as lack of oxygen due to high microbial activity, the accumulation of toxic compounds (organic acids), the immobilization of nitrogen with high C:N ratio, high ammonia concentration and the presence of heavy metals and mineral salts (Tiquia, 2010). These factors influence seed germination simultaneously and it is difficult to assess which parameter has the greatest influence. The immobilization of nitrogen at high C:N ratio and with high ammonia concentration contributes to compost phytotoxicity (Tiquia, 2010). A GI > 60 (the threshold limit for compost to show maturity) was reached as the composting time proceeded (Gaind, 2014). It is believed that composting strategies affected the speed of composting, time of maturation and disappearance of phytotoxicity (El Fels et al., 2014).

CONCLUSIONS

The effects of microwave pretreatment and inoculants on composting process in mesophilic condition were investigated. It was found that microwave-assisted NaOH pretreatment without inoculant could reduce the OM by about 15% more than without pretreatment. However, the pre-treatment might produce phytotoxin that could inhibit seed growth. The inoculant in this study seems not to work well in the pretreated environment. Microwave-assisted NaOH pretreatment could enhance the composting process with the addition of suitable inoculants. In addition, microwave-assisted pretreatment without NaOH should also be investigated in the future. The inoculants enhance the composting process for the untreated EFB. The pH increased from 6.5 to 8.6 and the electrical conductivity increased up to a final acceptable value of 3863.33 µS/cm. The germination index (GI) increased from 57% to 87%, which shows it is free from phytoxicity.

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