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Assessment of Compost Stability using Single and Mixed Culture in a Static Semi-Closed Fed-Batch Reactor

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Different microbial inoculums are expected to affect the degradation and stability of different compost materials. The results tend to vary more significantly in an open system due to the varied ambient conditions (temperature, moisture and air-borne microbial). To minimise such variation and avoid low reproducibility of the data, the present work was performed in a static semi-closed fed-batch reactor (SSCFBR) for better control of the composting process. The thermophilic *Bacillus coagulans* (BC) and the commercial effective microorganisms (EM) were used as the microbial inoculum (MI) in the SSCFBR. Distilled water was used in place of the MI for the control experiments. The mixture of chicken dung (CD), wooden husk (WH), and rice husk (RH) were used as the compost materials. The MI was inoculated into the compost bed in the SSCFBR (15 L working volume) at the same initial optical density (OD) of 0.8 and the initial volume of 800 mL with a forced aeration of 0.4 L/min. The parameters for the assessment of compost stability included the temperature, moisture content, oxygen uptake rate (OUR) and carbon dioxide emission rate (CER). BC showed comparable or better results to that achieved by the commercial EM. The temperature profile for both composts showed similar patterns where the highest peak of temperature was recorded within 1 d of composting (slightly higher for BC, 55 ± 0.8 °C and 47 ± 0.8 °C for EM). Such thermophilic temperature profile has not been observed in the control. The initial moisture content was set similar for all composts (44.5 ± 0.7 %). The moisture content declined slightly different for both MI compost (to 39.0 ± 0.6 % for BC and 36.6 ± 1.2 % for EM). Both composts showed very similar trend in the OUR. A higher evolution rate of carbon dioxide (CO_2) was observed for the compost with BC for the first three days (0.06 ± 0.014 g/mol) as compared to that by EM (0.02 ± 0.007 g/mol). On the 4th d of composting, the production of CO_2 in EM compost showed a near constant value (0.008 ± 0.002 g/mol) but the BC compost showed the rate of 0.06 ± 0.01 g/mol the 5th d and declined to 0.02 ± 0.004 g/mol on the 7th d. For both composting cases, the highest rate of CO_2 was observed at the highest peak of temperature. The SSCFBR has been designed and could be used to facilitate the assessment of compost stability in a closed system with improved reproducibility of the data for composting.

1. Introduction

Composting is widely operated in an aerobic system and represents a natural biological process by microbial to degrade the organic waste into valuable products. Rapid urbanisation and industrialisation have caused the degradation of soil and its microbial community. Addition of microbial inoculant (MI) is recommended for composting especially when degrading organic wastes that take longer to be degraded such as the hemicellulose, cellulose and lignin materials (El-Akshar et al., 2012). The parameters monitored during the open composting tend to vary more significantly due to the varied ambient conditions (temperature, moisture and air-borne microbial). To minimise such variation and avoid low reproducibility of the data, the present work was performed in a static semi-closed fed-batch reactor (SSCFBR). This study investigated the effectiveness of *Bacillus coagulans*, a thermophilic microbe, for the degradation of a model kitchen waste. The SSCFBR was designed to facilitate the study of the microbial communities including the microbial profiles, succession and

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competition during the biodegradation process at different thermal gradients (Narihiro and Hiraishi, 2005). The technique of feeding compost raw materials into a fed-batch reactor has been widely applied for the composting of kitchen residues, backyard composting and classroom composting (Mori et al., 1995). Fed-batch composting is a suitable mode for the daily introduction of waste where the operation is applied to small- and medium-scale of composting reactors (Nakasaki et al., 2000).
In this study, two types of MI were applied; the thermophilic bacteria *Bacillus coagulans* (BC) and the commercial Effective Microorganism (EM). The thermophilic microbe BC was preferred due to its advantageous of growing and fermenting at high temperature from 50 to 60 °C with simple nutrition requirement (Payot et al., 1999). An initial moisture content of compost at 35 to 55 % was preferable (US Composting Council, 2001) to ensure rapid degradation. Energy is formed during the degradation of organic materials expressed by the increase of temperature. High temperature stimulates the proliferation of thermophilic microorganism to increase the microbial activity and enhance the degradation of the organic matter. This study aims to assess the performance of the SSCFBR in evaluating the effectiveness of BC against the commercial EM for composting. The effectiveness of the MI was evaluated based on the temperature profile, moisture oxygen uptake rate (OUR) and carbon dioxide emission rate (CER).

2. Materials and Methodology

2.1 Composted Materials

The input materials for the composting consisted of wooden husk (WH), rice husks (RH) and chicken dung (CD) (Sarjani (M) Sdn. Bhd, Batu Pahat, Johor). The input materials have the mean sizes of 5 to 25 mm (Haug, 1993). Table 1 presents the main characteristics of the input materials used for this study. The initial C/N ratio for all experiments was fixed at 30. All input materials were autoclaved (150 L Selecta, Barcelona) for 15 minutes at 121 °C to eliminate anonymous indigenous microorganisms in dry composted material thus, ensured the process occurs in very control condition and less uncertainty disorder with known microorganisms to be inoculated during composting.

Table 1: The main characteristics of the input materials for the composting in this study

Parameter	Wooden Husk	Rice Husk	Chicken Dung
Organic matter (%)	44.8	34.3	27.1
C/N ratio	43.7	20.3	6.40
pH	7.3	5.4	8.1
Total organic carbon (%)	26.0	19.9	15.7
Total N (%)	0.79	1.3	3.26
Total C (%)	34.5	26.4	20.9
Total P (ppm)	6258	3939	4329

2.2 Microbial Inoculations

The B. coagulans ATCC 7050 was purchased from Arachem (M) Sdn. Bhd. It was stored at -20 °C before cultured in the incubator shaker (ST-250DR, Sastec, Malaysia) at 37 °C for 50 h in the yeast extract and glucose (YEG) (20 g yeast extract, 20 g glucose, 5 g sodium acetate, 2 g potassium phosphate, 0.2 g magnesium sulphate, 0.2 g manganese sulphate, and 1 L distilled water; at pH 4.8). The culture was then added to the composting materials to certain value of moisture content.
EM and molasses were purchased from EMRO (M) Sdn. Bhd (Johor, Malaysia). It was stored in the room temperature before used. 5 % of EM and 5 % of molasses were mixed in the distilled water and incubated for 7 d to form the EM activation solution where the pH dropped below 4.0.

2.3 Composting Experiments in the Static Semi Closed Fed-Batch Reactor (SSCFBR)

Figure 1 shows the schematic diagram of the Static Semi-Closed Fed-Batch Reactor (SSCFBR) used to conduct the aerobic composting in this study. The SSCFBR (0.25 m in width, 0.25 m in height and 0.30 m in length; working volume of 15 L) was modified and used as the model reactor. Five air-tight holes (sealed with rubber stoppers) were created to hold a thermometer, for inlet of air and air vent outlet of gas mixture, air ventilation and sampling outlet. The entire SSCFBR was insulated with the polystyrene foam (2 cm in thickness). Forced aeration using an air compressor (VT6271, Campbell Hausfeld, USA) was continuously operated at the air flow-rate of 0.4 L/min/kg compost for 7 d until the end of composting. The airflow rate was measured using an airflow

meter (Valve Ø 1.5 cm, Krohne, USA). The system was ventilated via the 1-cm air vent hole. Sampling was carried out through an outlet air-tight valve (6-cm in diameter) which allowed the manual sampling using a spatula (5 cm in diameter, 15 cm in length). The compressed air was continuously fed into the reactor, the formation of water vapour released during the composting was assumed to escape from the moist compost bed via the air vent outlet.

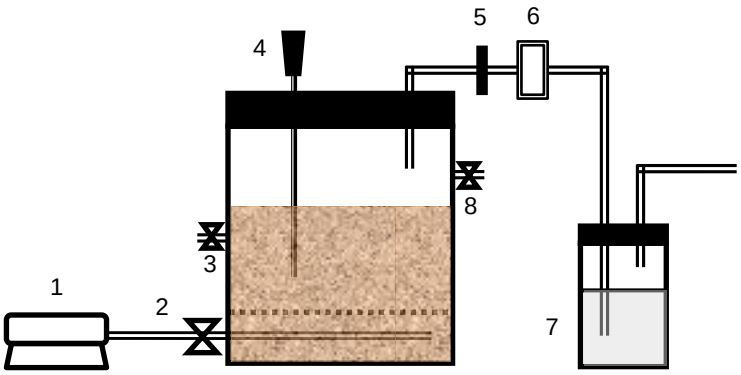

Figure 1: The schematic diagram of the static semi closed fed batch (SSCFB) reactor. (1. Air compressor, 2. Air flow meter, 3. Sampling outlet, 4. Thermometer, 5. Filter, 6. Gas analyser, 7. CO_2 trap with sodium hydroxide, 8. Air vent outlet)

One kg of sterilized composting input material was added with two types of microbial inoculants (MIs) (i.e. BC or the EM, the initial MI has an OD of 0.8), each has the volume of 800 mL. The mixture was homogenised manually before being transferred to the SSCFBR. The compost was sampled at 0, 1, 3, 5 and 7 d through the sampling port. The composting process was held over 7 d until the bed temperature reached the ambient temperature. All samples were analysed daily in triplicate, except for the bed temperature in the SCFBR which was recorded twice a day until the 7th d. The bed temperature was measured using the thermometer placed in the middle of the bed with the ambient temperature recorded to minimize the temperature gradient along the reactor (Ekinci et al., 2004). O_2 gas was measured using the gas analyser (Gas Alert Micro 5, BW Technologies, UK). The CO_2 gas was measured using the chemical standard analysis of CO_2 trap method (Cheng and Coleman, 1989) with modification using 100 mL of 0.5 M sodium hydroxide NaOH followed by titration using 0.5 M HCl as shown in Figure 1 and Eq(1). The CO_2 in gas was quantified by measuring the CO_2 gas that has reacted with the NaOH to produce carbonic acid as shown in Eq(1).

$$CO_2.C\ (mg) = \frac{HCl_b - HCl_s}{1000\ ml/L} \times 0.5\ HCl\ molarity\ \left(\frac{mol}{L}\right) \times 12\ g\frac{C}{mol} \times \frac{1000\ mg}{g} \quad (1)$$

where HCl_b is the mL of HCl used in the titration for the blank sample, HCl_s is the HCl used for the titration of the sample and CO_2.C is the mass of CO_2-carbon generated (mg) and 12 g/mol C represents the number of mole for carbon. The moisture content of the composted material was quantified based on the drying process at 100 °C for 24 h (El Zein et al., 2015) in the oven (UM 500, Memmert, Netherlands). The samples were cooled in a desiccator for 1 h until a constant weight. The samples were then burnt in a furnace (FN-1016, Constance, Germany) at 550 °C for 4 h, and cooled again in a desiccator for 4 h to a constant weight. The weight difference before and after the burning was used to calculate the organic matter (OM) content and the total organic carbon (TOC) (Nutongkaew et al., 2014). Each analysis was in triplicate with the mean value reported.

3. Results and Discussion

3.1 Bed Temperature Analysis

The temperature profiles for the compost samples inoculated with BC and EM are illustrated in Figure 2. The results show a significant effect for both the MI (BC and EM) to accelerate the temperature profile during composting. All input materials were initially autoclaved including the control. The filter installed in the SSCFBR has prevented the entrance of naturally occurring microorganisms into the bed. No lag phase was recorded for the temperature to rise to the highest peak within 1 d of composting. It was likely the initial compost was assisted with the MI and contained readily degradable material such as the cow dung (CD). Temperature rise in both composts showed that the aerobic composting was effectively conducted in the SSCFBR. Only a slight

temperature difference between both the composts was observed. The active thermophilic phase was considered ended within the compost bed when the temperature became stable and near ambient temperature. The compost inoculated with BC reached a slightly higher maximum temperature compared to that by EM inoculation (for BC, 55 ± 0.8 °C and for EM, 47 ± 0.8 °C). No increment of temperature was observed for the control composting without the MI.

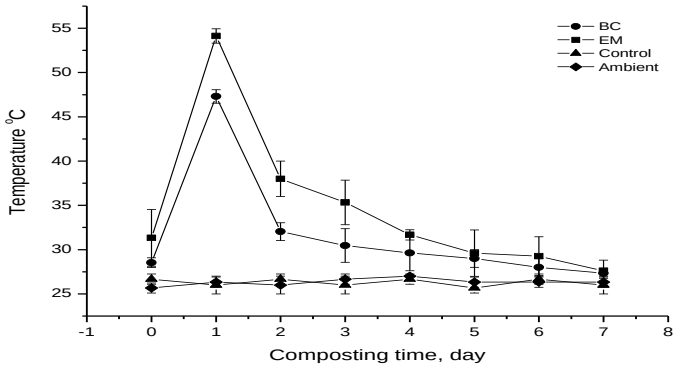

Figure 2: Temperature profiles for the composting inoculated with BC and EM

3.2 Moisture Analysis

The initial sterilized input material was added with 800 mL of MI (EM or BC inoculum, OD 0.8). No further liquid was added to the compost bed during the composting process. The moisture contents (%) for both composts, either with BC and EMare shown in Figure 3.

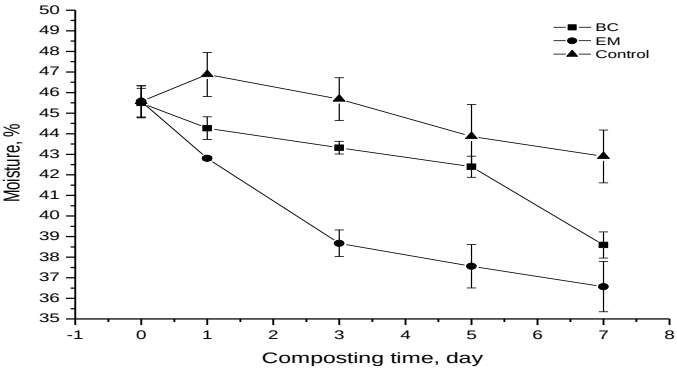

Figure 3: The moisture content profiles for the composting with BC and EM

The moisture content has reduced from 45 % to 39 %, 45 % to 36.6 % and 45 % to 43 % for composts inoculated with BC, EM and control, respectively along 7 d. Both composts with MI showed an enhanced level of moisture content reduction in tandem to the higher temperature profile achieved in Figure 2. Ideally the final compost should have the final moisture content closer to 30 % (Bari, 1999). Rapid moisture loss during composting will cause early dehydration that is possible to stop the biological process. Rapid drying provides a good physical stability to the compost but biologically rather unstable (Makan et al., 2013). Composting can proceeds well for rapid biological degradation at the initial moisture content between 35 to 55 % (US Composting Council, 2001). At moisture levels lower than 40 %, the microbial activity would be limited, while at higher levels (> 60 %), the process is likely to become anaerobic and foul-smelling. Intermittent addition of liquid was essential to promote the completion of the biodegradation process until a constant temperature profile close to the ambient temperature was finally achieved. This was however not the scope of the current study. The lowest moisture loss (2 %) was observed in the control compost due to the lack of microbial activity as evidenced by the low temperature profile in Figure 2.

3.3 Organic Carbon Content

The production of CO_2 is caused by the mineralization of carbon organic content in the composted materials (Bernal et al., 1998). The organic matter (OM) content of the composted materials decreased along the 7 d of composting. Figure 4 shows the evolution of the CO_2-C changes and TOC loss in the SSCFBR.

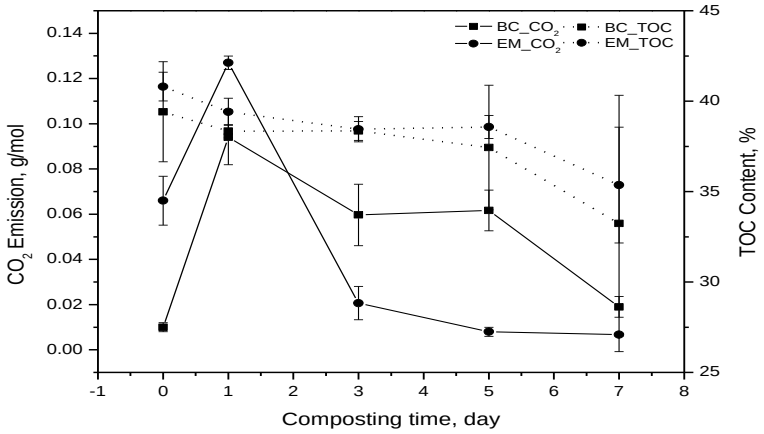

Figure 4: Evolution of CO_2 and TOC loss during composting

Figure 4 shows that both composts (BC and EM) recorded the highest mass loss of CO_2-C where the highest temperatures of the thermophilic phase were recorded in Figure 2. Easily degradable organic carbon compounds such as the CD were degraded during the first two to three days during the initial active phase with high mineralization rate. It was soon followed by a gradual decrease for both composts before the rate became more constant. The highest emission of CO_2 for both composts (slightly higher in EM (0.13 g/mol) compared to BC (0.09 g/mol) occurred after 1 d of composting. The CO_2 emission results in both composts corresponded well with the results reported by the TOC loss profiles (Figure 4). The emission of CO_2 reduced gradually until fairly constant at day 7. The relative high emission of CO_2 during the thermophilic phase indicates a high degree of OM or TOC degradation.

3.4 Oxygen Uptake Rate in Composting

Oxygen (O_2) can be supplied by the forced aeration to facilitate the growth of the aerobic bacteria to speed up the composting process. High decomposition rate was accomplished in a continuously and adequate O_2 supply to the bed. The O_2 concentration in the ambient air (21 %) is approximately four times higher than the 5 % needed for a reasonable composting rate (Cundiff & Mankin, 2003).
Figure 5 shows the consumption rate of O_2 for both composts with BC and EM. The O_2 consumption rate declined quite equivalently for the compost with EM (20.4 % to 17.6 %) and the compost with BC (20.3 % to 19.2 %) within the 1st d of composting. Both composts with MI showed a minimum of O_2 concentration higher than the threshold lower limit (12 to 14 %) as reported by Fraser and Lau (2000) for composting.

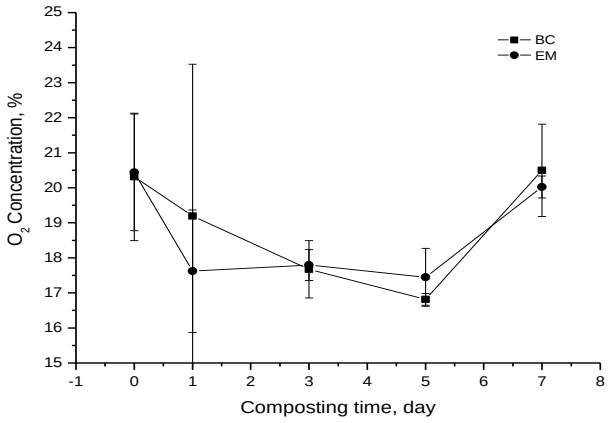

Figure 5: Consumption of O_2 during the composting with BC and EM

These results indicated that the composting has achieved a good level of stability. The high consumption rate of O_2 within the 1st day of composting corresponded well with the temperature profile and the emission of CO_2 and TOC loss during composting. However the increase trends of the O_2 consumption beyond 5 to 7 d have not corresponded well to other parameters as measured. It could be due to the pick-up of the mesophilic microbes; however more studies are needed to verify the results.

4. Conclusion

A composting system termed as the Static Semi-Closed Fed-Batch Reactor (SSCFBR) was designed and used to characterise the effectiveness of BC for composting as compared to the commercial MI, EM. Both the BC and EM showed reasonably reproducible results following 7 d of composting. The performance of the single strain of BC provided comparable results to the EM. Both composting with MI showed a more superior performance as compared to the control in the absence of MI. A conclusion might be difficult to be drawn under an open-composting system due to various interferences. The SSCFBR could be used to evaluate the complex performance of composting with improved reproducibility.

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