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Determination of Minimal Fractionation Period Required for Extracting Humic Acid from Paddy Husk Compost

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Abstract: The process of isolating humic substances, especially, humic acid (extraction, fractionation and purification) is time consuming, laborious and expensive. The objective of this study was to determine the minimum period required to fractionate humic acid from paddy husk compost. The rice straw compost was produced by mixing 80% of shredded rice straw, 10% of goat manure slurry, 5% of chicken feed and 5% of molasses. During the extraction process, 10 g of compost was placed in the centrifuge bottle, added with 100 mL of 0.5 M NaOH. The samples were equilibrated at room temperature for 24 h. After that, the mixture was centrifuged at 10, 000 G for 15 min. During the fractionation process, the pH of the solution was adjusted to 1.0 by using 6 M hydrochloric acid. Then the humic acid was allowed to equilibrate for 1, 3, 5, 7, 9, 12 and 24 h, respectively and the suspension was centrifuged. Purification was done subsequently to purify the humic acid.The yield of humic acid fractionated under 1 h was 0.26 g and was not statistically different from 3, 5, 7, 9, 12 and 24 h. This indicates the fractionation period of 1 h also had the same time taken as 3, 5, 7, 9, 12 and 24 h for the exchange sites of humic acid of compost to be saturated with H^+ions after acidification. Phenolic, carboxylic and total acidity were determined and falls within the range reported by literatures. The optimum fractionation period of humic acid from paddy husk compost was 1 h as the yield of humic acid under different fractionation periods were not significantly different. Therefore, by using 1 h of fractionation period it will absolutely save the consumption of chemicals, electricity and labor costs.

Key words: Paddy husk compost, fractionation period, humic acid, phenolic and carboxylic, total acidity, saturated

INTRODUCTION

Humic substances are defined as biogenic, naturally occurring, heterogeneous organic substances which generally can be characterized as being yellow to black in color of high molecular weight and refractory (Klavins and Purmalis, 2013; Pena-Mendez *et al.,* 2005). Humic substances are significantly altered macromolecules remaining in the soils that are not composed of easily known biomolecules such as carbohydrate, lignin, proteins and amino-sugars (DiDonato *et al.,* 2016; Tan, 2011). The International Humic Substances Society (IHSS) stated that humic substances are major components of natural organic matter in water and soil, as well as in geological organic deposits (Tan, 2000). The process that transform plant and microbial debris into humic substances is called, humification (DiDonato *et al.,* 2016; Tan, 2011).

Humic substances are divided into three groups depending on their solubility, namely Humic Acids (HA), humin and fulvic acids (Pena-Mendez *et al.,* 2005). Humic acid is insoluble in water under acidic conditions (pH 2) but is soluble in higher pH levels while humin is insoluble in water at any pH levels. Fulvic acid is soluble in water at any pH conditions (Raposo *et al.,* 2016). Humic acid is usually referred as the highest molecular weight fraction in streams (1.500-5.000 Da) and in sediments (50.000- 500.000 Da). Meanwhile, humin molecular weight is higher than FA and HA. Furthermore, humin is the main kerogen precursor because it has tendencies to deposit

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and accumulate. Fulvic acid has more acidic functional groups which is in the range from 600-10.000 Da in the streams but a little bit higher in soils which is 1.000-5.000 Da (Raposo *et al.*, 2016).

The increasing agriculture activities increase the uses of urea fertilizer, therefore will affect the climate change and also water quality. Humic acids may reduce the usage of urea fertilizer and also other chemical fertilizers which may absolutely affect the microorganisms in the soils. This is because HA can stabilize the structure of the microbial communities and buffer the changes in size or abundance of some microbial groups by chelating unavailable nutrients, thus, nutrients leaching from the applied fertilizers may be reduced and become available for the plant (Robert, 2014). Besides, Dong *et al*. (2008, 2012) stated that HA can also buffer soil pH.

The isolation (extraction, fractionation and purification) of HA can be done from various source of materials either natural sources such as peat (Rosliza *et al.*, 2009; Yusop *et al.*, 2007) or compost (Palanivell *et al.*, 2012; Ahmed *et al.*, 2005a, b). Usually, the isolation of HA from soils is very time consuming, laborious and expensive. It varies from 12 h-7 days (Rosliza *et al.*, 2009). Furthermore, the isolation period of HA will also differ in terms of source of materials (Palanivell *et al.*, 2013). For example, isolation period of composted pineapple (*Ananas comosus*) leaves was 24 h (Ahmed *et al*., 2005a, b) compared to isolation period from rice straw compost which was more than 24 h (Palanivell *et al.*, 2013). However, there is less information on the HA isolation duration of paddy husk compost. The objective of this study was to determine the minimum duration to fractionate HA from paddy husk compost.

MATERIALS AND METHODS

Description of paddy husk compost production and selected chemical properties characterization: Paddy husk with goat manure was composted at the open space of research area of Universiti Malaysia Kelantan Jeli Campus, Malaysia. Three composting bins with 425 mm (diameter) ×435 mm (height) were used for composting. Twelve holes with the holes size of 0.5 cm diameter each were perforated on the sides of the bins to enable good aeration during the composting process. The paddy husk compost was produced by mixing 80% of paddy husk, 10% of goat manure slurry, 5% of chicken feed and 5% of molasses. A total of 400 g of goat manure were dissolved in 3.0 L of water and filtered to produce goat manure slurry. Rice straw served as a substrate meanwhile the goat manure slurry provided nutrients, moisture and microbes for the composting process. The chicken feed was added to boost the microbe's energy. Besides, molasses was also added to provide the microbes with a source of carbohydrate. During mixing of paddy husk and goat manure slurry, the molasses and chicken feed were added bit by bit to obtain a uniform mixture. The composting material was turned when necessary for aeration and water was sprinkled when required to maintain the moisture content of 60%. Composting was done in triplicates to verify repeatability in minimizing error. Composting was stopped on the 60th day. A digital thermometer with accuracy of ±0.5 was used to determine the daily ambient temperature and compost temperature at 8 am and 5 pm.

The pH and Electrical Conductivity (EC) of the compost were determined by potentiometric method (Peech, 1965). Combustion method was used to determine the total organic matter and total C. The compost total P was extracted by using single dry ashing method (Tan, 2003a, b). The solution was analyzed by the molybdenum blue method (Murphy and Riley, 1962). A blue colour was developed and the P concentration was determined by UV-VIS spectrophotometer (DR 2800, USA) at 882 nm wavelength. The compost total cations (K, Ca, Pb, Mn, Mg, Na, Fe, Cu and Zn) were also extracted by using single dry ashing method (Tan, 2003a, b). An Atomic Absorption Spectrometer (AAS) (Pin AAcle 900F, USA) was used to determine the concentrations of the compost total cations. The N content of compost samples was analyzed using CHNS analyzer (TruSpec Micro Elemental Analyser (NCHS), LECO, USA) in the Department of Land Management, University Putra Malaysia Serdang Campus.

Isolation of HA (extraction, fractionation and purification): Method described by Stevenson (1994) was used to isolate the HA.

Extraction of HA: The extraction of HA was done by using the method described by Stevenson (1994). A 10 g of paddy husk compost was placed in the centrifuge bottle, added with 100 mL of 0.5 M NaOH and the bottle was closed tightly (Gracia *et al.*, 1993). The samples were equilibrated at room temperature on a mechanical shaker at 180 rpm for 24 h. After the extraction period, the side of the bottle was washed by using distilled water and the mixture was centrifuged at 10.000 G for 15 min. Afterwards, the dark color of supernatant liquors containing HA was decanted.

Fractionation and purification of HA: During the fractionation process, the pH of the solution was adjusted to 1.0 by using 6 M HCl. Then, the HA was allowed to equilibrate for 1, 3, 5, 7, 9, 12 and 24 h, respectively. After that, the supernatant which is FA was siphoned off from the acidified extracts (Aiken *et al.*, 1985). The remainder of the suspension was transferred to centrifuge bottles and the HA was centrifuged. Purification was conducted to purify the HA. It was done by using the method described by Ahmed *et al.* (2004). The HA was purified by suspending the HA into 50 mL of distilled water and centrifuged at 10. 000 G for 10 min till the supernatant was decanted. The procedures were repeated three times. The washed HA was oven-dried at 40°C until it reached constant weight. The yield of HA was expressed as the percentage of the weight of compost used.

Characterization of HA: Humification level was determined using E4/E6 ratio (465 and 665 nm) by UV-spectrophotometer (DR 2800, USA) (Stevenson, 1994). A 0.003 g of the HA was dissolved in 10 mL of 0.05 M $NaHCO_3$.

The carboxylic (-COOH), phenolic (-OH) functional groups and total acidity were determined by the method described by Inbar *et al.* (1990). A 0.02 g sample of HA was dissolved in 4 mL of 0.08 M NaOH and equilibrated at room temperature on a reciprocal shaker for 30 min. The initial pH was recorded. The solution was titrated with 0.10 M HCl-pH 2.5 within 15 min. Phenol content was calculated by assuming that 50% of the phenols were dissociated at pH 10. The acid consumption between pH 8 and 10 should represent half of the phenol. Carboxyl content was calculated based on the amount the acid required to titrate the suspension between pH 8 and the end point (pH 2.5). The total acidity was calculated by summation of the phenols and carboxyls.

Data analysis: The data obtained from the study was analyzed by using the Statistical Package for the Social Science (SPSS) Version 21. Analysis of Variance (ANOVA) was used to detect the significance difference between HA yields under different fractionation periods. Tukey's HSD test (p = 0.05) was used to separate the means between HA yields under different fractionation periods.

RESULTS AND DISCUSSION

Selected chemical properties of rice straw compost: The compost pH of this study was 6.48 (Table 1). This value was relatively lower than the pineapple leaves compost produced from a study conducted by Ch'ng *et al.* (2013) and millipede compost produced by Prabhas *et al.* (2011). This value was relatively similar with the compost produced from rabbit manure and horse mixed with fruit, grass cuttings and seaweed in a study conducted by Forjan *et al.* (2017). However, the pH of the compost in this study still falls within the accepted range of finished compost which is from 6.0-8.0 (Anonymous, 2017). The different pH values of both compost was because they were produced from different feed stocks (Stoffella and Khan, 2001). The pH is important to support microbial activity during composting (Bernal *et al.,* 2008). The EC of this study was 1.54 dS m^{-1} (Table 1) which was lower compared to study by Ch'ng *et al.* (2013) but higher than study by Prabhas *et al.* (2011). Electrical conductivity in this study was in the accepted range which is from 1-10 dS/m (Anonymous, 2017). Compost with high salinity may be toxic to some plants (Anonymous, 2017). Thus, this compost is suitable to be applied for plants.

Table 1: Selected physico- chemical properties of paddy husk compost

Property	Value obtained
pH (water)	6.48
Electrical conductivity (dS/m)	1.54
Total organic matter (%)	74.65
Total carbon (%)	43.30
Total N (%)	2.75
Total P (ppm)	393.29
Total Na (ppm)	2.264.972
Total K (ppm)	278.666.67
Total Ca (ppm)	278.466.67
Total Mg (ppm)	9.420.333.33
Total Pb (ppm)	16.23
Total Fe (ppm)	3.241.33
Total Zn (ppm)	61.83
Total Cu (ppm)	13.47
Total Mn (ppm)	1.628

Paddy husk compost contained 74.65% total organic matter and 43.30% of total C (Table 1). The value of total organic matter in this study was relatively lower but the value of total C was not different compared to data obtained from study by Ch'ng *et al.* (2013). However, the total C in this study was relatively higher than study by Zeynep and Coskun and data from study by Forjan *et al.* (2017). The value of organic matter shows the stability and maturity of the prepared compost. The higher value of organic matter shows that the compost is immature and less stable. The less mature and less stable compost may contain phytotoxins which can be harmful to plants. The stable compost means that it holds more nutrients without being used by microorganisms (Ameen *et al.,* 2016). The organic matter content will decrease during composting process until the compost reaches stability.

The total N of the compost used in this study was 2.71 % (Table 1) which was similar to the value obtained in study by Ch'ng *et al.* (2013) but relatively higher compared to a study conducted by Demir and Gulser (2015) who was conducted the study also on rice husk compost. This value was also relatively higher compared to studies conducted by Khater (2015), Al-Bataina *et al.* (2016) and Forjan *et al.* (2017) who conducted study on effect of compost on the recovery of a mine soil. The higher total N was due to the time taken for composting process was different. The total P in this study was 393.297 ppm (Table 1). This value was relatively lower compared to the study by Ch'ng *et al.* (2015) and Khater (2015). Phosphorous is important for

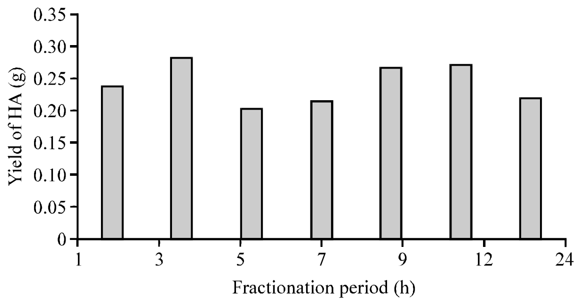

Fig. 1: Effect of different fractionation periods h on yield of HA

seed germination and root development. Phosphorous later will help to improve N absorption by crop when this compost applied to the crop.

Table 1 shows data of total cations (Na, Mg, K, Ca, Pb, Fe, Mn, Zn and Cu) in paddy husk compost. The total Na, K, Mg, Ca, Pb, Fe, Zn, Mn and Cu were 2,264,972, 278.666.67, 278,466.67, 9,420,333.33, 16.23, 3.241.33, 61.83, 13.47 and 1.628 ppm, respectively. The values of total Na, K, Ca and Mg were higher compared to data obtained from study by Ch'ng *et al.* (2015) and study by Forjan *et al.* (2017). Meanwhile, the value of total Fe, Zn, Cu and Mn were lower than the data obtained from study by Ch'ng *et al.* (2015). The significant reduction of total Fe, Zn, Cu and Mn in the compost could be due to the highest temperature during thermophilic phase which may have eliminated most of the heavy metals (Ahmed *et al.,* 2007).

Effect of different fractionation periods on yield of HA: Figure 1 shows the HA yield with different fractionation periods. The effect of different fractionation periods on HA yield obtained showed that: yield of HA fractionated under 5 h was lower compared to those fractionated under 1, 3, 7, 9, 12 and 24 h; Yield of HA fractionated under 1, 3, 7, 9, 12 and 24 h were not statistically different between each fractionation period except yield of HA fractionated under 5 h; 3) yield of HA fractionated under 1, 5, 7 and 9 h were not statistically different between each fractionation period except yield of HA fractionated under 3 and 12 h. The yield of humic acid fractionated under 1 h was 0.26 g and was not statistically different from 3, 5, 7, 9, 12 and 24 h. This indicates the fractionation period of 1 h also had the same time taken as 3, 5, 7, 9, 12 and 24 h for the exchange sites of HA of compost to be saturated with H ions after acidification. The yield of HA under fractionation period more than 24 h may not be economical time wise because the optimum fractionation period was estimated as 90% of the maximum extraction period (Palanivell *et al.,* 2012; Ahmed *et al.,* 2005a, b).

Table 2: Phenolic-OH, carboxylic -COOH, total acidity and E_4/E_6 of rice straw compost HA with those in literature

Variables	Fractionation duration/h (sec)	Humic acids	Range
Phenolic OH (mL/g)	1	267	100-200[a]
	3	467	30-220[b]
	5	400	
	7	333	
	9	400	
	12	300	
	24	367	
Carboxylic COOH (mL/g)	1	417	183-275[a]
	3	483	150-570[b]
	5	467	380-450[b]
	7	467	
	9	450	
	12	400	
	24	383	
Total acidity	1	683	300-450[a]
	3	950	430[c]
	5	867	
	7	800	
	9	850	
	12	700	
	24	750	
E_4/E_6	1	4.75	5.63-7.42[a]
	3	4.71	4.32-5.49[d]
	5	6.71	7-8[d]
	7	4.91	
	9	3.86	
	12	9.36	
	24	8.33	

[a]Hanisah *et al.* (2008), [b]Stevenson (1994), [c]Senesi *et al.* (1996), [d]Tan (2000)

The decreased of yield of HA under fractionation period of 24 h may be caused by the longer fractionation period and could cause chemical changes in HA (Stevenson, 1994). Since, there was no significant difference in the HA yield between the fractionation period of 1, 3, 5, 7, 9, 12 and 24 h it is recommended to opt for 1 h fractionation period as this will have significantly save the consumption of chemicals, electricity and labor costs.

Functional groups and humification level of HA: Phenolic,-OH, Carboxylic,-COOH and total acidity Table 2 were generally within the range with study by Palanivell *et al.* (2012) and Tan (2003a, b). Phenolic group of fractionation period of 12 and 24 h in this study were relatively higher when compared to phenolic group from study by Palanivell *et al.* (2012) but within the range with study by Tan (2003a, b). Meanwhile, the carboxyl group of fractionation period of 12 and 24 h were no different from the carboxylic group in the study by Palanivell *et al.* (2012). The values were still within the range in the study by Tan (2003a, b).

The total acidity in this study was relatively higher when compared to study by Palanivell *et al.* (2012) and Tan (2003a, b). The higher value of functional groups will absolutely causes higher value of total acidity. The presence of functional groups make the HA able to maintain the soil structure if this compost is applied to the soil it helps to chelate the heavy metals, adsorb pesticides and other toxic pollutants (Ahmed *et al.,* 2004). However, the values of E_4/E_6 in this study were also relatively higher compared to study by Palanivell *et al.* (2012) and Tan (2003a, b). The value of E_4/E_6 is the optical density value which is the absorbance at two arbitrary selected wavelength of 465 and 665 nm. It indicates the humification level of HA isolated in this study. The higher value of E_4/E_6 indicated that the lower molecular weights of HA in this study while the higher molecular weights produced lowest E_4/E_6 ratio (Palanivell *et al.,* 2013; Chen *et al.,* 1977). The higher value of E_4/E_6 also indicated lower humification level and condensation of aromatic compounds (Palanivell *et al.,* 2013).

CONCLUSION

The optimum fractionation period of HA from paddy husk compost was 1 h as the yield of HA under different fractionation periods were not significantly different. Therefore, by using 1 h fractionation period it will absolutely save the consumption of chemicals, electricity and labour costs.

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