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Determination of Extraction Period and Extractant Ratio for Extracting Humic Acid from Rice Straw Compost

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Abstract

The process of isolating humic substances especially humic acid consumes a lot of time. The isolation (extraction, fractionation and purification) processes of humic acid from different sources such as soils, organic fertilizers and so on vary from 12 hours to 7 days. Based on the previous findings reported by other researchers, the standard extractant ratio used for humic acid extraction is 1:10 because it gives the highest yield of humic acid isolated. Previous studies tend to focus on the isolation duration of humic acid but not extractant ratio. To date, there is also lack of information about the extraction period and extractant ratio required to extract humic acid from rice straw compost. This study aimed to assess the minimum duration and extractant ratio for extracting humic acid from rice straw compost. A ratio of 1: 5 and 1: 10 of rice straw compost sample to chemical used were performed in humic acid extraction. A 5 g of rice straw compost sample was mixed with 25 mL and 50 mL of 0.5 M sodium hydroxide, respectively, and were extracted under different extraction periods (1, 3, 5, 7, 9, 12, and 24 hours). There was significant difference in the extraction periods and extractant ratio on the yield of humic acid. This study revealed that an extractant ratio of 1:10 and 12 hours of extraction period are required to extract humic acid from rice straw compost and can be purified within 1 hour using distilled water. Humic acid yield was significantly lower when the extraction period was less than 12 hours because the time was not sufficient for the H^+ ions to displace Na^+ ions and other cations. Furthermore, the difficulty of HA extraction period of less than 12 hours was probably due to difficulty of wetting the air-dried compost. The significance of this study is humic acid from rice straw compost can be isolated within 12 hour of extraction period, 24 hours of fractionation period and 1 hour of purification period under extractant ratio of 1:10. Thus, this may help in reducing time and costs needed to produce the humate product from this compost. The extractant ratio of 1:10 is more preferred because of the higher humic acid yield obtained.

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Introduction

Humic substances are derived from organic matter, which are mainly formed through the microbial degradation of plant material and decayed organic matter that mainly found in manure, peat, lignite coal, and leonardite[1]. Humic acid (HA), fulvic acid, and humin are the main three components of humic substances. They are categorised based on their solubility[2,3]. The information, structure and function of humic substances are not well understood, although many researchers put a large research effort over the times[4]. It is very difficult to extract intact humic substances from soils because they are bound with mineral surfaces. Because of the expenditure and time involved with these extractions, many researchers are more preferred use commercial humic products for their studies.

Because of its molecular structure, it provides abundant benefits to plant growth and soil physio-chemical properties. Soil cation exchange capacity (CEC) can be improved with the presence of humic substances. Besides, it also reduces any drastic chemical reactions in the soil that will affect the fertility of soil and environment[2,5]. Toxic materials that are being introduced into the soil will be interrupted and adsorbed by HA application. Humic substances also improve plants' root growth and nutrient uptake, water and nutrients retention in the soil, and soil stability[5,6,7].

The process for isolating (extraction, fractionation, and purification) humic substances especially HA consumes a lot of time. The isolation process of HA varies from 12 hours to 7 days. Most of the studies are using 24 hours of extraction period and 24 hours of fractionation for their researches. However, there are various studies reported that the extraction, fractionation, and purification of HA can be reduced to less than 2 days, depending on types and sources of organic matter[8,9,10,11]. The highest HA yield isolated from a rehabilitated forest in Bintulu, Sarawak can be obtained within 4 hours of extraction period[12]. The extractant ratio is also one of the factors that affect HA yield production. The International Humic Substances Society (IHSS) recommends the extractant ratio of 1:10 because it gives the highest yield of HA isolated from humic substances[13]. However, a study concluded that the extractant ratio of 1:5 and 1:10 had no significant effect on the HA yield isolated from a compost[14].

There is a dearth information on the HA yield and extraction period under different extractant ratio. Most of the studies focused on the isolation duration of HA but not extractant ratio. To date, there is also lack of information about the extraction period and extractant ratio to extract HA from rice straw compost. Therefore, this study aimed to assess the minimum duration and extractant ratio for extracting HA from rice straw compost

Materials and Methods

Preparation of Compost from Rice Straw

Rice straw 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) x 435 mm (height) were used for composting. Twelve holes with the size of 0.5 cm diameter each were perforated on the sides of the bins to enable good aeration during the composting process. 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. 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 rice straw 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 a.m. and 5 p.m.

Different Properties of Compost

After the composting, pH and electrical conductivity of the finished rice straw compost was determined by a pH and EC meter, respectively in a 1:5 solution ratio

(dry compost and water)[15]. Combustion method[16] was used to determine the total organic matter and total C. Micro-Kjeldahl method[17] was used to determine the total N whilst total P was extracted using the method[18], and the P concentration was determined using the blue method[19]. Afterwards, C/N and C/P ratios were calculated. Total K, Ca, Mg, Zn, Cu, Fe, and Mn were also extracted and determined using the standard methods[18]. The method[20] was used to determine the ash content, ammonium (NH_4-N) and nitrate (NO_3-N) of the finished rice straw compost. All analyses were done in triplicate.

Isolation of HA

Extraction of HA

A 5 g of rice straw compost sample was mixed with 25 ml and 50 ml of 0.5 M sodium hydroxide, respectively, and were extracted under different extraction periods (1, 3, 5, 7, 9, 12 and 24 hours)[4]. The samples were shaken on an orbital shaker at 180 rpm for 1, 3, 5, 7, 9, 12, and 24 hours. After the period of extraction, distilled water was used to wash the side of the bottles. Next, centrifugation of the mixture was performed at 10, 000 rpm for 15 minutes. After that, dark colour of supernatant solution was poured out and filtered.

Fractionation and Purification of HA

The supernatant solution was adjusted to pH 1.0 by using 6.0 M hydrochloric acid (HCl)[4]. Then, the HAs were allowed to stand in room temperature for 24 hours. After that, the supernatant solution (fulvic acid) was separated from the acidified extracts[21]. The remains of the suspensions were poured into the centrifuge bottles and centrifuged for 15 minutes at 10, 000 rpm. After that, the suspended HA on the wall of centrifuge bottle was added with 50 ml of distilled water and centrifuged at 10,000 rpm for 10 minutes. Similar procedure was repeated three times[8]. Then, the HA was collected using a spatula, oven-dried at 40°C until constant weight was attained. The HA yield obtained was expressed as the percentage of weight of compost used.

Characterization of HA

To determine the humification level of HA isolated, E_4/E_6 ratio (465 and 665 nm) was determined by UV- spectrophotometer4. A total of 0.003 g of HA was weighed and 10 ml of 0.05 M sodium bicarbonate ($NaHCO_3$) was added to dissolve the HA. The model of spectrophotometer used was UV-VIS spectrophotometer (Thermo Scientific Genesys 20).

A method was used to determine the functional groups of the HA (carboxylic –COOH and phenolic –OH, and total acidity[22]. A total of 0.02 g of HA was weighed and then 4 ml of 0.08 M NaOH was added to it. The mixture was shaken on an orbital shaker at 180 rpm for 30 minutes. Before the titration, the pH of the solution was recorded. After that, titration of the solution was performed with 0.10 M HCl until the solution achieved a pH of 2.5 within 15 minutes. Phenol content was determined by assuming 50% of the phenols were dissociated at pH 10. The amount of acid consumed between pH 8 and 10 represents half of the phenol. Carboxyl content was determined based on the amount the acid to titrate with the suspension of the phenols and carboxyls. The total amount of carboxyl and phenolic gave the reading of HA total acidity.

Data Analysis

The data obtained from the study was analysed 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 extractant ratio and extraction periods. Tukey's test ($P \leq 0.05$) was used to separate the means between HA yields, extraction periods, and extractant ratio.

Results and Discussion

Chemical Properties of Compost

The humic acid and ash contents of the finished compost were 6.5% and 12.8%, respectively (Table 1). The C/N ratio of compost was 19.92. The C/N ratio is a good indicator to determine the degree of compost maturity. Compost having a value of C/N ratio below 20 indicates the compost is matured[23]. After composting, the C/N ratio of the compost was 19.92 (Table 1) and this indicates that the rice straw compost in this study had reached maturity[23]. The ammonium (NH_4-N) and nitrate (NO_3-N) contents of the compost was 37.5 and 24.5 mg kg^{-1}, respectively (Table 1). The pH of the rice straw compost was 7.54 (Table 1). The value was within the standard range for rice straw compost[24,25].

The compost samples contained 72.53% of organic matter and 42.65% of total C (Table 1). The values of organic matter and total C were within the range[26]. However, the concentration of organic matter and total C in the rice straw compost in this study was higher than that of rice straw compost without goat manure, chicken feed and molasses[26] because goat manure, chicken feed and molasses were high in organic matter and total C

The EC of rice straw compost was 1.53 dS m^{-1} (Table 1). This was due to the release of several ions during the mineralization of organic matter[23]. The contents of N, P, K, Na, Ca and Mg in the finished compost were 2.14, 0.34, 8.71, 0.55, 0.34, and 10.6%, respectively (Table 1). The percentage of the total N in compost sample was much higher than other studies[27,28]. This was because goat manure and chicken feed were high in N compared to rice straw. Therefore, when both animal and plant based organic matter were mixed together, total N became higher.

Table 1: Selected chemical properties of finished rice straw compost

Property	Value obtained
pH (water)	7.54
EC (dS m^{-1})	1.53
Humic acid (%)	6.50
Ash content (%)	12.80
Organic matter (%)	72.53%
Total carbon (%)	42.65%
Total N (%)	2.14%
Total P (%)	2351.1
C/N ratio	19.90
Total K (%)	150.70
Total Ca (%)	43.60
Total Mg (%)	230.10
Total Fe (µg g^{-1})	18.20
Total Cu (µg g^{-1})	0.90
Total Zn (µg g^{-1})	2.20
NH_4-N (mg kg^{-1})	37.50
NO_3-N (mg kg^{-1})	24,5

Effect of Extraction Period and Extraction Ratio on HA Yield

The data of HA yield are presented in Table 2. The means comparison ($P \leq 0.05$) of effect of different extraction periods on the yield of HA under different extractant ratios were consistent as the findings of other studies[29,10]. The observation from the result obtained in this study also revealed that: (1) yield of HA extracted under 1 hour in different extractant ratio were both significantly lower compared to other extraction period; (2) HA yield extracted under 12 and 24 hours with extractant ratio 1:5 and 1:10 were not statistically different in this study. This indicated that the HA yield was significantly lower when the extractant period was less than 12 hours because the time was not sufficient for the H^+ ions to displace Na^+ ions and other cations. Furthermore, the difficulty of HA extraction period of less than 12 hours was probably due to difficulty of wetting the air-dried compost.

Table 2: Different extraction periods and extractant ratio on HA yield

Extraction period	HA yield (%) Extractant ratio	
	1:5	1:10
1 hour	5.20 ± 0.2^{BC}	6.60 ± 0.12^{D}
3 hours	5.27 ± 0.18^{BC}	9.14 ± 0.13^{C}
5 hours	4.70 ± 0.30^{C}	9.53 ± 0.58^{C}
7 hours	6.06 ± 0.24^{B}	9.80 ± 0.12^{C}
9 hours	6.10 ± 0.50^{B}	13.20 ± 0.12^{B}
12 hours	8.90 ± 0.50^{A}	14.46 ± 0.29^{A}
24 hours	9.06 ± 0.27^{A}	13.40 ± 0.31^{AB}

Note: Means within column with different letter(s) indicate significant difference between means by Tukey's test at $P \leq 0.05$.

The decrease in the degree of high molecular-weight complexes depolymerization, and the degree in wetting and solubilization of the rice straw compost increased with increasing extraction period[29]. The exchange process between K from the extractant and the exchange sites mainly hydroxylic, phenolic and carboxylic functional groups in the compost progressed with extraction period until a maximum period is achieved when the maximum number of exchange sites might have been saturated with K ions[11]. Besides, this extraction process might have made the compost highly soluble. From the Table 2, extraction period under 12 and 24 hours

with both extractant ratio of 1:5 and 1:10 produced the highest HA yields. These results can be implied that the solubility and the maximum exchange sites were saturated with K ions.

Table 3: Different extractant ratio on HA yield under extraction periods of 12 and 24 hours

Extraction period	HA yield (%) Extractant ratio	
	12 hours	24 hours
1:5	8.90±0.50^{B}	9.06±0.27^{B}
1:10	14.46±0.29^{A}	13.40±0.31^{A}

Note: Means within column with different letter(s) indicate significant difference between means by Tukey's test at $P \leq 0.05$.

Since both extractant ratios obtained the same optimum extraction period, the ratio was analysed between the extractant ratio under 12 and 24 hours to detect any significant different by Tukey's test at $P \leq 0.05$. Table 3 shows that the HA yield extracted using 1:10 extractant ratio was significantly higher than that of 1:5 extractant ratio in both 12 and 24 hours extraction periods. Results were consistent with the IHSS recommended data[13].

Functional groups and humification level of HA

Generally, the ranges of phenolic -OH, carboxylic -COOH, and total acidity under 1:5 extractant ratio were within the ranges as reported in previous study30 (Table 4). The carboxylic groups were found to be between 483 to 950 cmol kg^{-1} under extractant ratio of 1:10 (Table 4) which were relatively higher than recorded in other study[30]. This might due to the unstable HA and caused the high value range of total acidity in that particular ratio. The high E_4/E_6 ratio of HA obtained mainly in compost samples indicates that the higher rate aliphatic structures were found in compost than the aromatic constituents, which suggest the early phase of humus formation[31]. Even though the E_4/E_6 ratio of both extractant ratio of HA were relatively lower than previous study, the values were still within the common range of E_4/E_6 ratio in HA, which is 2 – 5[4]. The low E_4/E_6 ratio obtained in the present study of HA samples could be due to intensive microbial activity. Usually, a low E_4/E_6 ratio reflects a high degree of aromatic condensation and high molecular weight[29].

Table 4: Phenolic -OH, carboxylic -COOH, total acidity, and E_4/E_6 of compost HA in literature

Variable	Extraction period (hours)	HA present study Extactant ratio		HA, literature
		1: 5	1:10	
Phenolic -OH (cmol kg^{-1})	1	233.33	166.67	240 – 54030
	3	200	266.67	
	5	200	266.67	
	7	300	200	
	9	200	266.67	
	12	266.67	233.33	
	24	233.33	133.33	
Carboxylic -COOH (cmol kg^{-1})	1	250	483.33	150 – 44030
	3	316.67	483.33	
	5	383.33	833.33	
	7	383.33	833.33	
	9	400	900	

	12	300	883.33	
	24	483.33	950	
Total acidity	1	483.33	650	500 – 70030
	3	516.67	750	
	5	583.33	1100	
	7	683.33	1033.33	
	9	600	1166.67	
	12	566.67	1116.66	
	24	716.66	1083.33	
E_4/E_6				
	1	5.163	5.479	2 – 54
	3	5.069	3.968	
	5	4.872	3.158	
	7	3.401	3.520	
	9	4.175	2.49	
	12	2.53	2.521	
	24	5.787	3.058	

Conclusions

The optimum yield of HA isolated from rice straw compost can be performed at an extraction period of 12 hours in both extractant ratio of 1:5 and 1:10. The process required approximately 12 hours to extract HA from compost and one hour of purification process using distilled water. However, the extractant ratio of 1:10 is better for higher HA yield. The significance of this study is HA from rice straw compost can be isolated within 12 hours of extraction period, 24 hours of fractionation period and 1 hour of purification period or less instead of existing range of 2 to 7 days. Thus, this may help in reducing time and costs needed to produce the humate product from the rice straw compost.

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