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Detection of Carbohydrate Compounds in Compost Made From Soybean Husk and Cow Manure

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ABSTRACT

Husk through composting techniques. However, information about the characteristics of soybean husk still needed to be studied more deeply. This caused coagulation of primary metabolites which had a very important biological functions as an energy source, fuel and intermediates for planting, the formation of RNA and DNA structural work (ribonucleic acid and deoxyribonucleic acid), this could be used to help explore the profile carried out in soybean husk degradation. Detection of carbohydrate compounds in this study used Gas Chromatography–Mass Spectroscopy (GC–MS) because it was a powerful tool in characterizing compost. The purpose of this study was to detect carbohydrate compounds in compost made from soybean husk and cow manure. Thus, further information obtained could be used as recommendations in handling soybean husk, especially as organic fertilizer through composting techniques. The study was conducted using a Randomized Complete Block Design (RCBD) with 5 composting arrangements with five replications. Related to payments were: C1: soybean husk (100 %), C2: cow manure (100 %), C3: soybean husk: cow manure (50 %: 50 %), C4: soybean husk: cow manure (75 %: 25 %) and C5: soybean husk: cow manure (25 %: 75 %). Data were analyzed using GC–MS and Least Significant Difference (LSD). Based on analysis using GC–\MS on compost, six sugar compounds were detected. While the treatment that showed the highest area of the curve was C5 (25 % soybean husk: 75 % cow manure), this result was directly proportional to the Least Significant Difference (LSD).

Keywords : GC-MS, Metabolite, Organic Waste

INTRODUCTION

Compost was a product of the thermophilic degradation process of organic waste which was stable, non–toxic and quickly available to plants [1]. Application of compost for several years on agricultural land, could have a positive impact which was to increase nutrient content and organic matter and improve physical, chemical and biological soil properties [2].This condition showed that composting in the long term could increase land productivity, prevent land degradation, and reduce environmental pollution, which meant an increase in land quality so that agricultural production of both quality and quantity could be achieved sustainably [3, 4]. The quality of compost could be determined not only from the ongoing degradation process, but also by the raw material or organic waste used. There were three main classes of organic waste suitable to be converted into organic fertilizer were animal, plant and industrial waste.Waste animals that were often used were cows [5, 6], horses [7], poultry [8, 9]. Plant wastes included fruit and vegetable waste [10, 11], whereas industrial waste which was widely used as raw material for composting was industrial white food waste and soybean husk [12, 13].

Soybean husk was a solid waste produced from the process of stripping soybeans on several processed products such as oil, flour, milk, soy sauce, tofu and tempeh [12, 14]. Soybean husks are a rich source of dietary fiber comprising 86 % polysaccharides, proteins, lipids, vitamins, minerals and polyphenols such as anthocyanidins, proanthocyanidins, and isoflavones [15]. In Indonesia, the availability of soybean husk was quite abundant, considering that 88 % of soybean allocation was used for the food processing industry, especially tempeh and tofu. From 967.876 t of Indonesian soybean production in 2016, 42.5 t of dry soybean husk produced and 85 t in the wet form.

Observing the availability and nutrient content of the soybean husk, this waste actually had the potential to be used as raw material in composting. This was due to soybean husk being organic waste, where all organic material could basically be used as organic fertilizer and although this waste was lignocellulosic, the relatively low lignin content (1 to 4 %) caused this soybean husk to be degraded. The potential of soybean husk as organic fertilizer had been demonstrated by the study of [12] which combined soybean husk with papaya waste through composting techniques. There was a significant increase in Ca (42.3 to 91.6 %), K (93.8 to 235 %), Mg (25.4 to 84.6 %) and P (37.1 to 129 %) and a decrease C N^{-1} ratio (21.4 to 52.8 %) after 9 w vermicomposting. On the other hand, the use of soybean husk as raw material in composting had not been done much especially in combination with livestock manure such as cow manure. Cow manure was widely used as a mixture in the process of degradation of organic waste. This was due to cow manure in addition to containing many microorganisms, also had a relatively high C N^{-1} ratio, where the high carbon content was needed by microorganisms as energy during composting [16–18]. Therefore, the researcher wanted to study further about the potential of soybean husk combined with cow manure through composting especially in detecting metabolite compounds in compost produced.

Detection of metabolite compounds contained in compost was very useful for evaluating the use of compost itself in the future. This was because metabolites could reflect cell mechanisms and metabolic profiles in understanding both physiological and biological functions and metabolic pathways of an organism [19]. Carbohydrates were one of the primary metabolites which were compounds found in all cells and played an important role in life. Carbohydrates are natural carbonyl compounds with several hydroxyl groups that produce these compounds when hydrolyzed. Included in the carbohydrate group were sugars (monosaccharides) and polymers, they were oligosaccharides and polysaccharides [20].

Detection of metabolites in this study used Gas Chromatography–Mass Spectroscopy (GC–MS), because it was a powerful tool in characterizing compost. GC–MS can be used to separate and detect polar acids (organic acids, amino acids, sugars and sugar alcohols) and non-polar compounds (fatty acids and sterols), and can show reproductive retention and high mass spectrum[21]. The purpose of this study was to detect the metabolites specifically carbohydrates contained in the compost made from soybean husk combined with cow manure in different proportions.Thus, it could be seen the potential of soybean husk as organic fertilizer and further information obtained could be used as a recommendation in soybean husk handling, especially as organic fertilizer through composting techniques

METHODS

Time and Place of Research

The study was conducted at the Experimental Garden of the University of Muhammadiyah Malang Indonesia. The materials used in composting consisted of soybean husk obtained from tempeh producers in Sanan, Malang, East Java Indonesia; cow manure, and earthworm *Lumbricus rubellus*.

Tools and Materials

The study methodology used Randomized Complete Block Design (RCBD) with 5 treatments and 5 replications.The treatment details were as follows: Composting: C_1: 100 % soybean husk, C_2: 100 % cow manure, C_3: 50 % soybean husk: 50v% cow manure, C_4: 75 % soybean husk: 25 % cow manure and C_5: 25 % soybean husk: 75 % cow manure. The experiment started with washing the soybean husk first until the water was clear, which aimed to reduce the acidity of the waste. Furthermore, soybean husk was dried by aerating. Whereas cow manure was taken from dairy farmers. Soybean husk that had been dried mixed with cow manure in accordance with the predetermined proportions of 50 %: 50 % (soybean husk: cow manure), 75 %: 25 % (soybean husk: cow manure) and 25 %: 75 % (soybean husk: cow manure), the proportion was calculated based on weight per weight. Furthermore, the treatment was fermented for two weeks anaerobically, as well as the 100 % treatment of soybean husk and cow manure.

Experimental Design

25 boxes of 40 cm × 40 cm × 20 cm were used for composting. Each box was filled with soybean husk and cow manure, or a combination of the waste according to the treatment of 10 kg and in each box given 1×10^3 g of *lumbricus rubellus* worms. In this study, composting was carried out aerobically and lasted for seven

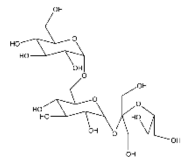
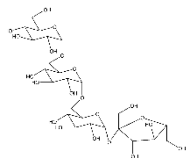
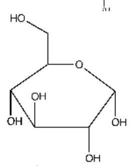
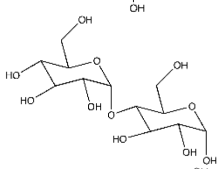
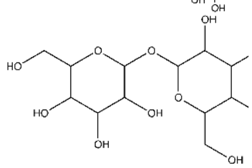
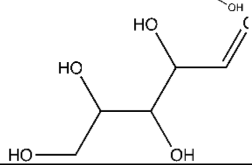
weeks and had produced all compost, which was characterized by changes in the material being smooth, odorless and blackish color.

Sampling for GC–MS analysis was taken from each box randomly on all replications. The compost sample was drained to a constant weight, then crushed using a blender and sieved with a mesh of 40. Then the sieved sample was weighed 5 g using an analytic scales, and placed into a 250 ml beaker then added 50 ml of absolute methanol [19] and stirred with a magnetic stirrer for 30 min. The stirring results were filtered and collected in a 250 ml erlenmeyer, then added 50 ml of absolute methanol to the sample residue and stirred with a magnetic stirrer for 30 min. Re–filtering was carried out on the stirring solution to get a clear filtrate. The filtrate was diluted to a volume of 250 ml using a measuring flask, then concentrated the filtrate solution using a rotary evaporator at 40ºC with a suction pressure of 150 mmHg to obtain a concentrated solution. 0.1 g of concentrated extract was taken and diluted with absolute methanol to 100 %. Homogenisation was then performed with vortex and filtered using 0.45 µm cellulose acetate membrane. Degassing of the sample solution was carried out to be injected into GC–MS. To find out the differences of each treatment, performed by Least Significant Difference (LSD).

RESULT AND DISCUSSION

Based on GC–MS analysis on compost, six sugar compounds were detected, they were Raffinose, Stachyose, Glucose, Maltose, Trehalose and Arabinose. From the five treatments tested, C_5 treatment (25 % soybean husk: 75 % cow manure) showed the highest chromatogram compared to other treatments, while C_1 treatment (100 % soybean husk) showed the lowest chromatogram. This condition was directly proportional to the results of the 5 % LSD test performed. In more detail, the results of the GC–MS analysis and the 5 % LSD test were presented in theTable 1 and Figure 1 to Figure 5 below:

Table 1. Metabolites detected at compost

Identification of Compound	Structure	Retention Time (Min)	Curves Area (%)				
			C_1	C_2	C_3	C_4	C_5
Raffinose		41.766	2218.625	2308.879	2439.625	2549.888	2664.604
Stachyose		68.692	4450.816	4149.726	3629.828	5009.817	4724.534
Glucose		5.511	2187.525	2309.862	3609.772	2509.771	2624.488
Maltose		15.276	4398.658	5068.817	4817.614	5087.816	5202.533
Trehalose		15.307	5948.382	6217.658	6484.821	6511.616	7226.333
Arabinose		4.711	2865.528	2997.758	3125.335	3509.817	3624.534
Total			**22069.534**	**23052.707**	**24106.995**	**25178.725**	**26067.026**

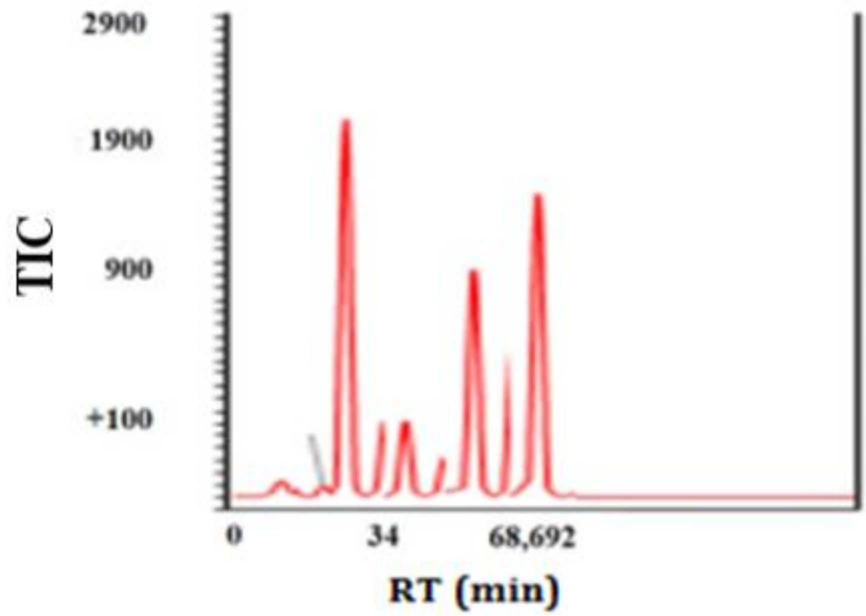


Figure 1. Curve Area Graph C1

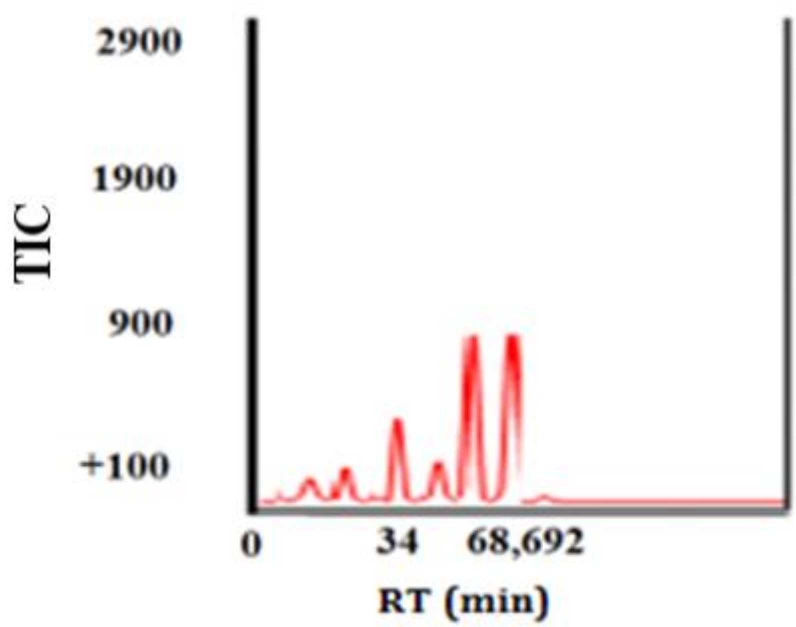


Figure 2. Curve Area Graph C2

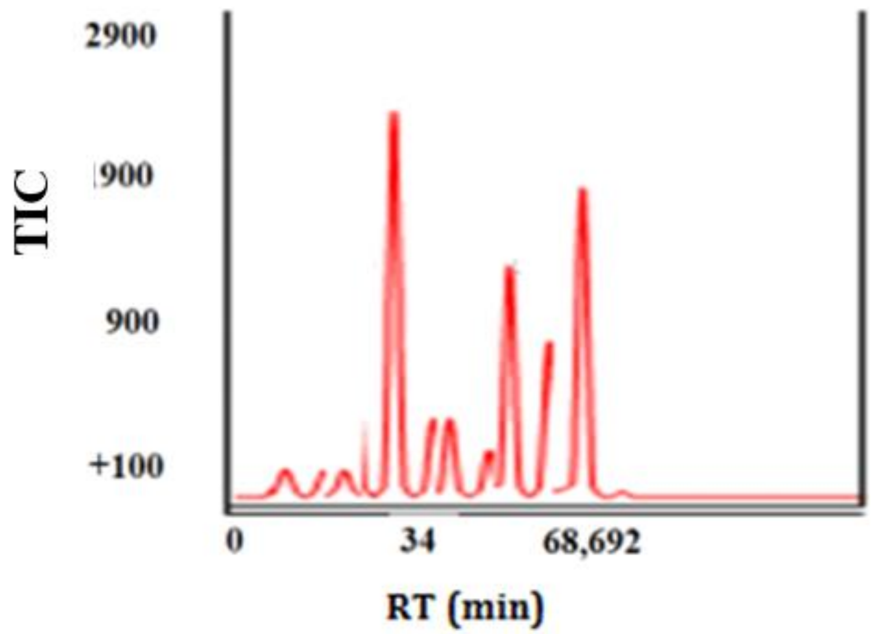


Figure 3. Curve Area Graph C3

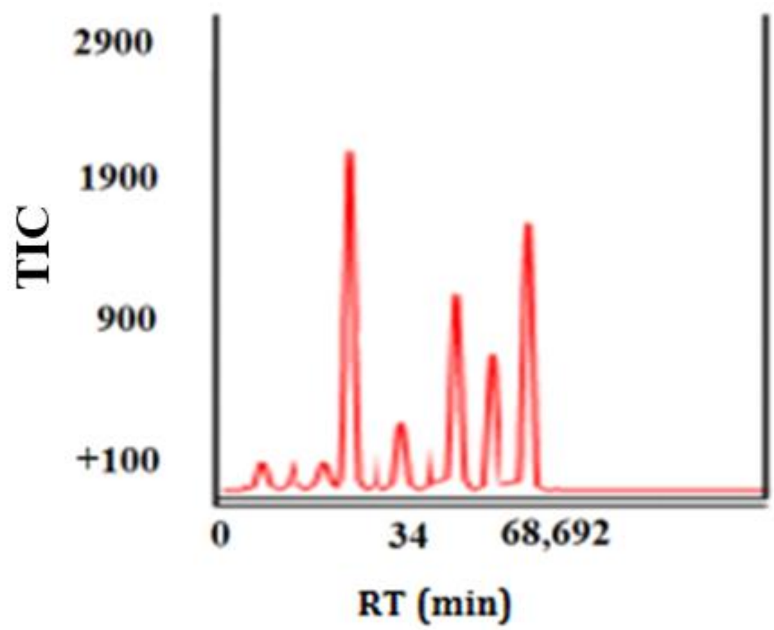


Figure 4. Curve Area Graph C4

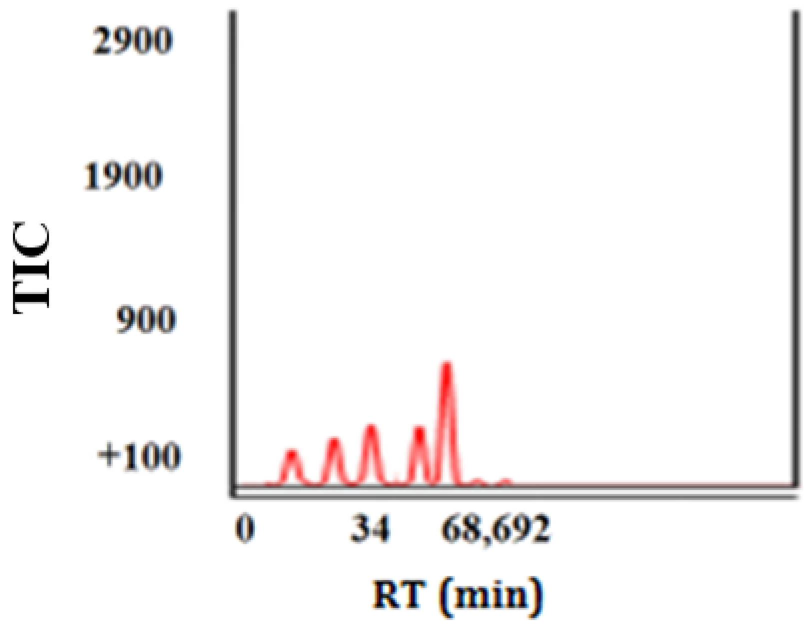


Figure 5. Curve Area Graph C5

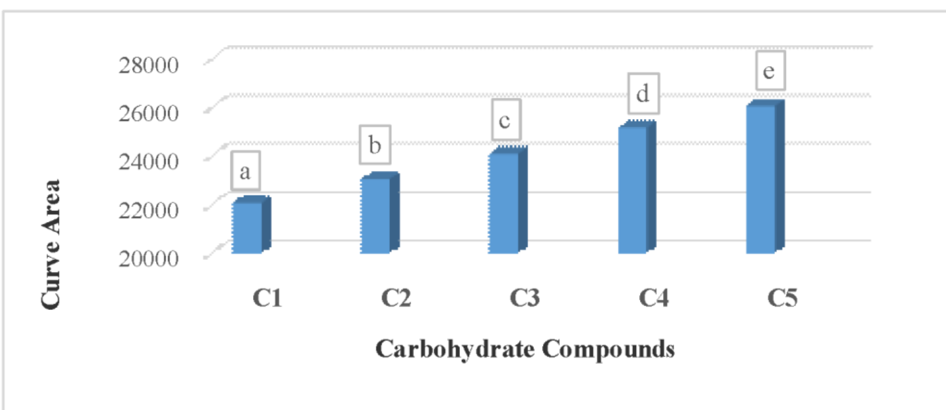


Figure 6. Carbohydrate Compounds Graph in LSD Test

Based on a LSD test for carbohydrate metabolite compounds showed a very significant difference between the treatments that were tested. The highest curve area occurred in C_5 treatment (25 % soybean husk: 75 % cow manure) and the lowest occurred in C_1 treatment (100 % soybean husk waste) (Figure 6).

Based on GC–MS and LSD test of α 5 % in soybean husk combined with cow manure, the C_5 treatment (25 % soybean husk: 75 % cow manure) was the best proportion compared to other treatments. This condition indicated that the C_5 treatment (25 % soybean husk: 75 % cow manure) was an ideal media condition in waste degradation activities.

Composting was a thermophilic process of waste degradation that involved various microorganisms under controlled environmental conditions. In this study, composting was carried out aerobically, where oxygen was absolutely needed by microorganisms to degrade the substrate and assimilate a number of carbon, nitrogen, phosphorus, sulfur and other elements for the synthesis of the protoplasm of the body's cells. In addition, the particle size of the substrate would greatly affect the activity of microorganisms and aeration. The finer the substrate particles, the more open surface area for microorganism activity and also the number of pores would increase so that the aeration becomes smooth. With a proportion of 25 % of soybean husk: 75 % of cow manure, where there was not too much proportion of soybean husk that was lignocellulosic, the activity of microorganisms in degrading the substrate could be faster, especially molecules with little carbon content such as sugar.This condition was shown by the high sugar compound formed in the C_5 treatment. In contrast, complex carbon molecules such as lignin,not only took longer but also required greater enzymes to degrade organic matter. Therefore, in the treatment of C_1 (100 % soybean husk) with media that entirely used soybean husk, the microorganisms needed more energy in degrading the waste so that it diverted most of the carbon and energy that was assimilated to the synthesis of organic molecules as an adaptation power to the compound lignocellulosics.

The ideal media was not only in accordance with the conditions of growth but also concerned the availability of adequate nutrients for microorganisms. Under these conditions, organism growth occurred exponentially which means there was a primary metabolism and produced metabolic products called primary metabolites such as carbohydrates, which were natural carbonyl compounds with several hydroxyl groups in the form of polymers, composed of sugar units Included in the carbohydrate group were sugars (monosaccharides) and polymers were oligosaccharides and polysaccharides [20].

Carbohydrates had a very important biological function as a source of energy, fuel and metabolic intermediates, the formation of structural frameworks of RNA and DNA (ribonucleic acid and deoxyribonucleic acid). Insoluble carbohydrate polymers acted as structural elements in bacterial cell walls (peptidoglycan or murein), plants (cellulose) and animals (chitin), associated with many proteins and lipids as well as in cell communication and in interactions between cells and elements another in a cellular environment [22].

In this study, two types of sugar were detected in compost, which were semi-complex sugar and simple sugar. Semi-complex sugar was oligosaccharide sugar consisting of more than 2 units of saccharide, while simple sugar was monosaccharide sugar and disaccharide sugar. Compounds included in semi-complex sugars in compost which were detected as chromatograms in GC–MS analysis were raffinose and stachyose, while chromatograms included in simple sugar compounds were glucose, maltose, trehalose, and arabinose.

Raffinose ($C_{18}H_{32}O_{16}$) was a trisaccharide consisting of galactose, glucose, and fructose. Found in nuts, vegetables and seeds. Especially in soybeans,raffinose accumulated a lot during the final stages of seed maturation and drying [23]. Whereas stachyose ($C_{24}H_{42}O_{21}$) was a tetrasaccharide consisting of two D–galactose units, one D–glucose unit, and one D–fructose unit connected in sequence. Naturally, stachyose was found in many vegetables and nuts such as green beans and soybeans.

In general, legumes contain more stachyose than raffinose [24]. This condition was also seen from the results of this study, where in all treatments tested, the area of the stachyose curve was higher than raffinose, it was in the C_1 treatment, the area of the stachyose curve was 33.47 % higher than raffinose, then the C_2 treatment 28.50 %, C_3 treatment 19.61 %, C_4 treatment 32.54 % and C_5 treatment 63.90 % also higher. This was possible because stachyose was one of the main carbohydrates that was soluble in seeds.

Trehalose was one of the simple sugars detected from the GC-MS chromatogram. Of all the sugars detected, both semi complex sugars and simple sugars, trehalose showed the highest broad curve. Trehalose is a non–reducing sugar formed from two glucose units joined by alpha bonds 1–1, named alpha–D–glucopyranose glucopyranosyl–1, 1–alpha–D–glucopyranoside [25]. This bond made trehalose very resistant to acid hydrolysis so it was more stable in high temperature solutions even under acidic conditions [26] and this chemical property was strongly suspected to provide higher trehalose compounds than other treatments.

Conclusion

Carbohydrate compounds in compost made from soybean husk waste and cow manure through composting techniques were successfully detected using GC–MS. Various proportions of soybean husk and cow manure tested in this study managed to get the most ideal proportion in the process of substrate degradation. The results of this study were expected to be used as a reference in handling soybean husk especially as raw material for making organic fertilizer.

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