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RESEARCH ARTICLE

Soluble phosphorus rich compost by aerobic solid-state fermentation of chicken bone-containing food waste with *Aspergillus niger*

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OBJECTIVES Food waste is a major issue in solid waste management due to its high volume and rapid accumulation. Without proper handling, it can cause foul odors, methane emissions, and health risks. Composting is a common solution but is often slow and limited to certain organic materials. Bones, a phosphorus-rich food waste, have potential for converting food waste into a valuable fertilizer. METHODS This study examines composting chicken bones and non-bone food waste through aerobic solid-state fermentation (SSF) using *Aspergillus niger*. The 28-day process at 28 °C was analyzed for pH and nutrient composition. RESULTS Results showed that *Aspergillus niger* effectively degraded organic matter and released phosphorus within a week, producing compost with high soluble P (2.47 mg P/g). Applying this compost to corn seeds for 7 days resulted in improved growth, including a 0.8 cm taller stalk, a 0.4 cm thicker stalk, two more leaves, and a more extensive fibrous root system compared to plants supplemented with naturally composted food waste. CONCLUSIONS Direct application of food waste led to stunted growth. These findings highlight *Aspergillus niger*-mediated SSF as an efficient method to convert food waste into high-quality compost, supporting sustainable agriculture and waste management.

KEYWORDS food waste; composting; *Aspergillus niger*; phosphorus; corn germination

1. INTRODUCTION

Food waste is a major type of waste that fills landfill sites in significant quantities. According to data acquired from the National Waste Management Information System (SIPSN) of Indonesia in 2022, food waste accounted for 40.7% of the 35,803,483.85 tons of solid waste produced by various sectors of society (Kementerian LHK 2024). About 55-60% of urban food waste in Indonesia consists of organic waste (Mulyadi 2019). In 2022, FAO reported that around 1.05 billion tons of wasted food was generated (UNEP 2024), contributing to 16.2 billion tons of carbon dioxide equivalent (Comission 2024). These greenhouse gas emissions are primarily in the form of methane (CH_4), which is produced during the decomposition of food waste piled up at landfill sites.

Compost is a stable end product resulting from the biological decomposition of organic material, a process known as composting. Compost contains a mixture of compounds commonly referred to as humus, which is typically dark-colored (brown or black), powdery in texture, and capable of retaining soil moisture. In addition to organic acids, the humus contains essential nutrients for plant growth, such as nitrogen (N), phosphorus (P), and potassium (K). The decomposition process through composting can also reduce the levels of compounds that inhibit seed germination, such as caffeine, tannins, and polyphenols (Zhu et al. 2023).

P is a vital element used in various industrial sectors, particularly in the agricultural sector. An adequate level of P is necessary during the seedling stage to promote root and shoot growth, increase leaf area and plant height, and prepare for early flowering and fruit yield through its involvement in many biochemical reactions (Abobatta and Abd Alla 2023). This becomes the primary reason for the global exploitation of phosphate minerals (Wahid et al. 2020). Further concern in the increasing demand for food to feed the growing human population resulted in P minerals depletion as early as in the 2090s (Reijnders 2014).

Recovering P from animal bone waste that potentially pollutes the environment can help meet a substantial portion of the demand for P fertilizers. High concentration of P

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can be found in animal bones as insoluble crystalline calcium phosphate minerals called apatite. Chicken is the most consumed meat in Indonesia compared to the other farmed animal meat (Tenrisanna and Kasim 2020). This could lead to unmanageable waste, particularly in the form of chicken bone. Compared to other animal bones, chicken bone was found to contain about 80 g P/kg bone, yet higher P availability than those from sheep and pig due to lower crystalline bioapatite formation (Ahmed et al. 2021).

Several studies have evaluated the utilization of *Aspergillus niger* in the composting process and the release of P from phosphate minerals. Heidarzadeh et al. (2019) reported the potential of *Aspergillus niger* to compost urban waste in just 18 days, which is faster than conventional composting processes that typically take around 1-2 months. *Aspergillus niger* can facilitate the dissolution of phosphate from tricalcium phosphate and iron phosphate minerals by 43.1% and 10.2%, respectively, after 7 days of incubation at 28°C through an organic acid leaching mechanism present in the culture solution of *Aspergillus niger* (Tian et al. 2021). Moreover, the secretion of lignocellulose-degrading enzymes accompanying the production of oxalic acid by *Aspergillus niger* has been shown to assist in the release of P up to 92.3% of the total P in rice straw soaked in *Aspergillus niger* liquid culture for 30 days at 28°C (Wang et al. 2022). However, studies on P release from solid-state fermentation (SSF) of food waste by *Aspergillus niger* have not yet been conducted. Furthermore, the characteristics of the resulting compost products need further evaluation to determine its applicability. Thus, this study aims to evaluate P release from chicken bone in food waste pile by SSF technique using *Aspergillus niger*. Resulting compost was characterized according to SNI 19-7030-2004 and corn seed germination test. The findings in this study could benefit urban communities in Indonesia to manage commonly found local food waste in the most efficient way as well as to demonstrate the potential benefit of using organic compost to the local farmers.

2. RESEARCH METHODOLOGY

2.1 Materials

Chicken bones and various organic (non-bone) food wastes, including spent coffee grounds, tea dregs, vegetable scraps, and fruit peels, were separately collected as daily food waste from local shops and vendors around the Tenggilis campus of the University of Surabaya. A P test kit with a detection range of 0.0025 - 5.00 mg/L PO4-P (Merck Millipore, Germany) was used to measure total P and insoluble P. *Aspergillus niger* culture was purchased from the Faculty of Technobiology at the University of Surabaya. Potato Dextrose Agar (PDA) for microbiology (Merck Millipore, Germany) was used to grow *Aspergillus niger*. Tween 80 (50 g/L) was obtained from Merck Millipore, Germany (CAS no. 9005-65-6). Soil for the germination test was collected from a garden patch at the University of Surabaya (Latitude: 7°19'20.6"S; Longitude: 112°46'05.8"E). ICP multi-element standard solution IV and HNO_3 (Merck Millipore, Germany) were used in multi-metal analysis contained in the ash sample.

2.2 Procedures

2.2.1 Waste pretreatment

Chicken bone and non-bone food wastes were dried in the oven at 60°C for 12 h, pulverized using a domestic grinder, and passed through a mesh no. 7 sieve. Any retained solids were re-ground until all powders passed through the screen. The chicken bone powder and non-bone food waste powder were then stored separately at room temperature in plastic containers for subsequent analyses, such as elemental analysis, moisture content, and ash content following ASTM D2974-14 standards (ASTM, 2017). The non-bone food waste powder was prepared by mixing sieved spent coffee grounds, tea dreg powder, vegetable waste powder, and fruit peel powder in their original weight proportions when collected (Figure 1).

2.2.2 Preparation of *Aspergillus niger* spore

Aspergillus niger spores were prepared by gently scraping the culture grown on slanted PDA media at 28°C for 8 days. To ensure uniform spore dispersion, the *Aspergillus* culture was moistened with 10 mL of 0.1% (v/v) Tween 80 before scraping. Microscopic inspection using a Neubauer counting chamber indicated a spore concentration of 2.59×10^7 spores/mL.

2.2.3 Composting procedure

Compost substrate was prepared by mixing non-bone food waste powder and chicken bone powder at varying mass ratios of 50:50, 75:25 and 90:10. Mixing was done in several petri dishes to facilitate replication. The homogeneous substrate mixture was then moistened to approximately 60% moisture content by adding 1 mL of spore suspension and few mL of sterile water. Compost fermented by wild microorganisms was prepared by similar steps excluding the addition of spore suspension. The mixture was incubated at 28°C for 1 day, 3 days and several days until up to 28 days. A glass beaker filled with 500 mL of deionized water was placed in the incubator to maintain the internal relative humidity at 33%, minimizing water evaporation from the substrate. The deionized water level was monitored daily to ensure it did not drop below 400 mL.

2.2.4 Compost analyses

At specific incubation time intervals, 10 g compost samples were collected for moisture content analysis. Then, the dried sample was divided into two equal parts for total P and insoluble P measurements. For total P analysis, 5 g of dried sample was placed in a furnace set at 600°C for 6 h. About 0.1 g of resulting ash then solubilized in 50 mL of 5 mM H_2SO_4 to measure total P content. The solution was then filtered and centrifuged at 3000 rpm for 5 minutes. This P-containing solution was further diluted to 100 mL using deionized water and mixed with the reagents according to the instruction provided in the analysis kit. Resulting blue solution was then analyzed using spectrophotometry technique at 430 nm.

For the analysis of insoluble P, 5 g of dried sample was introduced into deionized water (1:10 w/v) and stirred for 1 hour at room temperature to remove soluble P. After that, the leftover solid was recovered by filtration. The recovered solid was then ashed and solubilized in 50 mL of 5 mM H_2SO_4. The obtained solution was diluted to 100 mL by adding deionized water, mixed with reagent and put into P analysis for insol-

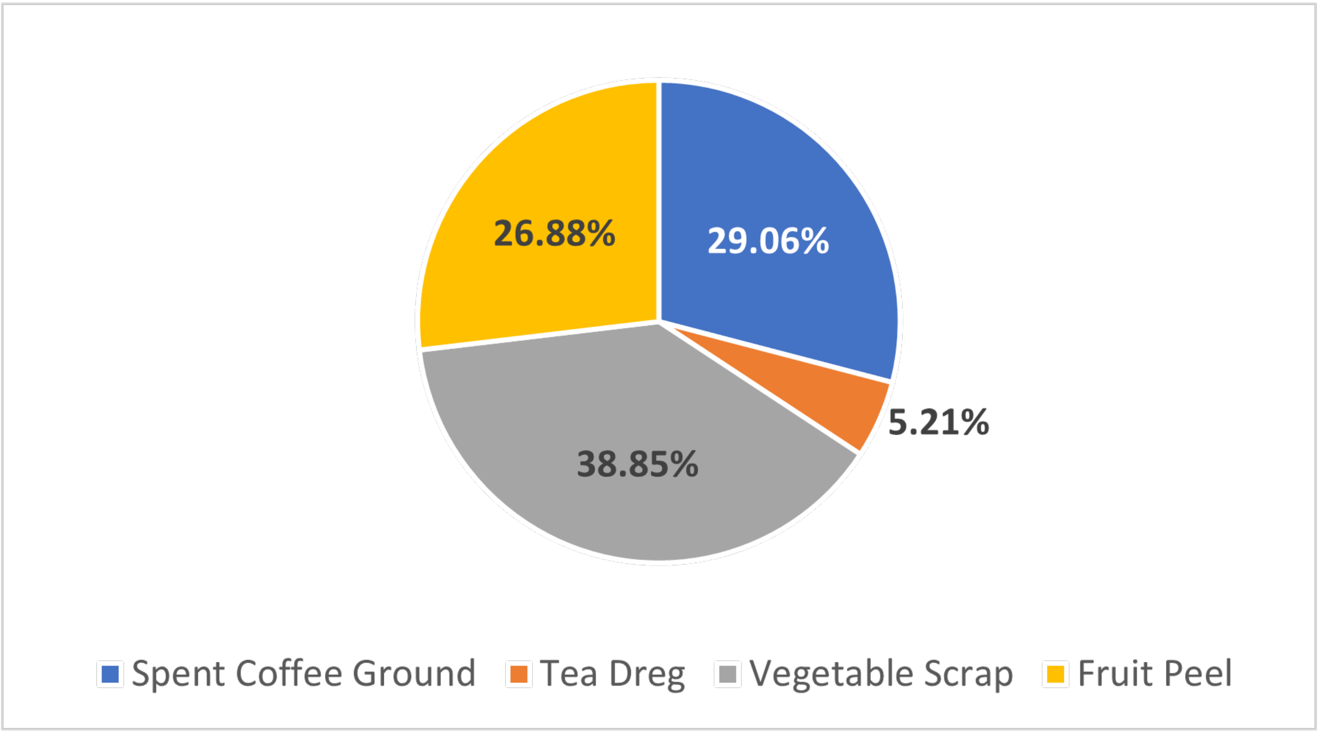

FIGURE 1. Composition of non-bone food waste powder (dry basis).

uble P measurement using spectrophotometry technique at 430 nm. Soluble P content in the sample was determined by subtracting the insoluble P from total P.

The compost sample with the highest soluble P content underwent further elemental analysis for total nitrogen (N) and the C/N ratio, as well as ash mineral analysis using ICP techniques. All analyses were performed at least in duplicate. The change of C and N profile prior and after composting was further investigated using paired t-test in Minitab 21.2. A confidence level (α) of 95% and p-value threshold at 0.05 were used. Failure in rejecting the null hypothesis (p-value > 0.05) indicates insignificant change in the tested parameter.

For the corn seed germination test, two negative controls were included: (1) an uncomposted food waste mixture and (2) food waste compost fermented by wild microorganisms. In this experiment, approximately 20 g of compost sample or uncomposted food waste mixture was mixed with 200 g of soil and placed in a plastic pot without compaction. A corn seed then was put into the soil mixture about 1 cm deep from the surface. Each pot was watered with 35 mL of deionized water every two days. On the 7th day, the entire plant was gently removed from the pot by tapping the pot bottom. The plant parts were subsequently measured using a ruler and caliper or counted manually where applicable.

3. RESULTS AND DISCUSSION

Figure 2 illustrates the profile of P release by *Aspergillus niger* in this study. The data revealed that the highest P release was observed after a 7-day incubation period across all ratios. The sharp increase in soluble P observed after the first day of incubation suggests that P release is primarily influenced by *Aspergillus niger* growth. This finding aligns with the study by Upton et al. (2017), which demonstrated that during the growth phase, *Aspergillus niger* produced an increased amount of citric acid to solubilize surrounding phosphate sources, thereby replenishing external phosphate levels to satisfy its growth requirements. The leaching of P from hydroxyapatite (the bone mineral) is represented by the following reaction (Misra 1996):

$$\begin{aligned} &Ca_{10}(PO_4)_6(OH)_{2(s)} + 4C_6H_8O_7(aq) \rightarrow \\ &\quad 2Ca_3(C_6H_5O_7)_{2(s)} + 2CaHPO_{4(aq)} + \\ &\quad\quad 2Ca(H_2PO_4)_{2(aq)} + 2H_2O_{(g)} \end{aligned} \tag{1}$$

After the first day of incubation, soluble P gradually increased to reach its highest value at the 7th day of incubation. This can be explained by a reduction in citric acid excretion due to the inhibition of pyruvate carboxylase activity in a high-P environment (Feir and Suzuki 1969). As the incubation period progressed, *Aspergillus niger* reached maturity and transitioned into its reproductive cycle, evident by the darker appearance of the substrate due to black conidia formation. At this phase, the uptake of external P was allocated mainly for reproduction purpose and polyphosphate storage by both the older and newer generations of *Aspergillus niger* (Upton et al. 2017). This led to the reduction in soluble P levels within the compost over extended incubation periods.

The highest soluble P was found in the compost made from a 50:50 non-bone to chicken bone ratio, indicating that a higher bone content resulted in more soluble P and any amount of non-bone was sufficient to supply the organic substrate needed for citric acid synthesis and the growth of *Aspergillus niger*. All quality parameters met the national standard for compost, except for C/N ratio (Table 1). This finding was further evaluated by looking at the C and N profile of the substrate and compost (Table 2). Table 2 showed that the substrate undergone an increase in C/N ratio, yet the compost still did not meet the standard, probably to the low C/N ratio in the process input (the substrate).

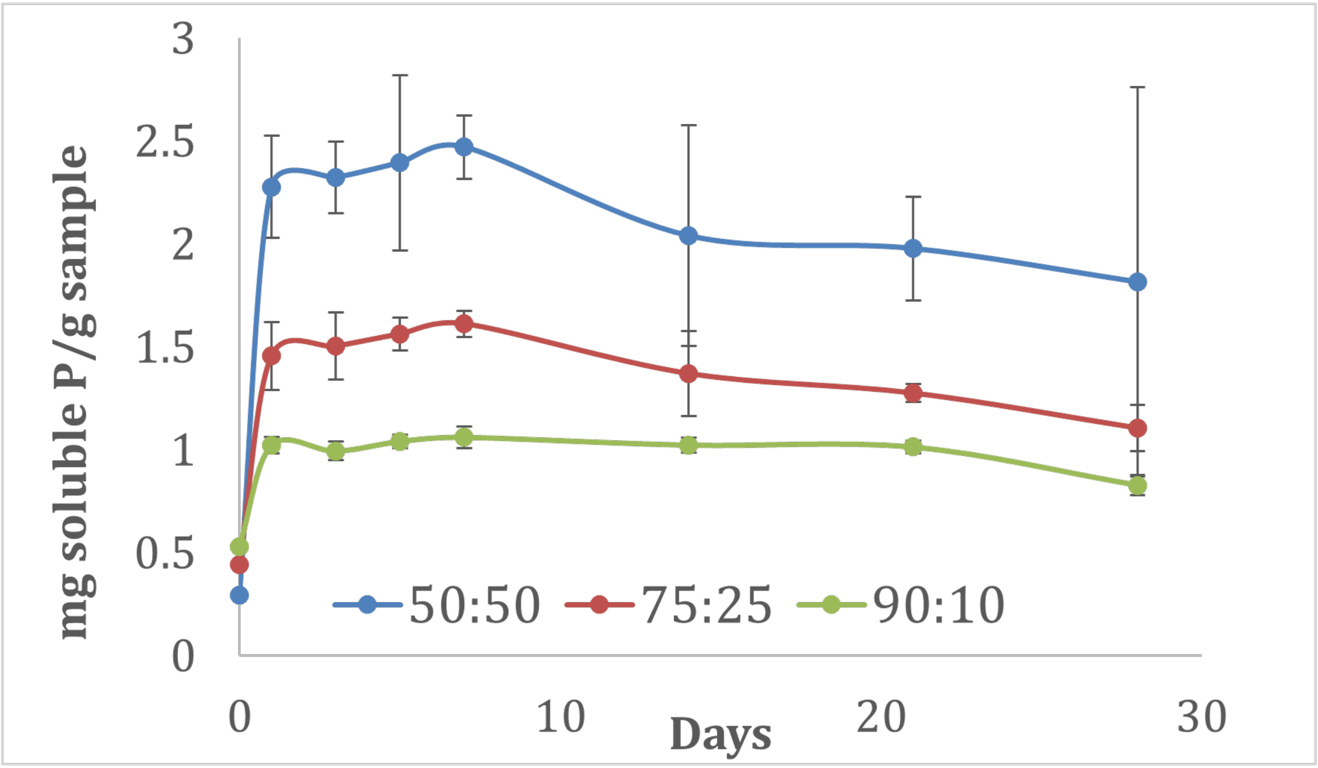

FIGURE 2. Time profile of soluble P in food waste compost samples with varying non-bone to chicken bone ratio.

Statistical analysis further elucidated insignificant change of C/N ratio (p-value = 0.088) and C content (p-value = 0.201). Meanwhile, the drop in N content could be considered marginally significant (p-value = 0.051). These statistic reports reflect carbon retainment and ammonification in compost pile during the composting process (Heidarzadeh et al. 2019). The impact of ammonification was obviously observed through the rise in pH from 6.50 to nearly 7.0 on the 7th day of incubation. This phenomenon was even more pronounced (pH 7.60-7.70 on the 7th day of incubation) at

TABLE 1. Properties of chicken bone: non-bone food waste (50:50) composted by Aspergillus niger for 7 days at 28°C (dry basis).

Quality Parameter	Compost by Aspergillus	SNI 19-7030-2004
pH	6.96 ± 0.50	6.80-7.49
Total N (%)	4.80 ± 0.05	min. 0.40
Total P (%)	0.52 ± 0.09	min. 0.10
Soluble P (mg P/g)	2.47 ± 0.15	n.a.
C/N ratio	8.53 ± 0.36	10-20
K (%)	2.14 ± 0.06	min. 0.20
Ca (%)	5.44 ± 0.54	max. 25.50
Mg (%)	1.80 ± 0.35	max. 0.60
Fe (%)	0.58 ± 0.13	max. 2.00
Cd (mg/kg)	n.d.	max. 3
Ni (mg/kg)	n.d.	max. 62

n.d.: not detected
n.a.: not available

TABLE 2. C, N and C/N ratio of compost substrate and resulting compost.

Sample	Element of Interest	
Compost substrate (Bone:Organic = 50:50)	%C	43.18 ± 1.42
	%N	7.18 ± 0.24
	C/N	6.01 ± 0.01
Compost by Aspergillus (Bone:Organic = 50:50, 28°C, 7 days)	%C	40.91 ± 2.16
	%N	4.80 ± 0.05
	C/N	8.53 ± 0.36

FIGURE 3. Results of corn seed germination for 7 days using (1) Aspergillus niger compost, (2) wild microbes compost, and (3) a mixture of chicken bone and non-bone food waste at a 50:50 ratio.

higher non-bone amounts (non-bone-to-bone ratios of 75:25 or 90:10), as the compost nitrogen was contained in the non-bone portion, such as spent coffee powder (Reijnders 2014). Hence, the unmet C/N ratio level may not prove the immaturity of compost since C/N ratio in the system was low to begin with and did not change significantly during composting. To meet the standard, the addition of carbonaceous substrate (i.e., biochar) into the compost can be suggested.

Adequate P levels are particularly important during the early growth stage to enhance shoot and root growth. Yet, most of the P in the soil is in the form of strongly bound solid mineral, rendering low amount of soluble P that is available to plants. A plant may become deficient in P when it requires more P than the soil can release at given temperature and surrounding situation. This was proved by direct application of food waste for corn seed germination yielded suboptimal growth, where the shoot appearance was far shorter than those fertilized with the composted food waste (Figure 3). Despite of almost similar total P content in all media, direct application of food waste into the planting media only contributed 0.03 mg P/g media, which was much lower than those enriched with the compost (0.26 mg P/g and 0.09 mg P/g for the media enriched with *Aspergillus niger*-compost and wild microbial-compost, respectively). It was obvious that corn seed planted in *Aspergillus niger*-compost featured 0.8 cm higher stalk, 0.4 cm thicker stalk, more leaves and more lush fibrous root systems than that grew in wild microbial-compost.

4. CONCLUSIONS

This study demonstrates that P present in chicken bone food waste can be effectively released with the aid of citric acid secreted by *Aspergillus niger*. The findings showed that as the bone ratio in compost increased, compost acidity became neutral and higher levels of soluble P was observed. Since excessive external P appeared to inhibit further organic acid synthesis by *Aspergillus niger*, soluble P did not increase after the 7th day of incubation. The compost with a 50:50 bone-to-non-bone ratio, fermented for 7 days by *Aspergillus niger* was the most effective in promoting corn seed growth as evidenced by a taller and thicker stalk, as well as more extensive fibrous root and leaf development, compared to plants grown in media supplemented with naturally composted food waste. *Aspergillus niger* is a potential candidate to transform food waste into high-quality compost with less water and time consumption, which may contribute to sustainable close loop agricultural practice. SSF by *Aspergillus niger* can be further explored as an eco-friendlier process of mineral leaching.

5. ACKNOWLEDGEMENTS

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