1. Bukti *submission* ke Jurnal Rekayasa Proses (26-09-2024)
2. Dokumen yang di-*submit*
3. Revisi pertama dan komentar *reviewer* (19-02-2025)
4. Hasil revisi
5. Notifikasi *submission* revisi (13-03-2025)
6. Notifikasi *acceptance* (08-04-2025)
7. *Final Paper*

1. Bukti submission ke
Jurnal Rekayasa Proses (26-09-2024)

UNIVERSITAS KRISTEN PETRA

ONG LU KI <ongluki@petra.ac.id>

[jrekpros] Submission Acknowledgement

1 message

Prof. Himawan Tri Bayu Murti Petrus, S.T., M.E., D.Eng. via Jurnal Ilmiah Universitas Gadjah Mada <noreply-ojs3@ugm.ac.id> Thu, Sep 26, 2024 at 11:38 AM
Reply-To: "Prof. Himawan Tri Bayu Murti Petrus, S.T., M.E., D.Eng." <bayupetrus@ugm.ac.id>
To: Lu Ki Ong <ongluki@petra.ac.id>

Lu Ki Ong:

Thank you for submitting the manuscript, "Soluble Phosphorus Rich Compost from Chicken Bone by Aerobic Solid-State Fermentation of Food Waste with Aspergillus niger" to Jurnal Rekayasa Proses. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Submission URL: https://jurnal.ugm.ac.id/v3/jrekpros/authorDashboard/submission/16629
Username: ongluki

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Prof. Himawan Tri Bayu Murti Petrus, S.T., M.E., D.Eng.

Jurnal Rekayasa Proses

2. Dokumen yang di-*submit*

Soluble Phosphorus Rich Compost from Chicken Bone by Aerobic Solid-State Fermentation of Food Waste with *Aspergillus niger*

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A B S T R A C T

Food waste has become a concern in solid waste management due to its high bulk volume and production rate. Without good management, food waste piling may result in foul odor, global warming by methane emission and even an epidemic. Composting is a method usually employed to process food waste. However, this method often took a long period and selective to organic waste such as leafy materials or fruit waste. Bone is a food waste that is rich in phosphorus, a chemical substance that is required to make various useful products such as fertilizer, battery, detergent, etc. This study investigates the characteristics of food waste compost containing chicken bone and various domestic organic food waste that was processed through aerobic solid-state fermentation using *Aspergillus niger*. The composting process was monitored over a 28-day period at 28°C. The resulting compost was characterized under various physicochemical parameters, including pH and nutrient composition. The findings demonstrated that *Aspergillus niger* effectively released phosphorus from bone while degraded organic matter in just a week, resulting in significant reductions in unprocessed waste volume and the production of a stable, nutrient-rich compost in a short time. Additionally, the compost was successfully induced better growth on corn seed compared to the food waste pile and naturally composted food waste. These results suggest that *Aspergillus niger*-mediated aerobic solid-state fermentation is a viable and efficient method for converting food waste into high-quality compost, promoting sustainable waste management and agricultural practices.

Keywords**:** food waste; composting; *Aspergillus niger*; phosphorus; corn germination

A B S T R A K

Limbah makanan telah menjadi isu pengelolaan limbah padat karena volume massanya yang besar dan tingkat produksinya yang tinggi. Tanpa pengelolaan yang baik, penumpukan limbah makanan dapat menghasilkan bau busuk, pemanasan global akibat emisi metana dan bahkan epidemi. Pengomposan adalah metode yang umum digunakan untuk memproses limbah makanan. Namun, metode ini sering memakan waktu lama dan selektif terhadap limbah organik seperti bahan daun atau limbah buah. Tulang adalah limbah makanan yang kaya akan fosfor, zat kimia yang diperlukan untuk membuat berbagai produk berguna seperti pupuk, baterai, deterjen, dan lain-lain. Studi ini menyelidiki karakteristik kompos limbah makanan yang mengandung tulang ayam dan berbagai limbah makanan organik domestik yang diproses *melalui fermentasi padat aerobik menggunakan Aspergillus niger. Proses pengomposan dipantau selama periode 28 hari pada suhu tetap (28°C). Kompos yang dihasilkan dikarakterisasi berdasarkan berbagai parameter fisik dan kimia, termasuk pH dan komposisi nutrisi. Temuan menunjukkan bahwa Aspergillus niger secara efektif melepaskan fosfor dari tulang sambil mendegradasi bahan organik hanya dalam waktu seminggu sehingga menghasilkan pengurangan volume limbah tak terolah yang signifikan dan produksi kompos yang stabil dan kaya nutrisi dalam waktu singkat. Selain itu, kompos tersebut berhasil meningkatkan pertumbuhan biji jagung dibandingkan dengan tumpukan limbah makanan dan limbah makanan yang dikomposkan secara alami. Hasil ini menunjukkan bahwa fermentasi padat aerobik yang dimediasi oleh Aspergillus niger adalah metode yang layak dan efisien untuk mengubah limbah makanan menjadi kompos berkualitas tinggi, yang mendukung pengelolaan limbah berkelanjutan dan praktik pertanian.*

*Kata kunci**:** limbah makanan; pengomposan; Aspergillus niger; fosfor; perkecambahan jagung*

1. Introduction

Food waste is a major type of waste that fills landfill sites in significant quantities. According to data from the National Waste Management Information System (SIPSN) of Indonesia in 2022, food waste accounts for 40.7% of the 35,803,483.85 tons of solid waste produced by various sectors of society (KLHK, 2022). FAO indicates that unprocessed and unconsumed food waste can reach up to 1.6 billion tons annually, with non-composted food waste contributing to greenhouse gas emissions equivalent to 3.3 billion tons of CO_2 per year (FAO, 2013). 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, humus contains essential nutrients for plant growth, such as nitrogen, phosphorus, and potassium. 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).

Phosphorus (P) is a vital element used in various industrial sectors, particularly in the agricultural sector. Adequate level of P is necessarily required during seedling stage for promoting root and shoot growth, increasing leaf area and plant height as well for preparing early flowering and fruit yielding stage through its involvement in many biochemical reactions (Abobatta and 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 phosphorus minerals depletion as early as in the 2090s (Reijnders, 2014).

Several studies have evaluated the utilization of *Aspergillus niger* in the composting process and the release of phosphorus from phosphate minerals. Heidarzadeh et al. 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 (Heidarzadeh et al., 2019). *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 phosphorus 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 phosphorus 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 phosphorus 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.

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. Phosphorus test kit with detection range at 0.0025 - 5.00 mg/L PO_4-P (Merck Millipore, Germany) were 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 IVand HNO_3 (Merck Millipore, Germany) were used in multi-metal analysis contained in the ashed sample.

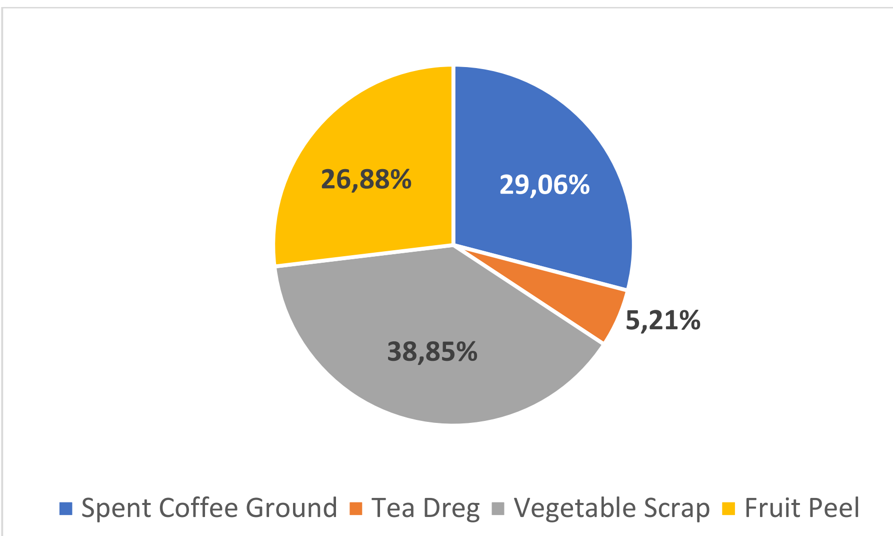

Figure 1. Composition of Organic Food Waste Powder (Dry Basis).

2.2 Procedures

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 organic 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 organic food waste powder was prepared by mixing the sieved spent coffee grounds, tea dreg powder, vegetable waste powder, and fruit peel powder according to their weight proportions as initially collected (Figure 1).

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.

Compost substrate was prepared by mixing organic (non-bone) food waste powder and chicken bone powder at various mass ratio of 50:50, 75:25 and 90:10. Mixing was done in several petri dishes to facilitate replication and sampling. The homogeneous substrate mixture was then moistened to approximately 60% moisture content using 1 mL of spore suspension and few mL of sterile water. The mixture was incubated next to a 500 mL container of deionized water in an incubator set at 28°C to maintain humidity. The deionized water level was monitored daily to ensure it did not drop below 400 mL. At specific incubation time intervals, 5 g of compost samples were collected for moisture content, ash content, and total phosphorus (P) measurement. Soluble P was extracted by stirring the sample in deionized water (1:10 w/v) for 1 hour at room temperature, followed by filtration and centrifugation at 3000 rpm for 5 minutes to separate the solid, which contained insoluble P. The solid phase was then ashed for P measurement. Soluble P was calculated as the difference between total P in original sample and insoluble P in the extracted sample.

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. For the corn seed germination test, approximately 20 g of compost was mixed with 200 g of soil and placed in a plastic pot without compaction. Two negative controls were included: (1) uncomposted food waste mixture and (2) naturally composted food waste mixture, which was prepared by moistening the substrate with sterile water and incubating for the same period that yielded the highest P release by Aspergillus niger. These controls were used to compare P release by Aspergillus niger with that by wild microorganisms. 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 bottom. The plant parts were subsequently measured using a ruler and caliper, or counted manually where applicable.

3. Results and Discussion

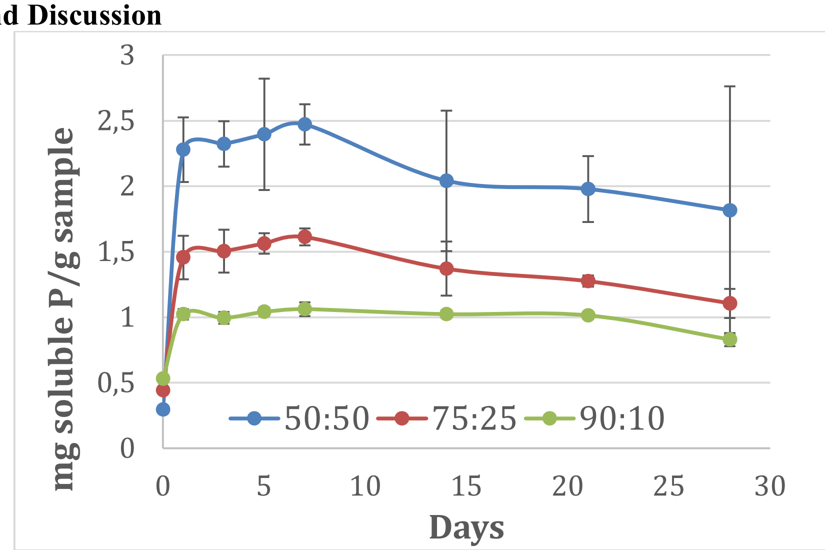

Figure 2. Time Profile of Soluble P in Food Waste Compost Samples with Varying Organic Waste-to-Chicken Bone Ratio.

Figure 2 illustrates the profile of phosphorus (P) release by Aspergillus niger in this study. The data reveal that the highest P release was observed gradually over a 7-day incubation period across all ratios, with the 50:50 organic-to-bone ratio yielding the most significant soluble P release. The sharp increase in soluble P observed after just 1 day of incubation suggests that P release was primarily influenced by *Aspergillus niger* growth, alongside its metabolic activities. 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):

$$Ca_{10}(PO_4)_6(OH)_{2(s)}+4C_6H_8O_{7(aq)} \rightarrow 2Ca_3(C_6H_5O_7)_{2(s)}+2CaHPO_{4(aq)}+2Ca(H_2PO_4)_{2(aq)}+2H_2O_{(g)}$$

Hydroxyapatite	Citric acid	Calcium citrate	Dicalcium phosphate	Calcium dihydrogen-phosphate	Water

The gradual increase in soluble P beyond the first day of incubation can be attributed to a reduction in citric acid excretion, likely due to the inhibition of pyruvate carboxylase activity in the high P environment (Feir and Suzuki, 1969). As the incubation period progressed, *Aspergillus niger* reached maturity, transitioning into its reproductive cycle, evident by the darker appearance of the substrate due to conidia formation. The uptake of external P for reproduction and polyphosphate storage by both the older and newer generations of *Aspergillus niger* (Upton et al., 2017) led to a reduction in soluble P levels within the compost over extended incubation periods.

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Table 1 presents the quality of compost produced from a 50:50 ratio of chicken bone to organic food waste, composted at 28°C for 7 days with the assistance of *Aspergillus niger*. This compost exhibited the highest soluble phosphorus (P) content. The carbon-to-nitrogen (C/N) ratio, a key indicator of compost maturity, was slightly lower than the minimum pH specified by composting standards. To assess the composting process, total nitrogen (N) content and the C/N ratio of the substrate were analyzed (Table 2). The decrease in total N and C content suggests the occurrence of nitrogen release, typically through ammonification, alongside the consumption of carbohydrates during composting (Heidarzadeh et al., 2019). Ammonification also contributed to a rise in substrate pH from 6.50 to nearly 7.0, a trend that was more pronounced when the organic waste content was higher (e.g., organic-to-bone ratios of 75:25 and 90:10). Under these conditions, the compost pH reached 7.60-7.70 by the 7th day. Although the data showed a low initial C/N ratio, this may not prove the immaturity of compost since the substrate had very low C/N ratio to begin with. Nevertheless, since these parameters closely align with the standard, agronomic performance should be considered when evaluating the compost's suitability for commercialization.

Table 1. Properties of Chicken Bone:Organic 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

Direct application of food waste for corn seed germination yielded suboptimal growth (Figure 3). The compost produced by wild microbes resulted in corn plant that was 0.8 cm shorter than that grown in *Aspergillus niger*-enriched compost. A significant difference was also observed in leaf number and fibrous root development; corn seed planted in soil enriched with *Aspergillus* compost produced two additional leaves and more extensive fibrous root systems, indicating better nutrient availability around the seed. Additionally, the stem diameter was slightly larger (approximately 0.4 cm). While the total P content of uncomposted food waste and naturally produced compost was similar to that of the *Aspergillus*-treated compost (around 0.3%), the soluble P levels in uncomposted food waste and natural compost were only 0.02 and 0.75 mg P/g, respectively. These findings suggest that soluble P in the growth medium plays a critical role in corn seed germination and leaf development.

Figure 3. The Results of Corn Seed Germination for 7 days using (1) *Aspergillus niger* Compost, (2) Wild Microbes Compost and (3) Mixture of Chicken Bone and Organic Food Waste at 50:50 Ratio.

4. Conclusions

This study demonstrates that phosphorus 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 the compost increases, compost with neutral pH and higher levels of soluble phosphorus was produced. Since excessive external phosphorus appeared to inhibit further organic acid production, the soluble phosphorus crept up to its peak at the 7th day of incubation. The compost with a 50:50 bone-to-non-bone ratio, fermented for 7 days, was most effective in promoting corn seed growth, as evidenced by taller and thicker stems, as well as wider fibrous root and leaf development compared to the control growth media. *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. Solid state fermentation by *Aspergillus niger* can be further explored as more eco-friendly process of mineral leaching.

Acknowledgements

The authors thank LPPM UBAYA for financial support through Internal Grant No. 180/ST-Lit/LPPM-01/FT/XII/2023. The authors also express their gratitude to Pra Cipta Buana Wahyu Mustika for his help in ICP analysis.

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3. Revisi pertama dan komentar *reviewer* (19-02-2025)

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[jrekpros] Editor Decision

2 messages

Ade Kurniawan, S.T., M.Eng., Ph.D. via Jurnal Ilmiah Universitas Gadjah Mada <noreply-ojs3@ugm.ac.id> Wed, Feb 19, 2025 at 10:39 PM
Reply-To: "Ade Kurniawan, S.T., M.Eng., Ph.D." <ade.kurniawan@ugm.ac.id>
To: Lu Ki Ong <ongluki@petra.ac.id>, Ricardo Angelio <ricardangelio213@gmail.com>, Clarissa Indrayani <indrayani.clarissa@gmail.com>, "Prof. Lieke" <lieke@staff.ubaya.ac.id>

Lu Ki Ong, Ricardo Angelio, Clarissa Indrayani, Prof. Lieke:

We have reached a decision regarding your submission to Jurnal Rekayasa Proses, "Soluble Phosphorus Rich Compost from Chicken Bone by Aerobic Solid-State Fermentation of Food Waste with Aspergillus niger".

Our decision is: Revisions Required

Ade Kurniawan, S.T., M.Eng., Ph.D.
Departemen Teknik Kimia, Fakultas Teknik, Universitas Gadjah Mada
ade.kurniawan@ugm.ac.id

Reviewer A:

1. Recommend proofreading the manuscript due to several grammatical errors.
2. In the introduction part, it is necessary to add the information of the chicken bone content which is potentially utilized for composting as well as why it is chosen to be used for SSF on this manuscript.
3. In the last paragraph of the introduction, it is recommended to add a statement to show the benefit of this study.
4. Figure 1 caption showed the organic food waste, which not includes the chicken bone in the graph. Meanwhile chicken bone is organic waste. It is recommended to change it into “non-bone food waste” to avoid the confusion. Please change through the manuscript for this context.
5. It is recommended to write the procedure into several sub section, such as

2.2.1 Pretreatment of the waste

2.2.2 Preparation of *Aspergillus niger* spore

etc

1. Please add the detail information in the procedure. For example, there is no clear information on how long the incubation for SSF, how the measurement of P content was done, how the compost substrate was made, wild microbes compost, etc.
2. There is no discussion about the effect of ratio of non-bone:bone waste to the different profile of soluble P (Figure 2). Please elaborate this result discussion.
3. Please do not break the table 1 on the different pages.

Recommendation: Revisions Required

Reviewer B:

This study presents an innovative approach to composting using *Aspergillus niger*, focusing on phosphorus release from chicken bones. While the methodology and results are promising, several areas could be improved to enhance clarity, logical flow, and consistency.

1. Abstract Clarity (Abstract, p.1, lines 5-15)
   The abstract is informative but somewhat dense. Simplifying sentence structures and clarifying key findings (e.g., exact improvements in corn germination) would improve readability.

2. Inconsistent Terminology (Throughout, e.g., p.3, lines 10-20)
   Terms like “solid-state fermentation” and “SSF” are used interchangeably without prior definition. Ensure consistency and define all abbreviations upon first use.

3. Methodological Detail (Methodology, p.6, lines 5-15)
   Details about moisture content adjustments during composting are insufficient. Specify how consistent moisture levels were maintained throughout the process.

4. Data Presentation (Results, p.9, Figure 2)
   Figure 2 lacks error bars, which are essential for interpreting data variability. Including them would improve data transparency.

5. Language Issues (Discussion, p.11, lines 5-15)
   Several sentences are awkwardly constructed, such as “The gradual increase in soluble P beyond the first day…” Rewriting for clarity and grammatical accuracy is recommended.

6. Statistical Analysis (Results, p.10, lines 10-25)
   The manuscript mentions results but lacks statistical significance testing (e.g., p-values, confidence intervals). Including this would validate the findings.

7. Logical Flow (Results and Discussion, p.12, lines 5-20)
   Without clear transitions, the discussion jumps between topics (e.g., phosphorus solubilization and corn germination). Improving flow would help maintain reader engagement.

8. Reference Updates (Introduction, p.3, lines 20-30)
   Some references (e.g., FAO, 2013) are outdated. Including more recent studies on food waste and composting would enhance the study’s relevance.

9. Proofreading (Throughout)
   Minor typographical errors (e.g., inconsistent use of italics for scientific names like *Aspergillus niger*) should be corrected throughout the manuscript.

Recommendation: Revisions Required

Jurnal Rekayasa Proses

A-Review results - Soluble phosphorus rich compost.pdf
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4. Hasil revisi

Reviewer A:	We thank Reviewer A for reviewing our manuscript.
1. Recommend proofreading the manuscript due to several grammatical errors.	We have checked and revised the manuscript.
2. In the introduction part, it is necessary to add the information of the chicken bone content which is potentially utilized for composting as well as why it is chosen to be used for SSF on this manuscript.	We have added some information regarding chicken bone and the reason to choose chicken bone for this study in the introduction part.
3. In the last paragraph of the introduction, it is recommended to add a statement to show the benefit of this study.	We have added a statement to show the benefit of this study.
4. Figure 1 caption showed the organic food waste, which not includes the chicken bone in the graph. Meanwhile chicken bone is organic waste. It is recommended to change it into “non-bone food waste” to avoid the confusion. Please change through the manuscript for this context.	We have revised the term in the caption of Figure 1 and through the manuscript.
5. It is recommended to write the procedure into several sub section, such as 2.2.1 Pretreatment of the waste 2.2.2 Preparation of *Aspergillus niger* spore etc	We have divided the method into several sub-sections.
1. Please add the detail information in the procedure. For example, there is no clear information on how long the incubation for SSF, how the measurement of P content was done, how the compost substrate was made, wild microbes compost, etc. 2. There is no discussion about the effect of ratio of non-bone:bone waste to the different profile of soluble P (Figure 2). Please elaborate this result discussion. Please do not break the table 1 on the different pages.	We have elaborated the procedures. We have added some brief discussion about the effect of non-bone to bone ratio on P solubilization. We have arranged the layout.
Reviewer B: This study presents an innovative approach to composting using *Aspergillus niger*, focusing on phosphorus release from chicken bones. While the methodology and results are promising, several areas could be improved to enhance clarity, logical flow, and consistency.	We thank Reviewer B for his/her recommendation and suggestions.
1. Abstract Clarity (Abstract, p.1, lines 5-15) The abstract is informative but somewhat dense. Simplifying sentence structures and clarifying key	We have re-written the abstract and added the key findings into the abstract.

findings (e.g., exact improvements in corn germination) would improve readability.	
2. Inconsistent Terminology (Throughout, e.g., p.3, lines 10-20) Terms like “solid-state fermentation” and “SSF” are used interchangeably without prior definition. Ensure consistency and define all abbreviations upon first use.	We have ensured the consistency of SSF abbreviation use in abstract and introduction part only.
3. Methodological Detail (Methodology, p.6, lines 5-15) Details about moisture content adjustments during composting are insufficient. Specify how consistent moisture levels were maintained throughout the process.	We have specified the relative humidity in the incubator to minimize water evaporation from compost.
4. Data Presentation (Results, p.9, Figure 2) Figure 2 lacks error bars, which are essential for interpreting data variability. Including them would improve data transparency.	We have displayed the error bars in Figure 2.
5. Language Issues (Discussion, p.11, lines 5-15) Several sentences are awkwardly constructed, such as “The gradual increase in soluble P beyond the first day...” Rewriting for clarity and grammatical accuracy is recommended.	We have revised the sentence.
6. Statistical Analysis (Results, p.10, lines 10-25) The manuscript mentions results but lacks statistical significance testing (e.g., p-values, confidence intervals). Including this would validate the findings.	We have processed elemental analysis data with t-test to validate the change of C and N content during composting.
7. Logical Flow (Results and Discussion, p.12, lines 5-20) Without clear transitions, the discussion jumps between topics (e.g., phosphorus solubilization and corn germination). Improving flow would help maintain reader engagement.	We have added some sentences to explain the relation between phosphorus solubilization and corn germination.
8. Reference Updates (Introduction, p.3, lines 20-30) Some references (e.g., FAO, 2013) are outdated. Including more recent studies on food waste and composting would enhance the study’s relevance.	We have renewed the reference to the latest findings of FAO.
9. Proofreading (Throughout) Minor typographical errors (e.g., inconsistent use of italics for scientific names like *Aspergillus niger*) should be corrected throughout the manuscript.	We have checked and revised the inconsistence writing of scientific names in the manuscript.

Soluble Phosphorus Rich Compost ~~from Chicken Bone~~ by Aerobic Solid-State Fermentation of Chicken Bone-Containing Food Waste with *Aspergillus niger*

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A B S T R A C T

Food waste ~~has become~~is a ~~concern~~major issue in solid waste management due to its high ~~bulk~~ volume and ~~production rate.~~rapid accumulation. Without ~~good management, food waste piling may result in~~ proper handling, it can cause foul ~~odor, global warming by~~odors, methane ~~emission~~emissions, and ~~even an epidemic.~~health risks. Composting is a ~~method usually employed to process food waste. However, this method~~ common solution but is often ~~took a long period~~slow and ~~selective~~limited to certain organic ~~waste such as leafy~~ materials ~~or fruit waste. Bone is a food waste that is rich in~~. Bones, a phosphorus~~,~~-rich food waste, have potential for converting food waste into a ~~chemical substance that is required to make various useful products such as~~valuable fertilizer~~, battery, detergent, etc.~~. This study ~~investigates the characteristics of food waste compost containing~~examines composting chicken bones and non-bone ~~and various domestic organic~~ food waste ~~that was processed~~ through aerobic solid-state fermentation (SSF) using *Aspergillus niger*. The ~~composting process was monitored over a~~ 28-day ~~period~~process at 28°C~~. The resulting compost was characterized under various physicochemical parameters, including~~ was analyzed for pH and nutrient composition. ~~The findings demonstrated~~Results showed that *Aspergillus niger* effectively ~~released phosphorus from bone while~~ degraded organic matter ~~in just~~and released phosphorus within a week, ~~resulting in significant reductions in unprocessed waste volume and the production of a stable, nutrient-rich compost in a short time. Additionally, the compost was successfully induced better~~producing compost with high soluble P (2.47 mg P/g). Applying this compost to corn seeds for 7 days resulted in improved growth ~~on corn seed~~, 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 ~~the food waste pile and~~ plants supplemented with naturally composted food waste. Direct application of food waste led to stunted growth. These ~~results suggest that~~findings highlight *Aspergillus niger*-mediated ~~aerobic solid-state fermentation is a viable and~~ SSF as an efficient method ~~for converting~~to convert food waste into high-quality compost, ~~promoting~~supporting sustainable agriculture and waste management ~~and agricultural practices.~~.

Keywords: food waste; composting; *Aspergillus niger*; phosphorus; corn germination

A B S T R A K

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Limbah makanan ~~telah menjadi isu~~ merupakan masalah utama dalam pengelolaan limbah padat ~~karena volume massanya yang besar dan tingkat produksinya~~akibat volumenya yang tinggi~~.~~ dan akumulasi yang cepat. Tanpa ~~pengelolaan~~penanganan yang baik, ~~penumpukan~~ limbah makanan dapat ~~menghasilkan~~menyebabkan bau ~~busuk, pemanasan global akibat~~ tak sedap, emisi metana, dan ~~bahkan epidemi.~~risiko kesehatan. Pengomposan adalah ~~metode yang~~solusi umum ~~digunakan untuk memproses limbah makanan. Namun, metode ini~~, tetapi prosesnya sering ~~memakan waktu lama~~kali lambat dan ~~selektif terhadap limbah~~ terbatas pada jenis bahan organik ~~seperti bahan daun atau limbah buah.~~tertentu. Tulang ~~adalah~~, sebagai limbah makanan yang kaya ~~akan~~ fosfor, ~~zat kimia yang diperlukan untuk membuat berbagai produk berguna seperti~~ berpotensi untuk dikonversi menjadi pupuk~~, baterai, deterjen, dan lain-lain.~~ yang bernilai tinggi.

Studi ini ~~menyelidiki karakteristik kompos limbah makanan yang mengandung~~ meneliti pengomposan tulang ayam dan ~~berbagai~~ limbah makanan ~~organik domestik yang diproses~~non-tulang melalui fermentasi padat aerobik (SSF) menggunakan Aspergillus niger. Proses ~~pengomposan dipantau~~ selama ~~periode~~ 28 hari pada suhu ~~tetap (~~28°C~~). Kompos yang dihasilkan dikarakterisasi~~ dianalisis berdasarkan ~~berbagai parameter fisik dan kimia, termasuk~~ pH dan komposisi nutrisi. ~~Temuan~~Hasil menunjukkan bahwa ~~Aspergillus~~A. niger secara efektif menguraikan bahan organik dan melepaskan fosfor ~~dari tulang sambil mendegradasi bahan organik hanya dalam~~ dalam waktu seminggu ~~sehingga~~, menghasilkan ~~pengurangan volume limbah tak terolah yang signifikan dan produksi~~kompos dengan kadar P terlarut tinggi (2,47 mg P/g).

Aplikasi kompos ~~yang stabil dan kaya nutrisi dalam waktu singkat. Selain itu, kompos tersebut berhasil~~ini pada benih jagung selama 7 hari meningkatkan pertumbuhan ~~biji jagung~~ dengan batang yang lebih tinggi 0,8 cm dan lebih tebal 0,4 cm, dua helai daun lebih banyak serta sistem akar serabut yang lebih rimbun dibandingkan dengan ~~tumpukan limbah makanan dan limbah makanan yang dikomposkan secara~~ jagung yang diberi kompos alami. Penggunaan langsung limbah makanan justru menghambat pertumbuhan. Hasil ini menunjukkan bahwa ~~fermentasi padat aerobik yang dimediasi oleh Aspergillus~~SSF berbasis A. niger ~~adalah~~merupakan metode ~~yang layak dan~~ efisien untuk mengubah limbah makanan menjadi kompos berkualitas tinggi, ~~yang~~ mendukung ~~pengelolaan limbah~~pertanian berkelanjutan dan ~~praktik pertanian~~pengelolaan limbah yang lebih baik.

*Kata kunci***:** *limbah makanan; pengomposan; Aspergillus niger; fosfor; perkecambahan jagung*

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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 ~~accounts~~accounted for 40.7% of the 35,803,483.85 tons of solid waste produced by various sectors of society (KLHK, 2022). ~~FAO indicates that unprocessed and unconsumed food waste can reach up to 1.6 billion tons annually, with non-composted food waste contributing to greenhouse gas emissions equivalent to 3.3 billion tons of CO_2 per year (FAO, 2013).~~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 (FAO, 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).

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~~Phosphorus (~~P~~)~~ is a vital element used in various industrial sectors, particularly in the agricultural sector. ~~Adequate~~An adequate level of P is ~~necessarily required~~necessary during the seedling stage ~~for promoting~~to promote root and shoot growth, ~~increasing~~increase leaf area and plant height~~ as well~~, and prepare for ~~preparing~~ early flowering and fruit ~~yielding stage~~yield through its involvement in many biochemical reactions (Abobatta and 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 ~~phosphorus~~P minerals depletion as early as in the 2090s ~~(Reijnders, 2014)~~(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 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 ~~phosphorus from phosphate minerals. Heidarzadeh et al.~~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~~ (Heidarzadeh et al., 2019).~~. *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).~~(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 ~~phosphorus~~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).~~(Wang et al., 2022). However, studies on ~~phosphorus~~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 ~~phosphorus~~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.

~~Phosphorus~~A P test kit with a detection range ~~at~~of 0.0025 - 5.00 mg/L ~~PO_4-~~PO4-P (Merck Millipore, Germany) ~~were~~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 ~~IVand~~IV and HNO_3 (Merck Millipore, Germany) were used in multi-metal analysis contained in the ~~ashed~~ash sample.

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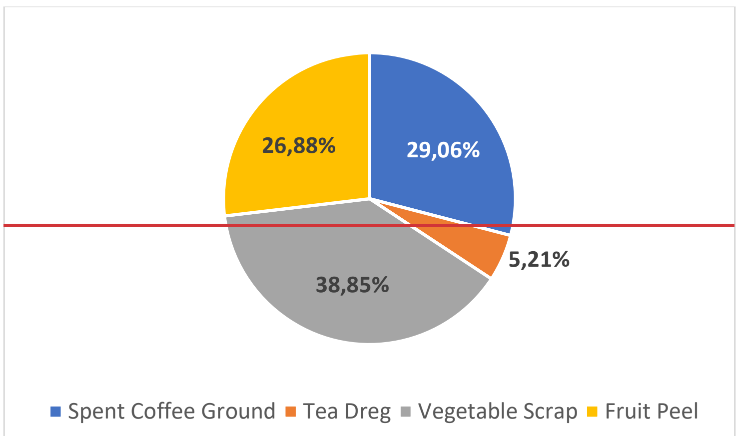


~~**Figure 1.** Composition of Organic Food Waste Powder (Dry Basis).~~

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 ~~organic~~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 ~~organic~~non-bone food waste powder was prepared by mixing ~~the~~ sieved spent coffee grounds, tea dreg powder, vegetable waste powder, and fruit peel powder ~~according to~~in their original weight proportions ~~as initially~~when collected (Figure 1).

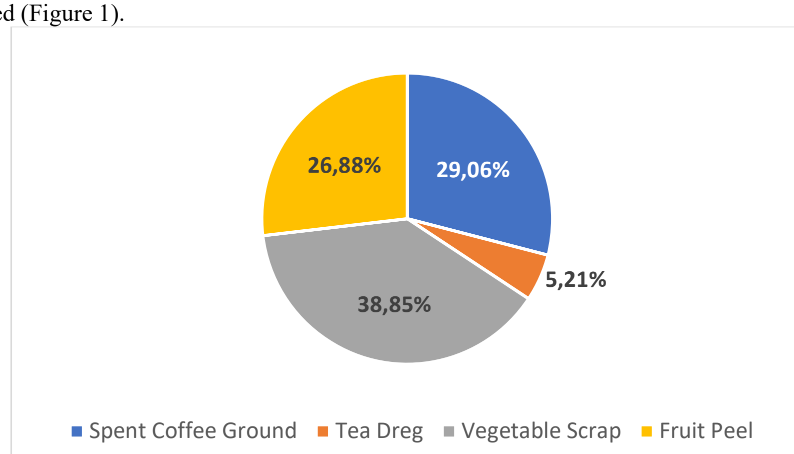


Figure 1. Composition of Non-Bone Food Waste Powder (Dry Basis).

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.

2.2.2 Composting Procedure

Compost substrate was prepared by mixing ~~organic (~~non-bone~~)~~ food waste powder and chicken bone powder at ~~various~~varying mass ~~ratio~~ratios of 50:50, 75:25 and 90:10. Mixing was done in several petri dishes to facilitate replication~~ and sampling.~~. The homogeneous substrate mixture was then moistened to approximately 60% moisture content ~~using~~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 ~~next~~at 28°C for 1 day, 3 days and several days until up to ~~a~~ 28 days. A glass beaker filled with 500 mL ~~container~~ of deionized water was placed in ~~an~~the incubator ~~set at 28°C~~ 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.

2.2.2 Compost Analyses

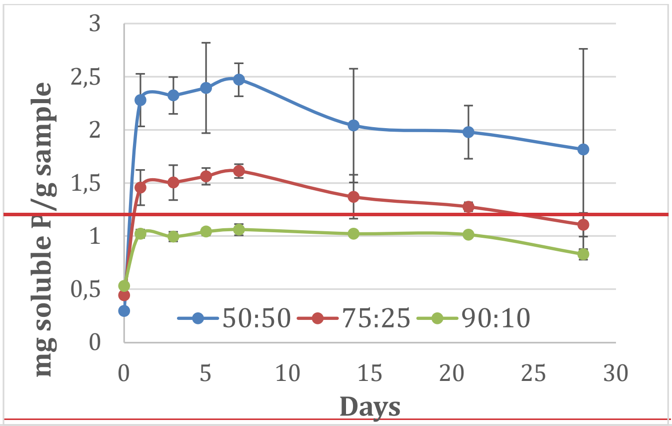
At specific incubation time intervals, ~~5~~10 g ~~of~~ compost samples were collected for moisture content~~,~~ analysis. Then, the dried sample was divided into two equal parts for total P and insoluble P measurements. For total P analysis, 5 g of dried sample was placed in a furnace set at 600°C for 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~~, and total phosphorus (P) measurement. Soluble P was extracted by stirring the sample in~~ . 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~~, followed~~ to remove soluble P. After that, the leftover solid was recovered by filtration~~–~~. The recovered solid was then ashed and ~~centrifugation at 3000 rpm~~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 ~~5 minutes to separate the solid, which contained~~ insoluble P~~. The solid phase was then ashed for P~~ measurement~~.~~ using spectrophotometry technique at 430 nm. Soluble P ~~was calculated as the difference between total P in original~~ content in the sample ~~and~~ was determined by subtracting the insoluble P ~~in the extracted sample~~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. ~~Two negative controls were included: (1) uncomposted food waste mixture and (2) naturally composted food waste mixture, which was prepared by moistening the substrate with sterile water and incubating for the same period that yielded~~A corn seed then was put into the ~~highest P release by Aspergillus niger. These controls were used to compare P release by Aspergillus niger with that by wild microorganisms.~~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



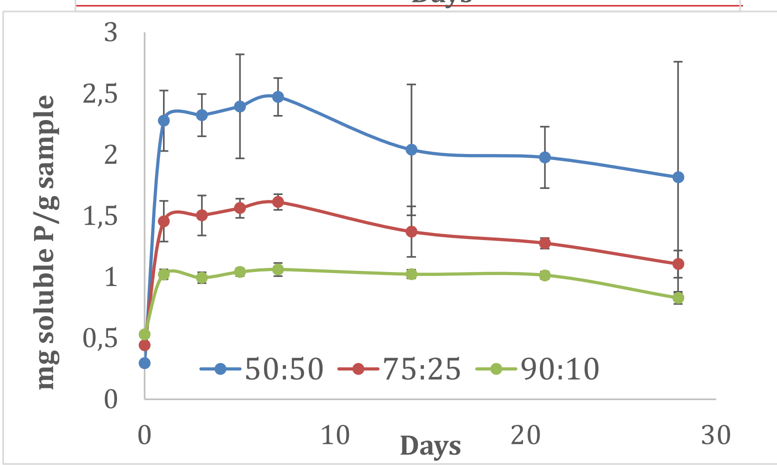


Figure 2. Time Profile of Soluble P in Food Waste Compost Samples with Varying ~~Organic Waste-~~Non-Bone to~~-~~ Chicken Bone Ratio.

Figure 2 illustrates the profile of ~~phosphorus (~~P~~)~~ release by *Aspergillus niger* in this study. The data ~~reveal~~revealed that the highest P release was observed ~~gradually over~~ after a 7-day incubation period across all ratios~~, with the 50:50 organic-to-bone ratio yielding the most significant soluble P release.~~. The sharp increase in soluble P observed after ~~just 1~~the first day of incubation suggests that P release ~~was~~is primarily influenced by *Aspergillus niger* growth~~, alongside its metabolic activities~~. This finding aligns with the study by ~~Upton et al. (2017)~~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):

$$Ca_{10}(PO_4)_6(OH)_{2(s)}+4C_6H_8O_{7(aq)} \rightarrow 2Ca_3(C_6H_5O_7)_{2(s)}+2CaHPO_{4(aq)}+2Ca(H_2PO_4)_{2(aq)}+2H_2O_{(g)}$$

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Hydroxyapatite	Citric acid	Calcium citrate	Dicalcium phosphate	Calcium dihydrogen-phosphate	Water

~~The gradual increase in soluble P beyond the first day of incubation can be attributed to a reduction in citric acid excretion, likely due to the inhibition of pyruvate carboxylase activity in the high P environment (Feir and Suzuki, 1969). As the incubation period progressed, *Aspergillus niger* reached maturity, transitioning into its reproductive cycle, evident by the darker appearance of the substrate due to conidia formation. The uptake of external P for reproduction and polyphosphate storage by both the older and newer generations of *Aspergillus niger* (Upton et al., 2017) led to a reduction in soluble P levels within the compost over extended incubation periods.~~

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~~Table 1 presents the quality of compost produced from a 50:50 ratio of chicken bone to organic food waste, composted at 28°C for 7 days with the assistance of *Aspergillus niger*. This compost exhibited the highest soluble phosphorus (P) content. The carbon-to-nitrogen (C/N) ratio, a key indicator of compost maturity, was slightly lower than the minimum pH specified by composting standards. To assess the composting process, total nitrogen (N) content and the C/N ratio of the substrate were analyzed (Table 2). The decrease in total N and C content suggests the occurrence of nitrogen release, typically through ammonification, alongside the consumption of carbohydrates during composting (Heidarzadeh et al., 2019). Ammonification also contributed to a rise in substrate pH from 6.50 to nearly 7.0, a trend that was more pronounced when the organic waste content was higher (e.g., organic-to-bone ratios of 75:25 and 90:10). Under these conditions, the compost pH reached 7.60-7.70 by the 7th day. Although the data showed a low initial C/N ratio, this may not prove the immaturity of compost since the substrate had very low C/N ratio to begin with. Nevertheless, since these parameters closely align with the standard, agronomic performance should be considered when evaluating the compost's suitability for commercialization.~~

~~**Table 1.** Properties of Chicken Bone:Organic Food Waste (50:50) Composted by *Aspergillus niger* for 7 days at 28°C (Dry Basis).~~

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.

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

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~~**Table 2.** C, N and C/N ratio~~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).

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*	%C	40.91 ± 2.16
	%N	4.80 ± 0.05

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(Bone:Organic = 50:50, 28°C, 7 days)	C/N	8.53 ± 0.36

~~Direct application of food waste for corn seed germination yielded suboptimal growth (Figure 3). The compost produced by wild microbes resulted in corn plant that was 0.8 cm shorter than that grown in *Aspergillus niger* enriched compost. A significant difference was also observed in leaf number and fibrous root development; corn seed planted in soil enriched with *Aspergillus* compost produced two additional leaves and more extensive fibrous root systems, indicating better nutrient availability around the seed. Additionally, the stem diameter was slightly larger (approximately 0.4 cm). While the total P content of uncomposted food waste and naturally produced compost was similar to that of the *Aspergillus*-treated compost (around 0.3%), the soluble P levels in uncomposted food waste and natural compost were only 0.02 and 0.75 mg P/g, respectively. These findings suggest that soluble P in the growth medium plays a critical role in corn seed germination and leaf development.~~

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 7^{th} day of incubation. This phenomenon was even more pronounced (pH 7.60-7.70 on the 7th day of incubation) at 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 (Ballesteros et al., 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.

Figure 3. ~~The~~ Results of Corn Seed Germination for 7 ~~days using~~Days Using (1) Aspergillus niger Compost, (2) Wild Microbes Compost, and (3) a Mixture of Chicken Bone and ~~Organic~~Non-Bone Food Waste at a 50:50 Ratio.

4. Conclusions

This study demonstrates that ~~phosphorus~~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 ~~the~~ compost ~~increases~~increased, compost ~~with~~acidity became neutral ~~pH~~ and higher levels of soluble ~~phosphorus~~P was ~~produced~~observed. Since excessive external ~~phosphorus~~P appeared to inhibit further organic acid ~~production, the~~synthesis by *Aspergillus niger*, soluble ~~phosphorus crept up to its peak at~~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 ~~stems~~stalk, as well as ~~wider~~more extensive fibrous root and leaf development, compared to ~~the control growth~~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

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5. Notifikasi *submission* revisi (13-03-2025)

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[jrekpros] Editor Decision

1 message

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Reply-To: "Ade Kurniawan, S.T., M.Eng., Ph.D." <ade.kurniawan@ugm.ac.id>
To: Lu Ki Ong <ongluki@petra.ac.id>, Ricardo Angelio <ricardangelio213@gmail.com>, Clarissa Indrayani <indrayani.clarissa@gmail.com>, "Prof. Lieke" <lieke@staff.ubaya.ac.id>

Lu Ki Ong, Ricardo Angelio, Clarissa Indrayani, Prof. Lieke:

We have reached a decision regarding your submission to Jurnal Rekayasa Proses, "Soluble Phosphorus Rich Compost from Chicken Bone by Aerobic Solid-State Fermentation of Food Waste with Aspergillus niger".

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7. *Final Paper*

JURNAL REKAYASA PROSES

VOLUME 19, NUMBER 1, 2025, 75–80

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-

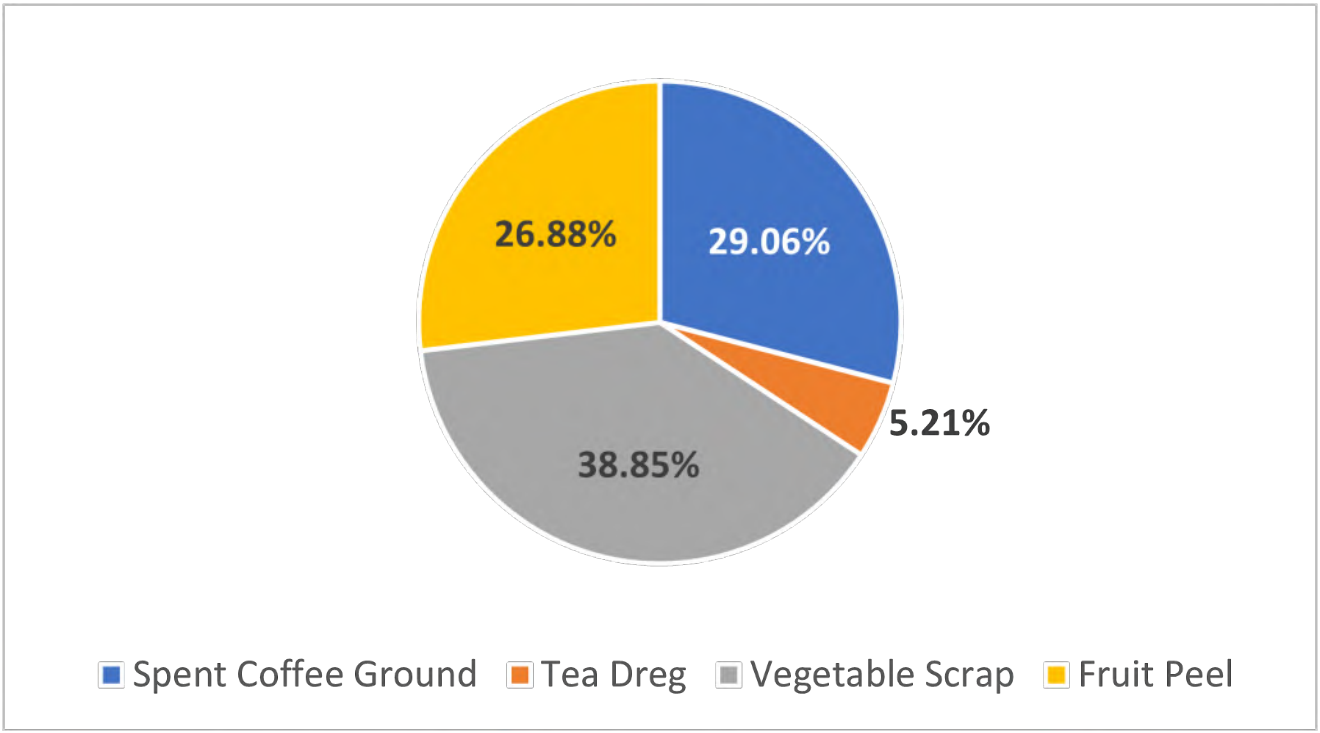

FIGURE 1. Composition of non-bone food waste powder (dry basis).

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):

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

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).

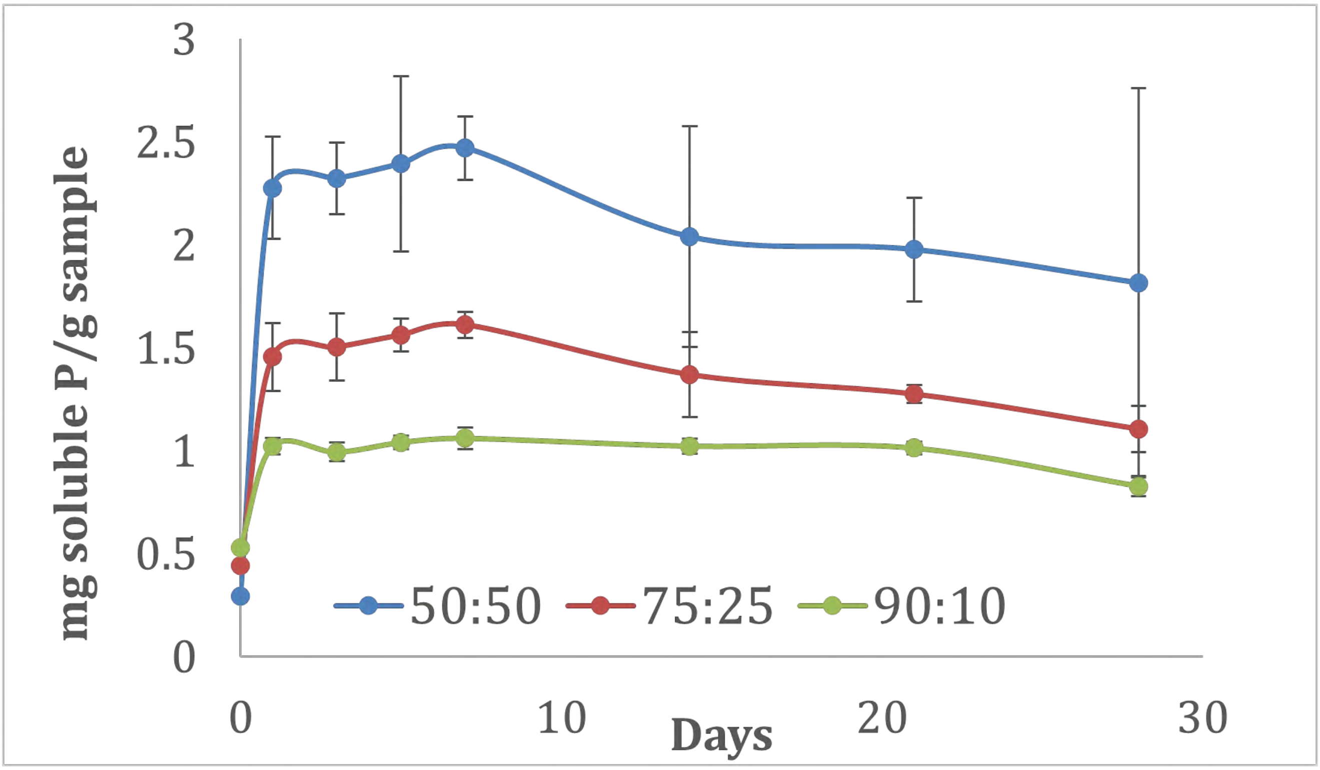

FIGURE 2. Time profile of soluble P in food waste compost samples with varying non-bone to chicken bone ratio.

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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