

Effect of Differences in Bio-activators and Fermentation Time on the Properties of Liquid Organic Fertilizers Based on Local Rabbit's Urine Waste

Muhammad Irfan Said^{1*}, Jamila Mustabi¹, Abdel Razzaq Al Tawaha²,
Sitti Nurani Sirajuddin¹, Nur Azizah¹, Raha Al-Assaf²

¹ Faculty of Animal Science, Hasanuddin University Jl. Perintis Kemerdekaan Km. 10 Makassar, South Sulawesi, 90245, Indonesia

² National Agricultural Research Center (NARC), Baq'a 19381, Jordan

* Corresponding author's email: irfanunhas@gmail.com; irfan.said@unhas.ac.id

ABSTRACT

Rabbits, which are commonly raised as livestock in many rural areas of Asian countries, produce liquid waste called urine that can have negative environmental impacts. In order to tackle this issue, the development of technology is necessary to effectively process this waste into a form that can be utilized without causing harm to the environment. Urine contains valuable organic compounds that can be used as raw materials for the production of liquid organic fertilizer (LOF). The quality of this fertilizer is dependent on the availability of microorganism substrates and the duration of fermentation. The objective of this study is to investigate the influence of different substrates and fermentation periods on the properties of liquid organic fertilizer. The raw material used in this research is locally sourced rabbit urine. The experiments were conducted in a laboratory at the Faculty of Animal Science, Hasanuddin University, Indonesia. Three types of substrates were employed: bioactivator animal substrate (BASb), bioactivator plant substrate (BPsb), and commercial microorganism (C-mic) as the control. Two different fermentation periods were tested: 2 weeks and 4 weeks. The data were analyzed using a completely randomized design (CRD) with factorial patterns. The results revealed that the type of bioactivator substrate had a significant impact ($p < 0.05$) on the N-organic, C/N ratio, P_2O_5 , and K_2O content. However, pH and C-organic showed no significant effect ($p > 0.05$). Additionally, fermentation time had a significant effect ($p < 0.05$) on C-organic, N-organic, and the C/N ratio, but pH did not have a significant effect ($p > 0.05$). Moreover, there was a significant interaction ($p < 0.05$) between the substrate type and fermentation time in the LOF-RU process. The production of N-organic content from local rabbit urine yielded promising results. For the LOF-RU production process with BASb or BPsb, fermentation periods of up to 4 weeks can be applied.

Keywords: bio activator, fermentation, rabbit urine, pH, nitrogen content.

INTRODUCTION

Over the past century, chemical fertilizers have been extensively utilized to maximize productivity in conventional agricultural systems due to the growing population (Jakhar et al., 2022). However, the excessive and continuous use of chemical fertilizers has had significant negative impacts on the environment, including diminished soil quality and disruption of microbial communities (Baghbani-Arani et al., 2021; Naz et al., 2022;

Richardson et al., 2023). Furthermore, the use of chemical fertilizers, particularly in agriculture, is increasing each year to meet the growing demand for food. This demand is driven by population growth and the improving social status of society. It is undeniable that chemical fertilizers play a critical role in agricultural production, making people, especially farmers, highly dependent on them (Zhi et al., 2022). However, the extensive use of chemical fertilizers can have several negative consequences including: (a) damage to soil

structure and this occurred because not all chemical fertilizers are fully absorbed by plants, leaving residue in agricultural land that can harm the soil structure, (b) acidification of soil due to excessive use of chemical fertilizers leads to the formation of acid compounds, which can harden the soil and hinder the growth of macro-organisms (worms) and microorganisms (c) water pollution because the chemical compounds from fertilizers can contaminate nearby water sources, such as rivers and lakes, as plants cannot fully absorb these substances, (d) uncontrolled growth of aquatic plants due to excessive use of chemical fertilizers can cause the unchecked growth of aquatic plants, reducing the oxygen levels available to aquatic organisms and (e) alkalinity of soil and this occurred due to excessive amounts of certain chemical components (K, Mg, and Ca) in fertilizers can increase soil alkalinity, depleting other crucial minerals and risking mineral deficiencies. Therefore, to mitigate the adverse impacts of chemical fertilizers, one alternative is the application of rabbit urine as a raw material for liquid organic fertilizer. This can help reduce the reliance on chemical fertilizers and alleviate their negative effects. Consequently, there is a pressing need for a new sustainable approach in agriculture, such as the utilization of organic fertilizers derived from animal waste. Organic fertilizers are gaining popularity in agriculture as they provide a sustainable and environmentally friendly alternative to chemical fertilizers. These fertilizers are typically sourced from organic materials, such as animal waste (Sorensen and Thorup-Kristensen, 2011; Berry et al., 2002), plant residues (Bergstrand, 2022), and compost.

Nowadays, animal waste, especially urine, is commonly used to produce organic fertilizer known as liquid organic fertilizer (LOF). Rabbit urine (RU) is particularly valuable for this purpose due to its high nutrient content, including nitrogen, phosphorus, and potassium, which are essential for organic fertilization (Mutai, 2020; Ferichani, 2024). RU is widely recognized for its superior nutrient composition compared to urine from other livestock. Using LOF derived from RU waste is considered a sustainable and cost-effective approach to enhancing soil fertility and promoting crop productivity (Diaz-Elsayed et al., 2020). LOF-RU contains several elements such as: N (2.7%); P (1.1%); K (0.5%) and Ca (1.2%) (Irawan et al., 2022). Furthermore, organic fertilizer produced from rabbit urine by applying different bio-activators has an N element content of 1.51–2.44%, P_2O_5 (2.09–3.12%); and K_2O (3.28–3.71%). As a

comparison, other researchers have produced liquid organic fertilizer from rabbit urine with nutrient content, namely: N (4%); P_2O_5 (2.8%); and K_2O (1.2%). The nutrient content in rabbit urine is relatively higher than the nutrient content in cow urine, namely: N (1.21%); P_2O_5 (0.65%); K_2O (1.6%) and in goats, namely: N (1.47%); P_2O_5 (0.05%); K_2O (1.96%) (Balittanah, 2006). Rabbit fertilizer contains organic matter C/N: (10±12%) and pH 6.47±7.52 (Sajimin et al., 2003). Other studies have also compared the chemical composition of rabbit urine with cow urine. The chemical composition of rabbit and cow urine respectively is: pH (9.14) and (8.74); organic C (0.62%) and (0.74%); total N (2.11%) and (1.79%); P_2O_5 (1.1%) and (0.005%) and K_2O (0.5%) and (1.68%) (Rosniawaty et al., 2015).

On the other hand, the properties of LOF produced from RU waste can vary depending on factors such as the type of bio-activators used and the duration of fermentation (Said et al., 2018). The choice of bio-activators during the fermentation process significantly impacts the nutrient content, microbial activity, and overall quality of the LOF (Said et al., 2018). Additionally, the duration of fermentation plays a crucial role in the production of liquid organic fertilizers (Said et al., 2018). Longer fermentation periods allow for a more thorough breakdown of organic matter, resulting in higher nutrient concentrations and increased microbial populations. These factors, combined with other variables like temperature and pH, can affect the stability, nutrient release kinetics, and overall effectiveness of liquid organic fertilizers derived from rabbit urine waste. Different bioactivators may contain specific strains of beneficial microorganisms that enhance nutrient cycling and improve nutrient availability for plants. Additionally, microorganisms significantly impact various processes, including de-nitrification, nitrification, and nitrogen fixation (Hsu et al., 2008; Philippot et al., 2011). Soil microorganisms also play a crucial role in the formation of both organic and inorganic compounds (Hu et al., 2011). As a result, the nutrient condition of the soil influences plant growth and the occurrence of disease in plants (Zhang et al., 2012). Fertilizers derived from livestock waste provide ample nutrients for food plants, enabling more efficient and environmentally friendly production (Leite et al., 2010; Ferichani, 2024).

Several indicators that can be used as parameters in determining soil quality include: the amount of microbial biomass in the soil and the activity and structure of these microbial groups (Nikitin et al., 2022; Maurya et al., 2020; Hermans et al., 2020).

Soil conditions for sustainable plant growth are influenced by adaptation processes and microbial structure (Hartmann, and Six, 2023; Das et al., 2022). In organic farming systems, the level of functional microbial diversity is more varied than in conventional farming systems. Land use patterns or seasons need attention and support in implementing organic farming systems (Wang et al., 2020)

The role of microorganisms in the formation of organic fertilizer is of utmost importance (Singh et al., 2020; Bamdad et al., 2022). However, the availability of microorganism resources is severely limited due to difficulties in accessing commercial sources, particularly for farmers residing in rural areas. Additionally, the cost of obtaining these resources is exorbitant. Consequently, harnessing the potential of local microorganisms presents a promising solution to this problem. Local microorganisms (L-mic) are microorganisms that thrive and adapt in open and spacious environments. They grow on specific substrates, and their remarkable adaptability is a notable advantage. Moreover, they are relatively inexpensive and easy to obtain and cultivate. The types of local microorganisms that flourish are influenced by the substrate they grow on. The substrate plays a vital role in providing nutrients for the metabolic processes of these microorganisms. Further research is warranted to comprehensively investigate the application of local microorganisms on different substrates in the formation of organic compounds. Therefore, farmers and researchers must conduct a thorough investigation into the impact of various bio-activators and fermentation durations on the characteristics of these fertilizers. By examining these factors, researchers can enhance the production process and ensure that the liquid organic fertilizers derived from rabbit urine waste satisfy the nutritional needs of crops, while also mitigating any potential adverse environmental effects. Consequently, the objective of this study is to assess the efficacy of liquid organic fertilizer derived from rabbit urine (LOF-RU) that utilizes natural components from animal and plant species as living substrates for L-mic.

MATERIALS AND METHODS

Experimental site

This study was conducted at the Beef Livestock Production Laboratory, Faculty of Animal Science, Hasanuddin University, Makassar, Indonesia.

Liquid organic fertilizer material

The main ingredient for liquid organic fertilizer of rabbit urine (LOF-RU) is obtained from a local rabbit farming business, Mattoanging Village, Lalabata District, Soppeng Regency, South Sulawesi Province, Indonesia.

Commercial microorganisms' source

Commercial microorganisms (C-mic) as a comparison (control) use the production of PT. Songgo Langit Persada, Indonesia. The C-mic contains *Lactobacillus sp* bacteria, *Rhodopseudomonas sp*, *Actinomyces sp*, *Streptomyces sp*, Yeast and Fungi.

Bio-activator animals and plants substrate materials

The bio activator animal substrate (BASb) material was extracted from Balinese cow dung obtained from the Beef Livestock Production Laboratory and the bio activator plant substrate (BPSb) was extracted from local banana roots obtained from the garden of the Faculty of Animal Science, Hasanuddin University, Makassar, Indonesia. Supporting materials in the LOF production process consist of lime farming (dolomite), brown sugar and wastewater from washing rice. Equipment supporting the production process such as urine fermentation installations, hoses, plastic bottles, measuring cups, thermometers, and pH meters. In order to maintain the consistency and standardization of all samples, the bioactivator substrate is prepared as a stock solution. This solution is carefully made by selecting raw materials that are clear and consistent, sourced from a single reliable supplier with controlled collection time and method. Similarly, the composition of ingredients is meticulously weighed to ensure accuracy. All steps, including material selection, weighing, collection times, and production processes, are carefully controlled and carried out with precise and equal measurements.

Preparation of bio-activator animal substrate (BASb)

Bio-activator animal substrate (BASb) was made from 1000 g of wet feces (< 3 hours after defecating) collected from the Bali cattle pen and then dissolved in 500 ml of water. Next, add

brown sugar in a 1:1 ratio. The solution was then stirred with a mixer at 100 rpm for 10 minutes. The solution was then put into a plastic container and fermented for 2 weeks at room temperature under anaerobic conditions.

Preparation of bio-activator plant substrate (BPsb)

Bioactivator plant substrate (BPsb) was prepared by grinding 1,000 g of local banana root pieces. Local banana roots are ground until they resemble pulp. Then 2 liters of wastewater from rice washing and 100 g of brown sugar were added to the mixture as a food source for microorganisms. The ingredient mixture is then placed in a plastic container and the fermentation process is carried out at room temperature under anaerobic conditions for two weeks.

Preparation of the composition of LOF-RU ingredients

Regarding the implementation of the research, the complete formula design and composition of the materials used in making LOF-RU are presented in Table 1.

The production process of LOF-RU

The composition of the ingredients used in the LOF-RU production process has been carried out according to the formula in Table 1. All ingredients have been put into the fermentation container. The fermentation process was carried out anaerobically at room temperature. Fermentation time was carried out according to the treatment (2 and 4 weeks) for each of the three different types of substrates (BAsb, BPsb and C-mic).

Experimental design and treatment

The study was conducted using an experimental research design based on a completely randomized design (CRD) with a $2 \times 3 \times 3$ factorial pattern. Three types of substrates were used in the study: bioactivator animal substrate (BAsb), bioactivator plant substrate (BPsb), and Commercial microorganism (C-mic) as the control. Furthermore, two fermentation times were implemented, specifically 2 weeks and 4 weeks. The use of fermentation periods of 2 and 4 weeks is based on several previous studies which have shown that the activity of microorganisms in breaking down organic compounds in the substrate lasts between 2 and 4 weeks. Additionally, the focus of ongoing research is to continuously improve the efficiency of the fermentation process, making it an important aspect to consider. Each treatment was replicated three times.

Statistical analysis

The data obtained was then analyzed for variance using SPSS software. The results were significant, then continued with a real difference test using Duncan's Multiple Range Test (DMRT) at the 5% level (17).

RESULTS AND DISCUSSION

pH value

The analysis of variance (ANOVA) results shows that there is no significant difference ($p > 0.05$) between treatments in both treatment substrate type and fermentation time as well as their interactions for pH. A comparison of the pH

Table 1. Formula design and ingredients composition in LOF-RU using different types of substrate and fermentation times

Ingredients of LOF-RU	Formula and combination of substrate types with fermentation times					
	BAsb-T ₂	BAsb-T ₄	BPsb-T ₂	BPsb-T ₄	C-mic-T ₂	C-mic-T ₄
Rabbit urine (RU) (mL)	1.000	1.000	1.000	1.000	1.000	1.000
Lime farming/Dolomite (%) (w/v)*	9	9	9	9	9	9
Brown sugar (%) (w/v)*	4.5	4.5	4.5	4.5	4.5	4.5
BAsb (%) (v/v)*	0.45	0.45	-	-	-	-
BPsb (%) (v/v)*	-	-	0.45	0.45	-	-
C-mic (%) (v/v)*	-	-	-	-	0.45	0.45

Note: BAsb (bioactivator animal substrate); BPsb (bioactivator plant substrate); C-mic (commercial microorganism); fermentation time (T₂ = 2 weeks; T₄ = 4 weeks); *) percentage amount is calculated based on the amount of RU as raw materials.

values of LOF made from RU, hereinafter referred to as LOF-RU, is clearly shown in Figure 1. The pH test results of all LOF-RU products show almost the same value, namely in the range of 6.40 to 6.76. The average pH in LOF-RU is close to neutral pH conditions. The results of this pH test are consistent with the pH in LOF required by the regulation of the Ministry of Agriculture of the Republic of Indonesia. The results show that the pH standard for LOF products should be in the range of 4–9. Therefore, the formula results obtained for LOF-RU still meet the requirements and eligibility for declaration as an organic fertilizer product. This result is also consistent with the pH LOF of dairy cattle according to the research results of (Singh et al., 2013), which is 7.8. Raden et al. (2017) obtained lower values. The results of the study show that the pH value is in the range of 5.45–5.64. In this research, researchers have used three types of bio activators, namely commercial

microorganisms (EM4), Boisca or shrimp paste. In general, microbes operate at a neutral pH. Some of them also work at low pH values (acidic) to above neutral, namely pH 5.5–8. During the fermentation process, the decomposition process of organic materials takes place. Finally, organic compounds are formed. In this state, microorganisms grow and develop in the form of fungi and bacteria. Organic acids are formed, the pH becomes neutral (pH 6–8) and then reaches the optimal pH (8.5–10).

C-organic

Based on the results of the statistical analysis of the data presented in Figure 2, it is evident that differences in fermentation time have a significant impact ($p < 0.05$) on the levels of C-organic in LOF-RU. However, no significant effect ($p > 0.05$) is observed for differences in substrate type. In general, the organic material content in

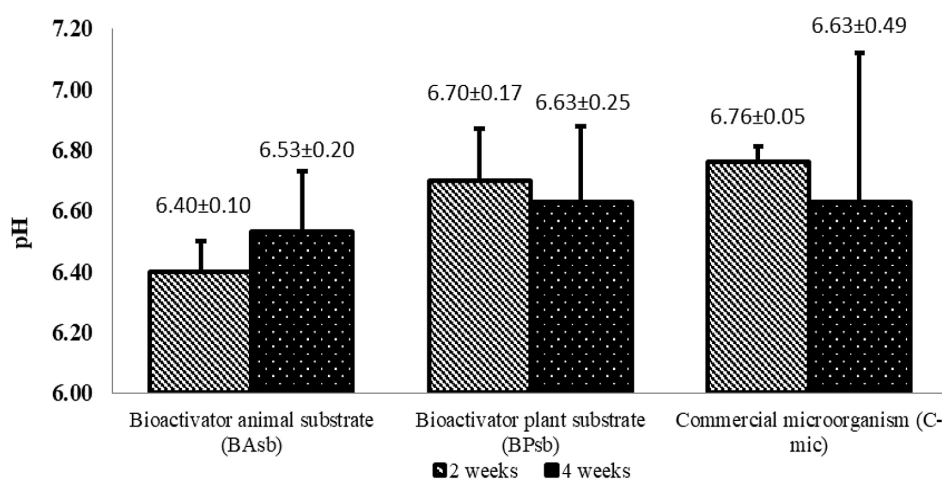


Figure 1. The effect of differences in substrate types and fermentation time in the production process of LOF-RU on the pH value

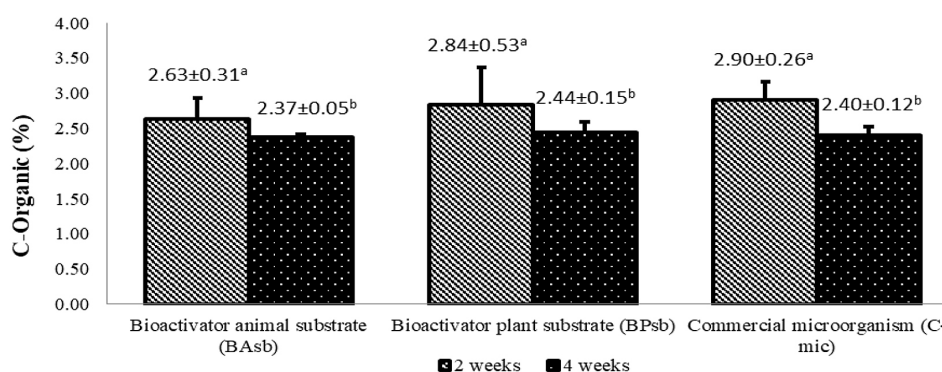


Figure 2. Graph of the effect of differences in substrate types and fermentation time and in the production process of liquid organic fertilizer of rabbit urine (Lof-Ru) on the C-organic (%) content; ^{a,b}Different superscripts for each treatment indicate significant differences ($p < 0.05$)

fertilizer plays a crucial role in determining its effectiveness in providing nutrients to plants. C-organic levels serve as one of the key indicators for assessing the quality of solid and liquid fertilizers (Reeves, 1997). Organic material functions to bind primary grains together, forming secondary grains. Similarly, in the formation of soil aggregates, C-organic is essential. This condition significantly influences porosity, water storage and supply, soil aeration, and soil temperature. Figure 2 provides a comprehensive comparison of the C-organic content of LOF-RU under different substrate types and fermentation times. The test results indicate that the average C-organic levels in LOF-RU are still higher (2.37–2.90%) compared to the findings reported by Raden et al. (2017), which ranged from 0.67–0.86%. The researchers utilized three types of bioactivators, namely EM4, boisca, and shrimp paste, as sources of microorganisms. Dordas et al. (2008) have previously reported that organic fertilizer materials derived from livestock possess the potential to yield high levels of C-organic, particularly in the topsoil. The C-organic levels could reach up to 18%, particularly on the soil surface. Errors in application, timing, and harvest methods may lead to the loss of nutrient elements, especially through leaching processes, nitrates, ammonia volatilization, increased soil salinity, as well as the transmission of pathogens and weeds. The research results showed that the urine C-organic levels produced were higher than the research results reported by (Rosniawaty et al., 2015). In rabbit and cow urine, the average C-organic levels were 0.62% and 0.74% respectively. The differentiating factor between organic and inorganic fertilizers is the presence of a high amount of C-organic content. If the C-organic content is still low in an LOF product, then the LOF product cannot yet be categorized as an organic fertilizer but is called a soil ameliorant. Therefore, several efforts can be made to increase the productivity of a LOF. One of them is formula changes and process improvements. Plant productivity can start from efforts to improve roots and air (Izaurrealde et al., 2000).

N-organic

The element nitrogen (N) is one of the elements that compose plant fertilizer. Plants require this element for the formation of tissues and organs, as well as for the synthesis of chlorophyll, proteins, and amino acids (Hawkesford et al.,

2023; Shah et al., 2024). The quality of organic fertilizer, which serves as a nutrient source, is also influenced by the presence of nitrogen in its composition. Figure 3 presents a comparison of the nitrogen-organic composition of LOF-RU across different substrate types and fermentation times. Statistical analysis of the data in Figure 3 reveals that differences in substrate type, fermentation time, and their interactions significantly affect the nitrogen-organic levels of LOF-RU ($p < 0.05$). As shown in the graph, the nitrogen-organic content ranges from 1.51% to 2.22%. The nitrogen levels increase with longer fermentation times, which is a natural process as the duration of fermentation affects the metabolic product. Different substrate types provide varying conditions for the growth and development of microorganisms, resulting in different nitrogen requirements and fermentation outcomes. Based on Figure 3, it is apparent that LOF-RU with BAsb exhibits the highest nitrogen content. This is logical because BAsb is derived from cow dung, which contains ammonia gas produced during the metabolic processes in the cow's digestive tract. The metabolic byproducts of rumen microorganisms, in the form of ammonia, are transported into the substrate. This is consistent with the findings of a study by Roten et al. (2017) which reported the production of NO_3^- and N_2O gases during the digestive metabolic processes of cattle, particularly those grazed in pasture areas. The resulting metabolites are feces and urine. Moreover, Lambert et al. (2011) reported that nitrogen is crucial for the growth of forage plants in grazing lands, whereas it becomes a limiting factor in intensively managed lands. Di et al. (2002) stated that nitrogen is produced from the urine of grazing livestock. Additionally, Ledgard et al. (1999) highlighted that nitrogen contributes to the production of NO_3^- gas. Therefore, using fertilizers with high nitrogen content on livestock-occupied lands should be reduced to prevent nitrogen excess resulting from livestock urine (Draganova et al., 2015; Moir et al., 2007). This excess could potentially increase nitrous oxide emissions due to nitrification and denitrification processes driven by microbial activity (Barneze et al., 2014).

The element nitrogen is one of the elements that forms fertilizer in plants. This element is needed by plants as a building material for tissues and organs. The process of forming chlorophyll, protein and amino acids certainly requires these elements. The quality of organic fertilizer as

a source of nutrient supply is also influenced by the availability of N in its composition. A comparison of the N-organic composition of Lof-Ru produced using different substrate types and fermentation times is presented in Figure 3.

C/N Ratio

Nutritional requirements are essential for the life processes of microorganisms, ensuring their metabolic processes and overall existence. These requirements are primarily obtained from carbon (C) and nitrogen (N) elements present in their environment. Therefore, it is important to pay attention to the availability and balance of C and N

elements when providing organic fertilizer products. Figure 4 shows a comparison of the C/N ratio in LOF-RU using different substrate types and fermentation times. Figure 4 shows that different substrate types and fermentation times give different C/N ratio values for the LOF-RU product ($p < 0.05$). The resulting average C/N ratio falls within the range of 0.97–1.87. This outcome is logical considering that different substrate types lead to distinct metabolic processes in microorganisms. Moreover, the nutritional requirements (C and N) differ as fermentation time increases, resulting in variations in the produced LOF-RU content. Different microorganism ecosystems require different C and N elements (Ishiwatari et

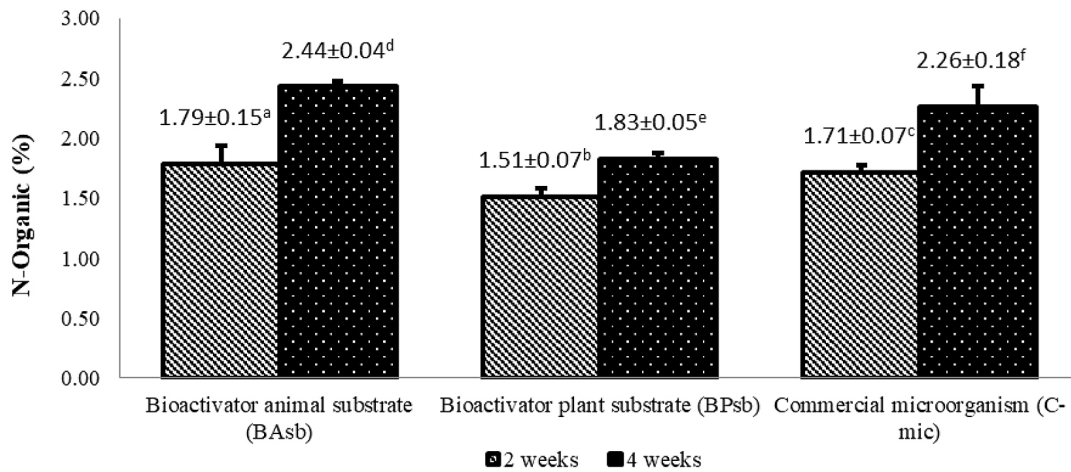


Figure 3. Graph of the effect of differences in substrate types and fermentation time in the production process of LOF-RU on the N-organic (%) content; ^{a-f} Different superscripts for each treatment indicate significant differences ($p < 0.05$)

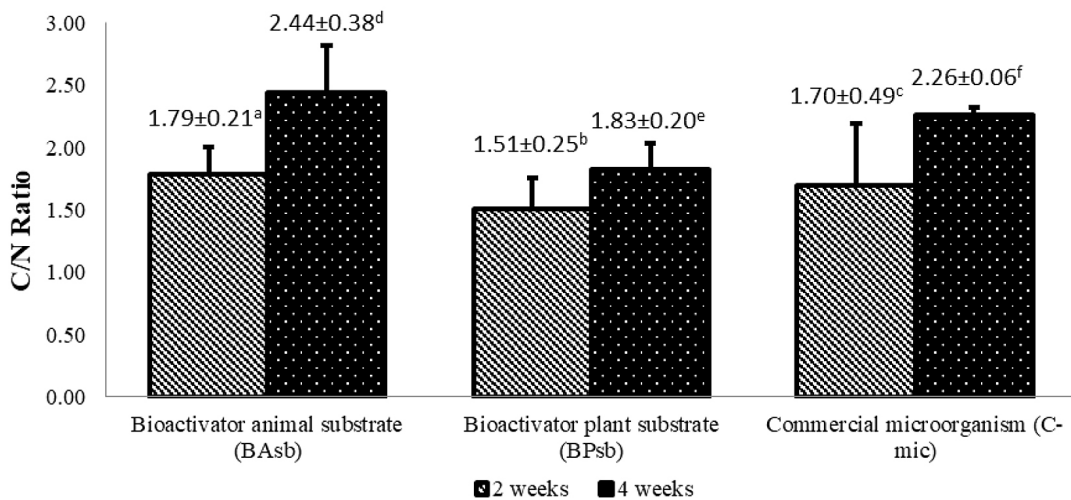


Figure 4. Graph of the effect of differences in substrate types and fermentation time in the production process of LOF-RU on the C/N ratio value; ^{a-f} Different superscripts for each treatment indicate significant differences ($p < 0.05$)

al., 1987). The C/N ratio conditions influence the speed of the fermentation process, particularly in LOF (Prah et al., 1994).

The C/N ratio is a useful tool for studying the presence of plants in a given location, as nitrogen is absorbed by both plants and microorganisms. When plants and microorganisms decompose, carbon sediments are left behind. The disparity between nitrogen and carbon also determines the type of ecosystem that previously existed (Ishiwatari et al., 1987). A higher C/N ratio slows down the fermentation process in liquid fertilizer (Prah et al., 1994). According to Lugo-Presez and Lloyd (2009), the availability of nitrogen is influenced by the content of organic fertilizer, as microorganisms' activity affects the concentration of nitrogen elements. The C/N ratio plays a crucial role in this regard.

P₂O₅ content

One of the chemical elements needed by living creatures is phosphorus (Bhatla and Lal, 2023). Excessive phosphorus elements can cause a process called eutrophication. The eutrophication process has the connotation of a pollutant so that the excess of this element is a concern (Cordell et al., 2011). The availability of phosphorus elements in LOF-RU needs to be carefully considered so that it does not cause negative impacts, both for plants and the environment. The P₂O₅ compound is a compound that contains phosphorus as a constituent element. A comparison of P₂O₅ compound levels in LOF-RU treated with different substrate types and fermentation times

is presented in Figure 5. The results of the statistical analysis of the data in Figure 5 show that differences in substrate type and fermentation time, as well as their interactions, have a significant effect ($p < 0.01$) on P₂O₅ levels in LOF-RU. The P₂O₅ content value for the entire treatment was in the range of 2.09–3.12%. Based on the data in Figure 5, it can be seen that there is a tendency for P₂O₅ levels to increase with increasing LOF-RU fermentation time. The P₂O₅ levels on BAsb were on average higher than Psb and C-mic. The elements potassium (K) and nitrogen (N) which are insoluble in water are related to the availability of phosphate. As much as 4–42% of the P₂O₅ content comes from phosphate elements (Ashley et al., 2011). According to (Elser and Bennett, 2011), phosphate sources need to continue to be utilized as a source of nutrition for plants. Phosphate compounds that dissolve in water affect the balance pattern of an ecosystem, especially aquatic ecosystems. Low phosphate levels (< 0.01 mg P/L) cause a decrease in algae growth. In excessive conditions, algae can grow rapidly. This can have an impact on the availability of oxygen in these waters.

K₂O content

The element potassium (K) is one of the elements required for plant nutrition. This element plays an important role in the process of regulating plant physiological functions. Several physiological characteristics of plants that need to receive adequate nutritional intake include photosynthesis reactions, the process of distributing water in cells and tissues, the process of opening stomata and several

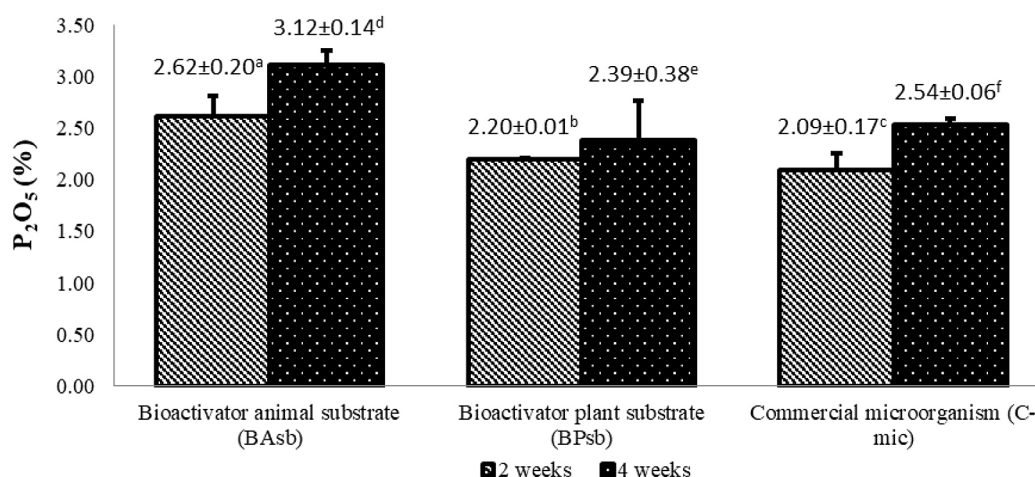


Figure 5. Graph of the effect of differences in substrate types and fermentation time in the production process of liquid organic fertilizer of rabbit urine (LoF-Ru) on the P₂O₅ (%) content; ^{a-f} Different superscripts for each treatment indicate very significant differences ($p < 0.01$)

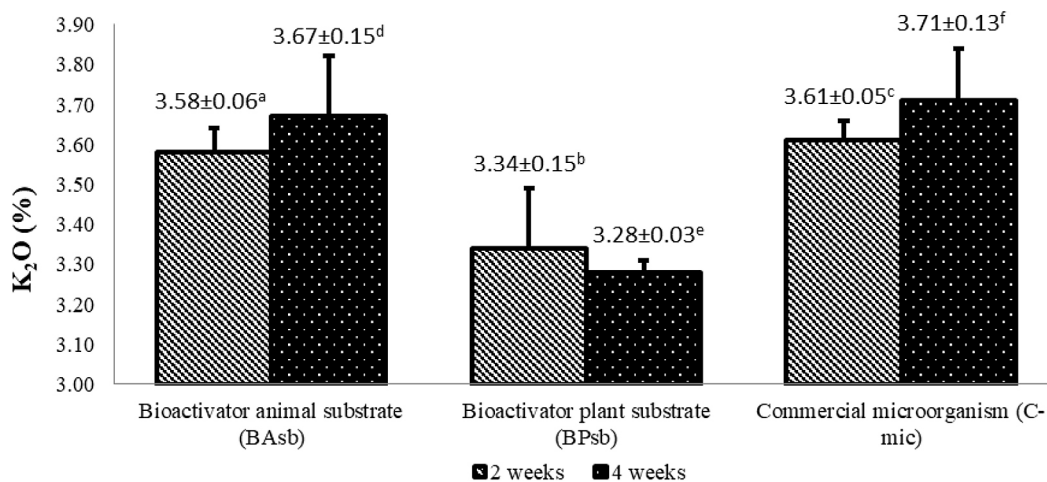


Figure 6. Graph of the effect of differences in substrate types and fermentation time in the production process of LOF-RU on the K₂O (%) content; ^{a-f}Different superscripts for each treatment indicate significant differences ($p < 0.05$)

transportation processes in plant tissues. The availability of element K in organic fertilizer is needed as a nutrient provider. The K element in organic fertilizer can be in the form of the K₂O compound. The availability of these compounds is related to the processes that occur during organic fertilizer production. A comparison of the K₂O compound content in Lof-Ru when applying different types of substrates and fermentation times is completely presented in Figure 6. Statistical analysis of the data (Figure 6) shows that the application of different substrate types and fermentation times has a significant effect ($p < 0.05$) on K₂O levels in LOF-RU products. Test result data shows that K₂O levels are in the range of 3.28–3.71%. The results of this test are still higher than the research results reported by (Raden et al., 2017), namely 0.47–0.59%. This research used 3 types of decomposers, namely EM4, boisca, and shrimp paste in the fermentation process. The use of K₂O compounds is not always needed for a long period. This depends on the needs of the plant at that time (Maguire et al., 2009). The process of growth and strengthening plant resistance requires a supply of nutrients, especially element K. Availability in plant tissues ranges from (1.7–2.7%). This value is based on the dry weight of normally growing leaves (Johnston, 2003).

CONCLUSIONS

The fermentation time affects various aspects of the composition. It increases the N-organic content and the C/N ratio, as well as

the levels of P₂O₅ and K₂O. However, it reduces the C-organic content. The pH value generally remains constant, regardless of the fermentation time or the type of substrate used. The application of different substrates results in varying N-organic content, C/N ratio, and levels of P₂O₅ and K₂O. Utilizing rabbit's urine (Ru) as the main ingredient shows potential for developing a commercial product that meets government standards. In the LOF-RU production process, local microorganisms can be used as an alternative to commercial microorganisms (C-mic) on bio activator animal substrate (BAsb) or bio activator plant substrate (BPsb). Fermentation times of up to 4 weeks can be employed in the LOF-RU production process.

The future research on temperature and humidity will play a significant role in ensuring the continuity of the fermentation process and the activity of microorganisms. This is particularly important in tropical and sub-tropical areas, where the conditions may vary. Therefore, we highly recommend taking these suggestions into consideration. Additional research on the impact of temperature and humidity would be beneficial to supplement the existing data.

Acknowledgements

The research team hereby expresses many thanks, especially to the Ministry of Research, Technology and Higher Education, Republic of Indonesia, the Head of the Indonesian Institute of Sciences and the Rector of Hasanuddin

University for their financial support through the “IPTEKDA LIPI” scheme (contract number: 203/33/SP/DATTGP2UBTD/II/2014 date February 03, 2014). Thanks are also extended to St. Nur Azizah and Rismawaty Rasyid for their assistance in research in the laboratory and field.

REFERENCES

- Ahmad, R., Jilani, G., Arshad, M., Zahir, Z. A., Khalid, A. 2007. Bio-conversion of organic wastes for their recycling in agriculture: an overview of perspectives and prospects. *Annals of Microbiology*, 57, 471–479. <https://doi.org/10.1007/BF03175343>
- Ashley, K., Cordell, D. and Mavinic, D. 2011. A brief history of phosphorus: from the philosopher’s stone to nutrient recovery and reuse. *Chemosphere*. 84, 737–746. <https://doi.org/10.1016/j.chemosphere.2011.03.001>
- Baghbani-Arani, A., Modarres-Sanavy, S.A.M., Poureisa, M. 2021. Improvement the soil physicochemical properties and fenugreek growth using zeolite and vermicompost under water deficit conditions. *Journal of Soil Science and Plant Nutrition*, 21, 1213–1228. <https://doi.org/10.1007/s42729-021-00434-y>
- Balittanah. 2006. Pupuk Organik dan Pupuk Hayati (Organic Fertilizer and Biofertilizer). Balai Besar Litbang Sumberdaya Lahan Pertanian Badan Penelitian dan Pengembangan Pertanian. Bogor.
- Bamdad, H., Papari, S., Lazarovits, G., Berruti, F. 2022. Soil amendments for sustainable agriculture: Microbial organic fertilizers. *Soil Use and Management*, 38(1), 94–120. <https://doi.org/10.1111/sum.12762>
- Barneze, A.S., Mazzetto, A.M., Zani, C.F., Misselbrook, T. and Cerri, C.C. 2014. Nitrous oxide emissions from soil due to urine deposition by grazing cattle in Brazil, *Atmospheric Environment*. 92, 394–397.
- Bergstrand, K.J. 2022. Organic fertilizers in greenhouse production systems—a review. *Scientia Horticulturae*, 295, 110855. <https://doi.org/10.1016/j.scienta.2021.110855>
- Berry, P.M., Sylvester-Bradley, R., Philipps, L., Hatch, D.J., Cuttle, S.P., Rayns, F.W., Gosling, P. 2002. Is the productivity of organic farms restricted by the supply of available nitrogen?. *Soil Use and Management*, 18, 248–255.
- Bhatla, S.C., Lal, M.A. 2023. Essential and Functional Mineral Elements. In *Plant Physiology, Development and Metabolism*, 25–49. Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-99-5736-1_2
- Cordell, D., Rosemarin, A., Schröder, J.J and Smit, A.L. 2011. Towards global phosphorus security: a systems framework for phosphorus recovery and reuse options. *Chemosphere*, 48, 747–758.
- Das, P.P., Singh, K.R., Nagpure, G., Mansoori, A., Singh, R.P., Ghazi, I.A., Singh, J. 2022. Plant-soil-microbes: A tripartite interaction for nutrient acquisition and better plant growth for sustainable agricultural practices. *Environmental Research*, 214, 113821. <https://doi.org/10.1016/j.envres.2022.113821>
- Di, H.J. and K.C. Cameron. 2002. Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. *Nutr. Cycl. Agroecosyst.* 64, 237–256.
- Dordas, C.A., Lithourgidis, A.S., Matsi, T. and Barbayiannis, N. 2008. Application of liquid cattle manure and inorganic fertilizers affect dry matter nitrogen accumulation, and partitioning in maize. *Nutr. Cycl. Agroecosyst.* 80, 283–296.
- Draganova, I., Yule, I., Stevenson, M., Betteridge, K. 2015. The effects of temporal and environmental factors on the urination behaviour of dairy cows using tracking and sensor technologies. *Precision Agric.* 17(4), 1–15.
- Elser, J and Bennett, E. 2011. Phosphorus cycle: a broken biogeochemical cycle, *Nature*. 478, 29–31.
- Ferichani, M. 2024. The potential of rabbit urine in converting household waste into fertilizer as the resilience of farmer family economics in sub-urban. In *IOP Conference Series: Earth and Environmental Science*, 1292(1), 012035. IOP Publishing.
- Hartmann, M., Six, J. 2023. Soil structure and microbiome functions in agroecosystems. *Nature Reviews Earth & Environment*, 4(1), 4–18. <https://doi.org/10.1038/s43017-022-00366-w>
- Hawkesford, M.J., Cakmak, I., Coskun, D., De Kok, L.J., Lambers, H., Schjoerring, J.K., White, P.J. 2023. Functions of macronutrients. In *Marschner’s mineral nutrition of plants* 201–281. Academic Press. <https://doi.org/10.1016/B978-0-12-819773-8.00019-8>
- Hermans, S.M., Buckley, H.L., Case, B.S., Curran-Cournane, F., Taylor, M., Lear, G. 2020. Using soil bacterial communities to predict physico-chemical variables and soil quality. *Microbiome*, 8(1), 79. <https://doi.org/10.1186/s40168-020-00858-1>
- Hsu, S.F. and Buckley, D.H. 2008. Evidence for the functional significance of diazotroph community structure in soil. *ISME J.* 3, 124–136.
- Hu, J., Lin, X., Wang, J., Dai, J. and Chen R. 2011. Microbial functional diversity, metabolic quotient, and invertase activity of a sandy loam soil as affected by long-term application of organic amendment and mineral fertilizer. *J Soil Sediment.* 11, 271–280.
- Irawan, S., Tampubolon, K., Karim, A., Musri, M.A., Suhelmi dan E. Sitepu. 2022. Kesuburan tanaman dengan menggunakan urine kelinci dengan penambahan air kelapa dan probiotik em 4 dengan

- minuman yakult dengan cara fermentasi. *Journal Liaison Academia and Society (J-LAS)*. 2(4), 63–83.
23. Ishiwatari, R and M. Uzaki. 1987. Diagenetic changes of lignin compounds in a more than 0.6 million-year-old lacustrine sediment (Lake Biwa, Japan). *Geochimica Et Cosmochimica Acta*. 51(2): 321-28.
24. Izaurrealde, R.C., McGill, W.B. and Rosenberg, N.J. 2000. Carbon cost of applying nitrogen fertilizer. *Science*. 288, 811–812.
25. Jakhar, A.M., Aziz, I., Kaleri, A.R., Hasnain, M., Haider, G., Ma, J., Abideen, Z. 2022. Nano-fertilizers: A sustainable technology for improving crop nutrition and food security. *NanoImpact*, 27, 100411.
26. Johnston, A.E. 2003. Understanding potassium and its use in agriculture, European Fertilizer Manufacturers Association (EFMA), Belgium.
27. Lambert, M.G., Clark, D.A. and Litherland, A.J. 2011. Advances in pasture management for animal productivity and health. *New Zealand Vet. J*. 52, 311–319.
28. Ledgard, S.F. Penno, J.W. and Sprosen, M.S. 1999. Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitrogen fertilizer application. *J. Agric. Sci.* 132, 215–225.
29. Leite, L.F.C., Oliveira, F.C., Araújo, A.S.F., Galvão, S.R.S., and Lemos Soil, J.O. 2010. Organic carbon and biological indicators in an Acrisol under tillage systems and organic management in north-eastern Brazil. *Soil Res*. 48, 258–265.
30. Lugo-Presez, J and Lloyd, J.E. 2009. Ecological Implications of organic mulches in arboriculture: a mechanistic pathway connecting the use of organic mulches with tree chemical defences. *Arboriculture & Urban Forestry*. 35(4), 211–217.
31. Maguire, R., Alley, M., Wysor W.G. and Flowers, W. 2009. Fertilizer types and calculating application rates. Virginia Cooperative Extension. Publication 424–035, Virginia State University.
32. Maurya, S., Abraham, J.S., Somasundaram, S., Toteja, R., Gupta, R., Makhija, S. 2020. Indicators for assessment of soil quality: a mini-review. *Environmental Monitoring and Assessment*, 192, 1–22. <https://doi.org/10.1007/s10661-020-08556-z>
33. Moir, J.L. Cameron, K.C. and Di, H.J. 2007. Effects of the nitrification inhibitor dicyandiamide on soil mineral N, pasture yield, nutrient uptake and pasture quality in a grazed pasture system. *Soil Use Manage*. 23, 111–120.
34. Mutai, P.A. 2020. The potential use of rabbit urine as a bio fertilizer foliar feed in crop production. *Africa Environmental Review Journal*, 4(1), 138–147.
35. Naz, M., Dai, Z., Hussain, S., Tariq, M., Danish, S., Khan, I.U., Du, D. 2022. The soil pH and heavy metals revealed their impact on soil microbial community. *Journal of Environmental Management*, 321, 115770.
36. Nikitin, D.A., Semenov, M.V., Chernov, T.I., Ksenofontova, N.A., Zhelezova, A.D., Ivanova, E.A., Khitroc, N.B., Stepanov, A.L. 2022. Microbiological indicators of soil ecological functions: a review. *Eurasian Soil Science*, 55(2), 221–234. <https://doi.org/10.1134/S1064229322020090>
37. Nygaard Sorensen, J., Thorup-Kristensen, K. 2011. Plant-based fertilizers for organic vegetable production. *Journal of Plant Nutrition and Soil Science*, 174(2), 321–332.
38. Philippot, L., Andert, J., Jones, C.M. Bru D., and Hallin, S. 2011. Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N₂O emissions from soil. *Global Change Biol*. 17, 1497–1504.
39. Prah, F.G. Ertel, J.R., Goni, M.A., Sparrow, M.A. and Eversmeyer, B. 1994. Terrestrial organic-carbon contributions to sediments on the washington margin. *Geochimica Et Cosmochimica Acta*. 58(14), 3035–48.
40. Raden, I., Fathillah, S.S., Fadli, M. and Suyadi. 2017. Nutrient content of liquid organic fertilizer (LOF) by various bio-activator and soaking time. *Nusantara Bioscience*. 9(2), 209–213.
41. Reeves, D.W. 1997. The role of soil organic matter in maintaining soil quality in continuous cropping systems. *Soil Till Res*. 43, 131–167.
42. Rosniawaty, S., Sudirja, R. dan Afrianto, H. 2015. Pemanfaatan urin kelinci dan urin sapi sebagai alternatif pupuk organik cair pada pembibitan kakao (*Theobroma cacao* L.). *Jurnal Kultivasi*. 14(1), 32–36.
43. Rosniawaty, S., Sudirja, R. dan Afrianto, H. 2015. Pemanfaatan urin kelinci dan urin sapi sebagai alternatif pupuk organik cair pada pembibitan kakao (*Theobroma cacao* L), *Jurnal Kultivasi*. 14(1), 32–36.
44. Roten, R.L., Fourie, J., Owens, J.L., Trethewey, J.A.K., Ekanayake, D.C., Werner, A., Irie, K., Hagedorn, M. and Cameron, K.C. 2017. Urine patch detection using LiDAR technology to improve nitrogen use efficiency in grazed pastures, *Computers and Electronics in Agriculture*. 135, 128–133.
45. Said, M.I., Asriany, A., Sirajuddin, S.N., Abustam, E., Rasyid, R., Al-Tawaha, A.R.M. 2018. Evaluation of quality of liquid organic fertilizer from rabbit's urine waste fermented using local microorganisms as decomposers. *The Iraqi Journal of Agricultural Science*, 49(6), 990.
46. Sajimin, Y.C. Raharjo, N.D. Purwantari dan Lugiyo. 2003. Produksi tanaman pakan ternak diberi pupuk feses kelinci. *J Online Agroekoteknologi*. 2(3), 15–161.
47. Shah, I.H., Jinhui., W. Li., X., Hameed., M.K., Manzoor., M.A., Li., P., Zhang, Y., Niu., Q., Chang, L. 2024. Exploring the role of nitrogen and potassium in photosynthesis implications for sugar: Accumulation and translocation in horticultural crops. *Scientia Horticulturae*. 327, 112832.
48. Singh, J., Kunhikrishnan, A., Bolan, N.S. and Sagar, S. 2013. Impact of urease inhibitor on ammonia

- and nitrous oxide emissions from temperate pasture soil cores receiving urea fertilizer and cattle urine. *Science of the Total Environment*, 465, 56–63.
49. Singh, T.B., Ali, A., Prasad, M., Yadav, A., Shrivastav, P., Goyal, D., Dantu, P.K. 2020. Role of organic fertilizers in improving soil fertility. *Contaminants in Agriculture: Sources, Impacts and Management*, 61–77.
50. Wang, Y., Liu, L., Yang, J., Duan, Y., Luo, Y., Taherzadeh, M.J., Li, Y., Li, H., Awasthi, M.K., Zhao, Z. 2020. The diversity of microbial community and function varied in response to different agricultural residues composting. *Science of the Total Environment*, 715, 136983.
51. Zamora, P., Georgieva, T., Ter-Heijne, A., Tom, H.J.A., Sleutels, A.W. Jeremiasse, Saakes, M., Buisman, C.J.N., and Kuntke, P. 2017. Ammonia recovery from urine in a scaled-up microbial electrolysis cell. *Journal of Power Sources*. 356, 491–499.
52. Zhang, Q.C., Shamsi, I.H., Xu, D.T., Wang, G.H. and Lin, X.Y. 2012. Chemical fertilizer and organic manure inputs in soil exhibit a vice versa pattern of microbial community structure. *Appl Soil Ecol*. 57, 1–8.
53. Zhi, J., Qiu, T., Bai, X., Xia, M., Chen, Z., Zhou, J. 2022. Effects of nitrogen conservation measures on the nitrogen uptake by cotton plants and nitrogen residual in soil profile in extremely arid areas of Xinjiang, China. *Processes*, 10(2), 353.