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Evaluation of rumen bacteria bioactivator consortium from Bali cattle for enhancing agricultural waste composting

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Abstract The significant differences in cellulose and lignin content were observed in week four, with oil palm waste compost showed the highest content, followed by coffee husk waste and Melastoma. Microbial activity led to notable reductions in cellulose and lignin on week 8 across all waste types, although oil palm waste still retained the highest content. The significant reduction in the C/N ratio indicated continuing microbial decomposition and nitrogen mineralization. At week 8, the greatest nitrogen content was observed in oil palm waste, while both Melastoma and coffee husk waste exhibited similar nitrogen content. There was insignificant effect of LMO concentration on cellulose, lignin, pH, C, N, C/N ratio, or micronutrient content, indicating the effectiveness of Rumen Bacteria Bio-activator Consortium (RBBC) for organic matter decomposition. The pH for all waste types increased during the decomposing with Melastoma compost had the highest pH. In general, the study showed that the RBBC effectively increased the activity of microbes and the decomposition of lignocellulosic materials, even though LMO concentration primarily had negligible effect on the composting results. This study results highlight the contribution of waste type and microbial consortia for composting. Also, RBBC has high potential in agricultural waste management.

Keywords: Coffee husk waste, Consortium Bioactivator, LMO, Oil palm waste, Waste management

Introduction

For decades, one of the main challenges in sustainable agriculture is to improve soil quality and fertility (Gruhn *et al.*, 2000). Also, increase in the agricultural waste worldwide occurs continuously. Millati *et al.* (2019) reported that agricultural waste generated globally reached approximately 2 billion tons each year. These wastes have various content of cellulose, hemicellulose, and

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lignin. Study by Broto *et al*. (2019) indicated that Bengkulu Province produced large amount of agricultural wastes such as oil palm fronds, coffee husks, and weeds. The accumulation of undecomposed agricultural wastes can contaminate the environment and cause soil degradation unless properly managed (Dewilda *et al*., 2021). Consequently, raw materials for composting using agricultural wastes become an effective solution to increase soil quality and fertility. This effort also reduces environmental risk (Etesami and Maheshwari, 2018). Agriculture wastes as raw material for composting becomes a sustainable practice to converts the waste into organic fertilizer which can enhance soil health and diminish environmental contamination (Sharma *et al*., 2019). It is essential to use a bio-activator to speed up organic material decomposition.

A lot of studies have suggested that bio-activators improved organic matter quality (Allaily *et al*., 2022; Saidi *et al.,* 2023; Sharma *et al*., 2019; Yanqoritha, 2023). Incorporating bio-activator with organic material can accelerate its decomposition, therefore, several treatments are necessary to improve the composting process. A number of factors should be considered to speed up the composting process, including the selection of the proper bio-activators. Local Microorganisms (LMO) can be used as bio-activators. LMO is a liquid from natural organic materials and contains beneficial microorganisms. These microorganisms facilitate the faster organic matter decomposition. The use of LMO is applicable solution for composting since it is easy to prepare and inexpensive (Broto *et al*., 2019). LMO solutions contain high both macro- and micronutrients as well as bacteria which have the potential to decompose organic matter. Therefore, LMO can function as a biological decomposer and as an organic pesticide. Using LMO for composting of agricultural wastes can increase nutrient content of organic fertilizer (Indasah and Fitriani, 2021). Therefore, LMO serves as an effective agent for enhancing the quality of agricultural waste compost.

Local microorganism (LMO) from cow rumen bacterial consortium is another type of bio-activator used for composting. The rumen consists of specialized microorganisms which are able to break down lignocellulosic plant materials. The microbial consortium is able to speed up composting process and increase essential plant nutrients (Gharechahi *et al*., 2023). Using the consortium can produce high quality, eco-friendly and cost-effective organic amendment for supporting local farmer need.

The present study used Bali cattle rumen as the source bacterial consortia, since their bacterial population were effective in decomposing fibrous plant materials. According to Anggraeny *et al*. (2025), Bali cattle is well-known for its resilience, adaptability, and efficient use of local feed resources and has a unique rumen microbial community. The community has exhibited strong potential in decomposing complex organic materials, such as lignocellulose which is a main element of agricultural waste (Gharechahi *et al*., 2023; Xing *et al*., 2020; Yu *et al*., 2007). Furthermore, various researches have suggested that integrating rumen microbial consortia in composting process substantially enhances the quality of organic fertilizer (Dewilda *et al*., 2021; Rusli et al., 2022). This consortium significantly improves fermentation and decomposition processes. The objective of the present study was to determine compost quality generated from oil palm frond waste, coffee husks, and Melastoma weeds using bio-activator from Bali cattle rumen bacterial consortium.

Materials and methods

Experimental design

The study was carried out in the greenhouse of Department of Crop Production, Faculty of Agriculture, University of Bengkulu from November 2023 to June 2024, using a completely randomized design (CRD) with 2 factors. Type of agricultural waste (L) as first factor, consisted of oil palm fronds (L1), coffee husks (L2), and Melastoma weeds (L3). Concentration of LMO (M) was the second factor which comprised 5% (M1), 10% (M2), and 15% (M3). The treatment combination was replicated 3 times. The treatment combination were as follows:-

L1M1: Oil palm fronds waste + LMO 5%
L1M2: Oil palm fronds waste + LMO 10%
L1M3: Oil palm fronds waste + LMO 15%
L2M1: Coffee husks waste + LMO 5%
L2M2: Coffee husks waste + LMO 10%
L2M3: Coffee husks waste + LMO 15%
L3M1: Melastoma + LMO 5%
L3M2: Melastoma + LMO 10%
L3M3: Melastoma + LMO 15%

Compost materials collection, preparation and component analysis

The study was initiated by collecting different organic waste materials. Coffee husks were provided by coffee mill waste in Kepahiang Regency, Bengkulu Province, Indonesia. Oil palm frond waste was obtained from oil palm plantations in Seluma Regency, whereas Melastoma were collected from oil palm plantations in Bengkulu City. The bacterial consortium was prepared using the rumen fluid of Bali cattle. A nutrient solution- mixture of 15 liters of coconut water and 4 kilograms of molasse, was prepared to enrich the consortium. The bacterial consortium had various valuable bacteria, such as cellulolytic, nitrogen-fixing, potassium-solubilizing, phosphorus-solubilizing, and indole-3-acetic acid (IAA)-producing microorganisms.

The composting was initiated by chopping the collected waste in to approximately 5 cm pieces. Ten kg of chopped waste was homogeneously mixed with 1 kg chicken manure, then placed in composting bin. The bacterial consortium bio-activator was inoculated to the mixture and moistened to 60-65% which was the optimum range for microbial activity. The bin was tightly covered and attached with gas release pipes for aeration. During the composting, temperature was measured every 2 days using a digital thermometer. Compost was sampled at week 4 and 8 to determine changes in temperature, pH and compost quality. The composting process ended when the temperature was approximately 30°C, designating a reduced microbial activity. At the end of the incubation, compost was sieved using 5 mm screen for further laboratory analysis. The content of cellulose and lignin were analyzed according to the method by Van Soest (1994). Total organic carbon was measured using the Walkley and Black method (Walkley and Black, 1934). Kjedahl method was used to analyzed nitrogen content whereas pH was measured using a pH meter at the ratio of compost to distilled water 1:5. Likewise, the content of Cu, Fe, and Zn was assessed with Atomic Absorption Spectrophotometry (AAS).

Statistical analysis

Data were analyzed using Analysis of Variance (ANOVA) at a 5% significance level using SPSS software. Treatment means were separated using Duncan's Multiple Range Test (DMRT) at the same significance level.

Results

Lignin and cellulose content of compost

Table 1 indicates the influence of waste type and LMO concentration on the content of cellulose and lignin at week 4 and 8. Across waste types, both cellulose and lignin prominently decreased from week 4 to week 8 ($p<0.05$), suggesting that during the composting, microorganisms actively broke down complex lignocellulosic materials. At week 4, compost from oil palm fronds had the highest content of cellulose and lignin, followed by coffee husks and Melastoma. The trend remained consistent at week 8, indicating higher content of lignocellulose in oil palm fronds caused slower decomposition. On the other hand, lower initial content of lignocellulose in Melastoma accelerated decomposition during the composting.

Notably, LMO concentration (5%, 10%, and 15%) did not influence on cellulose and lignin (p>0.05). Nonetheless, there was a significant interaction between waste type and LMO concentration on cellulose content at week 4 and week 8, and lignin content at week 8. These findings suggest that certain combinations of waste type and LMO concentration significantly affect the decomposition of lignocellulose during the composting.

Tabel 1. The Influence of Waste Type and LMO Concentration on the content of Cellulose and Lignin in Compost

Waste Types	Initial content		Week-4		Week-8	
	Cellulose (%)	Lignin (%)	Cellulose (%)	Lignin (%)	Cellulose (%)	Lignin (%)
LI	41.54	24.19	33.27^{a}	11.42^{a}	19.48^{a}	7.28^{a}
L2	21.77	8.55	20.72^{b}	8.34^{c}	8.76^{b}	4.66^{b}
L3	11.49	5.18	11.08^{c}	4.79^{b}	5.12^{c}	2.87^{c}
LMO (%)						
5	-	-	23.84^{a}	8.32^{a}	11.36^{a}	5.71^{a}
10	-	-	23.64^{a}	8.18^{ab}	11.17^{b}	4.71^{b}
15	-	-	17.59^{b}	8.05^{b}	10.83^{c}	4.39^{c}
Interaction:			**	ns	**	**

LI: Oil Palm frond waste, L2: Coffee husk waste, L3: Melastoma compost. Numbers followed by the same letter within a column is not significantly different at 5%

Nutrient content of compost

The influence of waste type and LMO concentration on pH, carbon, nitrogen, and the C/N ratio at weeks 4 and 8 is presented in Table 2. Across all compost, pH constantly increased from week 4 to week 8. At week 4, the highest pH was observed in the compost from coffee husk, followed by oil palm, while the lowest pH was achieved by Melastoma compost. The differences in pH indicated the different chemical composition among waste materials, mainly organic acid and phenolic compound content. By week 8, pH increase was related to the formation of alkaline substance due to microbial activity.

Table 2 also shows that at both weeks, various waste types did not significantly affect carbon content. However, carbon content decreased significantly at week 8 as compared to week 4. On the other hand, by week 8, nitrogen content significantly increased across all waste types. The highest nitrogen content was observed in compost derived from oil palm waste, followed by Melastoma, which was not significantly different from coffee husks. Increase in nitrogen indicated nitrogen compound mineralization during the composting.

Moreover, the reduction of C/N ratio from week 4 to week 8 across all treatments indicates the progression of compost maturity.

The study also resulted that LMO concentrations (5%, 10%, and 15%) did not significantly influence all measured parameters—pH, carbon (C), nitrogen (N), and the C/N ratio—at either week 4 or week 8 (Table 2). Likewise, there were no significant interaction between waste type and LMO concentration for all variables. These results suggested that in this study, the decomposition process was mainly driven by the inherent chemical composition of the waste materials, rather than by the LMO concentrations.

Tabel 2. The content of C, N, C/N and pH of compost as influenced by Waste Type and LMO Concentration

Waste types	Weeks-4				Weeks-8			
	pH	C (%)	N (%)	C/N	pH	C (%)	N (%)	C/N
LI	5.37[b]	37.30[a]	1.53[a]	25.20[a]	6.82[ab]	23.37[a]	2.05[a]	11.88[a]
L2	6.34[a]	36.83[a]	1.47[a]	26.23[a]	6.72[b]	25.40[a]	1.91[b]	14.08[a]
L3	4.98[c]	35.15[a]	1.21[b]	24.61[a]	7.07[a]	24.27[a]	1.96[b]	13.11[a]
LMO (%)								
5	5.67[a]	35.99[a]	1.33[a]	28.73[a]	7.05[a]	23.37[a]	2.45[a]	9.82[a]
10	5.58[a]	38.39[a]	1.43[a]	27.74[a]	6.72[a]	25.05[a]	1.67[a]	15.21[a]
15	5.43[a]	34.92[a]	1.45[a]	24.61[a]	6.84[a]	24.28[a]	1.81[a]	14.03[a]
Interaction:	ns	ns	ns	ns	ns	ns	ns	ns

LI: Oil Palm frond compost, L2: Coffee husk compost, L3: *Melastoma* compost. Numbers followed by the same letter within a column is not significantly different at 5%

The study also revealed that micronutrients (Fe, Cu, and Zn) at week 4 and 8 significantly differed among waste type ($p<0.05$) (Table 3). At week 4, compost derived from Melastoma (L3) had the greatest content of Fe (2461.67 mg/kg) compared to those from oil palm waste (L1) (971.67 mg/kg) and coffee husk waste (L2) (253.11 mg/kg). The trend was consistent at week 8 where L3 compost (2660.00 mg/kg) remained higher than those L1 (980.22 mg/kg) and L2 (180.11 mg/kg). These findings designated that Melastoma contains more Fe than other sources.

Likewise, at week 4, the type of waste material significantly influenced copper (Cu) content. Compost from coffee husk exhibited the highest Cu content (6.67 mg/kg), followed by oil palm waste (3.66 mg/kg), being Melastoma compost exhibiting the lowest content (2.89 mg/kg). Nonetheless, by week 8, compost from all waste types had comparable Cu content (Table 3).

A similar trend was detected in zinc (Zn) content. By week 4, the highest Zn 4.88 mg/kg) was found in compost derived from Melastoma, significantly higher than that of coffee husks (3.55 mg/kg) and oil palm waste (2.22 mg/kg)

(Table 3). By week 8, Melastoma compost still had the highest Zn concentration (4.89 mg/kg), followed by coffee husks (4.33 mg/kg) and oil palm waste (4.00 mg/kg).

On the other hand, LMO concentration (5%, 10%, and 15%) had no significant effect on iron (Fe), copper (Cu), and zinc (Zn) concentrations at either week 4 or week 8 (Table 3). Also, there was no significant interaction between waste type and LMO concentration were found in any of the micronutrients. These findings highlight that the type of organic waste was the key factor affecting micronutrient, while LMO concentration did not affect under the conditions of this study.

Tabel 3. The content of micronutrients (Fe, Cu, Zn) in compost as influenced by various Waste Types and LMO Concentration

Waste types	Weeks-4			Weeks-8		
	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
LI	971.67 ^{b}	3.66^{b}	2.22^{c}	980.22^{b}	3.11 ^{a}	4.00^{c}
L2	253.11 ^{c}	6.67 ^{a}	3.55 ^{b}	180.11^{c}	3.33 ^{a}	4.33^{b}
L3	2.461.67^{a}	2.89 ^{b}	4.88^{a}	2660.00^{a}	2.22 ^{a}	4.89^{a}
LMO (%)						
5	1029.22 ^{a}	4.89^{a}	3.56^{a}	1326.22 ^{a}	3.67 ^{a}	4.33^{b}
10	1356.00 ^{a}	4.44 ^{a}	3.56^{a}	1259.44 ^{a}	2.89 ^{a}	4.33^{b}
15	1301.22 ^{a}	3.89 ^{a}	3.56^{a}	1234.66 ^{a}	2.11 ^{a}	4.56^{a}
Interaction:	ns	ns	ns	ns	ns	ns

LI: Oil palm frond compost, L2: Coffee husk compost, L3: Melastoma compost. Numbers followed by the same letter within a column is not significantly different at 5%

Discussion

The study results emphasized a complex interaction effect between agricultural waste types and LMO concentration on vital composting parameters, including cellulose, lignin, pH, C, N, and C/N ratio. Compost from oil palm waste steadily exhibited the greatest content of lignin and cellulose at week 4 and week 8 while the second largest was coffee husk waste and the lowest was found in compost from Melastoma. The highest content of cellulose and lignin in compost from oil palm waste is attributable to the nature of its lignocellulose which is more resistant to microbial decomposition.

The significant decrease in lignin and cellulose in week 8 as compared to week 4 suggests the breakdown of lignocellulosic compound. Previous studies confirmed that complex lignocellulosic compound was effectively degraded by microbial activity(Astuti *et al.*, 2023; Listya *et al.*, 2020). This result indicates that the microbial consortium from RBBC derived from Bali cattle provided significant contribution to the decomposition process. Studies by Chukwuma *et al.* (2021) and Thapa *et al.* (2020) underscored the vital role of cellulolytic bacteria in breaking down lignocellulosic biomass. On the other hand, Melastoma biomass decomposed faster due to its lower lignocellulose content. This result is supported by previous study where organic materials with simpler chemical structures are more easily degraded by microbes without the aid of additional bio-activators (Vikman *et al*., 2002).

Likewise, cellulose and lignin content were not significantly different at various LMO concentration (5%, 10%, and 15%) ($p > 0.05$). Nonetheless, there was a significant interaction between waste type and LMO concentration on the content of cellulose at both week 4 and week 8, and on content of lignin at week 8. This result suggested that sole LMO concentration did not constantly affect the degradation of lignocellulose, but its effectiveness depends on particular combinations of waste types and LMO concentration.

The study also resulted that compost pH increased from week 4 to week 8 for all waste types. Melastoma compost exhibited the greatest increase in pH (41.79%), followed by oil palm waste compost (24.34%) while the lowest increase was found in coffee husk compost (5.99%). Increase in pH during composting process is associated with microbial activity which breakdown organic materials, releasing basic compounds (Nozhevnikova *et al*., 2019; Sundberg *et al.*, 2013). The lower pH increase in coffee husk compost might have been related to its relatively high content of phenolic compounds including chlorogenic and flavan-3-ols. These compounds can inhibit the activity of microorganism (Esquivel *et al*., 2020; Rebollo-Hernanz *et al*., 2021). This finding is in line with results suggested by Dembinska *et al*. (2025), where phenolic compounds can hinder microbial decomposition. Meanwhile, Melastoma compost has pH within the optimal range for microbial activity (6.5–7.5), as outlined by Soobhany (2018). In this study, RBBC-derived microbes might assist to neutralize organic acids and support favorable condition for decomposition.

At week 4, carbon content was not significantly different across waste types, however, it decreased significantly by week 8. The greatest carbon content was consistently achieved by oil palm waste compost, followed by coffee husk and Melastoma. The decline in carbon indicates the organic matter breakdown by microbes during composting process where carbon is transformed into simpler compound and discharged CO_2 (Chen *et al*., 2011; Insam and De Bertoldi, 2007). Additionally, the persistent highest content of carbon in oil palm waste compost indicates a slower decomposition rate, possibly because of its high content of lignocellulosic complex. According to Oluwatosin (2018), lignocellulose has high amounts of lignin and hemicellulose. These compounds possess complex structure, thereby more resistant to microbial breakdown. Jeffries (1994) noted that lignin generates physical and chemical barriers that hamper enzymatic access to cellulose and hemicellulose.

On the other hand, Melastoma compost exhibited the smallest carbon content by week 8, designating a faster breakdown of organic materials. This result is associated with its lower lignin content. Moreover, Rusli *et al*. (2022), reported that Melastoma has high metabolic activity and strong antioxidant properties. These characteristics increase microbial activity in the surrounding environment, contributing to the accelerated carbon decomposition observed in Melastoma waste. This phenomenon also explains its lower C content than other waste types at the end of composting.

Additionally, significant increase in nitrogen (N) content was observed across all treatments, particularly from week 4 to week 8 across, suggesting effective mineralization of organic nitrogen compound. During the mineralization, organic nitrogen was converted into inorganic forms, including ammonium (NH_4^+) and nitrate (NO_3^-) (Yu *et al*., 2019). This conversion is a primary indicator of microbial activity during composting. This result is in accordance with those reported by Qiu *et al*. (2021) and Sánchez *et al*. (2017), where nitrogen content in compost increases due to intensified microbial activity during organic matter decomposition.

At the end of the composting process, oil palm waste compost had the greatest nitrogen content, followed by those of Melastoma and coffee husk. The high nitrogen content in oil palm waste compost might be related to its initially higher nitrogen concentration. Furthermore, its high lignocellulose causes a slower nitrogen release.

On the other hand, even though Melastoma and coffee husk had lower nitrogen content at week 4, both exhibited prominent increases in nitrogen content by week 8, indicating that microbial activity was still able to effectively release nitrogen in these substrates. Specifically, Melastoma substrates could maintain high microbial activity, possibly due to its bioactive compounds and more readily degradable structure (Rusli *et al*., 2022).

The C/N ratio at week 4 was similar across all treatments, but it significantly decreased by week 8. The reduction of C/N ratio is primary indicator of composting process, reflecting the breakdown of carbon-rich compound and mineralization of organic nitrogen. Reduction of C/N ratio also designates successful transformation of complex organic compounds into simpler inorganic forms (Azis *et al*., 2023). The decline in C/N ratio also demonstrates that microorganisms are actively converted carbon organic materials into CO_2 and to release nitrogen in forms available to plants (Cai *et al*., 2024).

The study also revealed that the concentration of LMO (5%, 10%, and 15%) did not significantly influence pH, carbon, nitrogen, or the C/N ratio at week 4 and week 8. This finding indicates that the decomposition of organic matter is mainly dependent on the physicochemical properties of the waste and the microbial activity contributed by the RBBC, rather than the concentration of LMO. The possible reason for this phenomenon is that at the lowest concentration (5%), LMO already provided a sufficient population of microbes, leading to higher dosages unnecessary (Mishra and Yadav, 2021). In addition, composting effectiveness is dependent on the number of inoculum and various factors including substrate compatibility, oxygen availability, temperature, moisture content, and microbial competition (Bernal *et al.*, 2009; Insam and Bertoldi, 2007). Li *et al*. (2019) reported that when the population of microbes had reached a thershold, further inoculation has no effect, since the composting condition reaches biological saturation. The current findings indicate that RBBC-derived LMO was effective at all tested concentrations, with no significant differences observed in key parameters such as pH, carbon, nitrogen, and the C/N ratio, in spite of the dosage applied.

Regarding micronutrient, concentration of Fe, Cu, and Zn was significantly different among waste types. At both observations, the greatest concentration of Fe was consistently observed in compost derived from Melastoma, exceeding those from oil palm waste and coffee husk. This finding might be related to the higher Fe content in Melastoma biomass or a higher capability to retain Fe during composting. On the other hand, at week 4, the highest Cu content was found in compost from coffee husk, but by week 8, Cu content was not significantly different among sources, likely due to microbial transformation or immobilization process. Zn content followed similar pattern where at the 2 observations, the highest was achieved by Melastoma compost compared to the other two waste types. These results are in-line to previous studies where micronutrient dynamic is dependent on types and chemical composition (Gao *et al*., 2023; Wang *et al*., 2019).

On the other hand, the content of Fe, Cu and Zn at any points of observation was not significantly different among LMO concentration. These results support the conclusion that the dominant factor determining micronutrient content in final compost is waste types rather than LMO concentration. LMO effectiveness in speeding up the composting process might be associated with its functional microbial population such as cellulolytic and nitrogen-fixing bacteria, contributing to breakdown lignocellulose and nitrogen mineralization. In this study, the insignificant effect of LMO concentration is likely related to that even at the lowest concentration, LMO already contains sufficient microbes, causing the higher concentration did not provide the differences. In general, the results underscore the importance in selecting appropriate organic materials and using microbial inoculant such as RBBC for composting purposes. While varying LMO concentration did not provide significant improvement under current conditions, its effects could be more distinct at higher dosages when composting period is extended or when combined with other bio-activators.

In summary, the study concluded that the quality of final compost was highly dependent on the type of agricultural waste. Oil palm waste, containing high lignocellulose content, decomposed more slowly than Melastoma which degraded faster. The Rumen Bacteria Bio-activator Consortium (RBBC) was effective in speeding up cellulose and lignin degradation, enhancing N content and declining the C/N ratio, both are vital indicator of composting process. Furthermore, the highest content of micronutrients, especially Fe and Zn was found in Melastoma compost. Nonetheless, the quality of compost was not affected by different LMO concentration tested in this study. Overall, these findings emphasized that RBBC has convincing potential as easy to make and cost effective bio-activator for sustainable management of agricultural waste.

Conflicts of interest

The authors declare no conflict of interest.

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