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Assessement of Several Lignocellulolytic Isolates for Sugarcane Trash Decomposition

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In the sugar industry, sugarcane trash is produced with the characteristics of high lignin and cellulose content. Naturally, litter can be decomposed, but the activity of lignocellulolytic and cellulolytic enzymes excreted by lignocellulolytic microbes will accelerate decomposition. This experiment was carried out to obtain microbes capable of decomposing sugarcane trash in situ in the field. Exploration activities have been carried out by collecting samples in the form of soil, trash, and mushroom fruiting bodies from several sugarcane plantation areas. Next, the selected isolates were combined to obtain a consortium of selected lignocellulolytic microbes as active ingredients for sugarcane trash decomposers. For this purpose, a series of tests on the effectiveness of isolates in decomposing sugarcane trash in glass jars, sacks, in beds, Petri dishes, bag logs and in the field have been carried out. The observations made were temperature, humidity, level of friability, C, and N content. The results of the study showed that based on the parameters of friability level, pH and color change of sugarcane litter, 10 (ten) microbial isolates were obtained consisting of 6 cellulolytic bacterial isolates and 2 lignolytic bacterial isolates, one selected cellulolytic fungi, and one lignolytic fungi. In the next activity, the ten isolates were formulated into one formula which had the potential to degrade sugarcane trash in less than 8 weeks.

Keywords: sugarcane trash, lignocellulolytic microorganism, consortium decomposer

Sugarcane is an important crop globally, especially in tropical regions, due to its role as a sugar producer and a source of energy. In one growing season, more than 1.6 million tons of trash or biomass are generated. The decomposition of sugarcane trash is crucial for ensuring the sustainability of sugarcane productivity because through the decomposition of trash, nutrients are produced, and in its application, it can improve soil structure and health. This process is known as mineralization, which involves the role of lignocellulolytic microbes.

Sugarcane trash as a lignocellulosic biomass, cellulose and hemicellulose are wrapped by lignin, forming a complex structure that is not easily degraded by microbes. Since the high content of lignin and cellulose, only lignocellulolytic microbes are capable to degrade. Although the use of lignocellulolytic microbes in the decomposition of sugarcane trash is still a matter of debate, some research results indicate the role of lignocellulolytic microbes in accelerating the mineralization process of sugarcane trash. Cellulolytic microbes are microorganisms with specific abilities to degrade carbon compounds in organic matter due to their ability to produce lignocellulolytic enzymes that can hidrolyze lignocellulose biomass.

Sugarcane fields are habitats that have long been inhabited by cellulolytic microbes due to continuous pruning of trash and the accumulation of trash remnants in the fields. With this assumption, the isolated microbes from sugarcane plantations are considered to be selected or adapted isolates. Microbial exploration is a method to obtain microbes needed for specific purposes. Therefore, the utilization of native lignocellulolytic microbes in sugarcane trash management will facilitate the

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acceleration of composting time and improvement of compost characteristics. Rahayu et al. (2022) reported that the physicochemical characterization of 5 composts, including the C/N ratio of compost, is below 12-17, macroelements N, P, K available in compost are relatively high compared to the minimum requirement of SNI. Compost 4, which comprises a lignocellulolytic bacteria consortia, has enormous potential for development as a novel type of decomposing for sugarcane waste processing. Karnchanawong and Nissaikla (2014), reported that the addition of mature compost from an optimized composting process using exploration-derived inoculum resulted in better compost compared to the addition of commercial inoculum. However, Formowitz et al. (2007) noted similar results with inoculation that did not improve compost quality because the inoculum was unable to outcompete the native microbial community thriving in compost under optimal conditions.

The use of decomposer microbes for the management of non-burning land is crucial. This is because in non-burning land management (ZB), trash needs to be rapidly decomposed so that the nutrients contained in the trash can be utilized by sugarcane plants. Sugarcane trash contains 10-22,9% lignin, 35% cellulose, and 23-35,50% hemicellulose (Jutakanoke et al., 2017). One ton of trash contains around 5,4 kg N, 1,3 kg P_2O_5, 3,1 kg K_2O, which is equivalent to 0,35% N, 0,13% P_2O_5, and 0,65% K_2O (Yulianti et al, 2023; Singh and Solomon, 1995; Sivaraman, 2014). However, despite research on lignocellulolytic microbes, implementation in the field has not been widespread. One issue is the lack of data on the impact of sugarcane trash management, especially through the use of decomposer microbes, on the enhancement of sugarcane trash utilization for growth and productivity. In this research, lignolytic and cellulolytic microbes were isolated from various agroclimates and laboratory selection was carried out, as well as limited field testing, as an initial stage in the development of sugarcane trash decomposers.

MATERIAL AND METHODS

The experiment consists of six activities as outlined below:

Testing The Effectiveness of Several Decomposer Isolates in The Laboratory. The isolates used in this activity are cellulolytic and lignolytic bacteria isolates obtained through isolation using CMC and methylene blue media, respectively. The medium used in this experiment is liquid CMC medium (1L) prepared by adding 2g CMC, 0.5g K_2HPO_4, 0.25g $MgSO_4.7H_2O$, 2g gelatin, 0.2g congo red and 1000mL distilled water. Methylene blue medium is prepared by adding 25mg methylene blue, 5g NaCl, 10g tripton, 5g yeast extract, and 1000mL distilled water. Meanwhile, liquid alkali lignin medium is prepared by adding 1g K_2HPO_4, 0.2g $MgSO_4.7H_2O$, 0.2g KCl, 2g $NaNO_3$, 0.2g yeast extract, 1g malt extract, 2 pellets of KOH, 0.4mL guaiacol, 1g alkali lignin, 0.5g chloramphenicol, and 4mg benomyl.

In the initial stage, the isolates were subcultured on slant agar medium and incubated for 2-3 days. Subsequently, they were cultured on liquid CMC and methylene blue media, each incubated for 5 days. For fungal isolates, after subculturing on PDA medium, the isolates were then cultured on PDB medium and incubated for 7 days. At the end of the incubation period, the isolates were inoculated into sugarcane trash placed in glass jars.

In preparing the trash, the trash was first soaked, then drained, and placed in glass jars at a quantity of 80g. Subsequently, each isolate was inoculated at 10% of the weight of the trash, and the mixture was incubated for 6 weeks. After the 6-week incubation period, the trash's decomposition was observed.

Figure 1. Sugarcane trash in glass jars that has been inoculated with selected isolates

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Table 1. Origin and function of each decomposer isolate

No	Isolate code	Origin of the isolate	Isolate function
1	Lemongrass litter	Lemongrass litter, N9	Cellulolytic (bacteria)
2	Sugarcane leaf	Sugarcane leaf litter, N9	Cellulolytic (bacteria)
3	SRC1	Sugarcane leaf litter RC, Buma, Lampung	Cellulolytic (bacteria)
4	SRC2	Sugarcane leaf litter RC, Buma, Lampung	Cellulolytic (bacteria)
5	SRC3	Sugarcane leaf litter RC, Buma, Lampung	Cellulolytic (bacteria)
6	SPC1	Sugarcane leaf litter PC, Buma, Lampung	Cellulolytic (bacteria)
7	SPC2	Sugarcane leaf litter PC, Buma, Lampung	Cellulolytic (bacteria)
8	SPC3	Sugarcane leaf litter PC, Buma, Lampung	Cellulolytic (bacteria)
9	SPC4	Sugarcane leaf litter PC, Buma, Lampung	Cellulolytic (bacteria)
10	SPC5	Sugarcane leaf litter PC, Buma, Lampung	Cellulolytic (bacteria)
11	LRC1	Sugarcane leaf litter RC, Buma, Lampung	Lignolytic (bacteria)
12	LPC1	Sugarcane leaf litter PC, Buma, Lampung	Lignolytic (bacteria)
13	LPC2	Sugarcane leaf litter PC, Buma, Lampung	Lignolytic (bacteria)
14	*Trichoderma* sp.	North Sumatra	Cellulolytic (fungi)
15	*Omphalina* sp.	North Sumatra	Lignolytic (fungi)
16	*Paecilomyces lilacinus*	North Sumatra	Lignolytic (fungi)

Testing The Effectiveness of Decomposers in Limited Field Conditions (Sacks). In this test, 9 selected isolates were used, consisting of 6 cellulolytic bacteria isolates (Lemongrass, SPC1, SPC3, SPC4, SPC5, SRC1), 2 lignolytic bacteria isolates (LRC1 and LPC2), and 1 cellulolytic fungal isolate. Cellulolytic bacteria were cultured using CMC medium. After subculturing, they were inoculated into liquid CMC medium and incubated for 5 days. Meanwhile, lignolytic bacteria isolates were cultured on liquid methylene blue medium (incubated for 5 days) after subculturing on solid methylene blue medium. The fungal isolate used was *Trichoderma* sp., which was initially grown on sterile PDA medium and incubated for 5 days, and then further incubated using PDA medium for 7 days.

The preparation of the trash for composting involves soaking the trash overnight, then draining and placing it in sacks at a quantity of 10 kg. The decomposer consortium isolate is inoculated at 10% of the wet weight of the trash. The mixture is then incubated for 6 weeks. Observations include temperature and decomposition level each week.

Table 2. Origin and function of each decomposer consortium isolate

No	Isolate code	Origin of the isolate	Isolate function
1	Lemongrass	Lemongrass litter, N9	Cellulolytic (bacteria)
2	SPC1	Sugarcane leaf litter PC, Buma Lampung	Cellulolytic (bacteria)
3	SPC3	Sugarcane leaf litter PC, Buma Lampung	Cellulolytic (bacteria)
4	SPC4	Sugarcane leaf litter PC, Buma Lampung	Cellulolytic (bacteria)
5	SPC5	Sugarcane leaf litter PC, Buma Lampung	Cellulolytic (bacteria)
6	SRC1	Sugarcane leaf litter RC, Buma Lampung	Cellulolytic (bacteria)
7	LRC1	Sugarcane leaf litter RC, Buma Lampung	Lignolytic (bacteria)
8	LPC2	Sugarcane leaf litter PC, Buma Lampung	Lignolytic (bacteria)
9	*Trichoderma* sp.	North Sumatra	Cellulolytic (fungi)

Testing The Effectiveness of Decomposer Isolates in The Field (Treatment Without Control). In this test, 9 isolates were used, including 6 cellulolytic bacteria isolates (Lemongrass, SPC1, SPC3, SPC4, SPC5, SRC1), 2 lignolytic bacteria isolates (LRC1 and LPC2), and 1 cellulolytic fungal isolate. Cellulolytic bacteria were subcultured using CMC medium. After subculturing, they were inoculated into liquid CMC medium and incubated for 5 days. Meanwhile, lignolytic bacteria isolates were cultured on liquid methylene blue medium (incubated for 5 days) after initial subculturing on solid methylene blue medium. The fungal isolate used was *Trichoderma* sp., which was initially grown on sterile PDA medium and incubated for 5 days, and then further incubated for 7 days using PDA medium.

The preparation of trash for composting involves soaking the trash overnight, then draining and spreading it on the ground within enclosed square boundaries made of red bricks, with each box measuring 1x1m. The weight of the sugarcane trash used is 5 kg, and it is inoculated with decomposer isolates at 10% of the wet weight of the trash. The mixture is then incubated for 6 weeks. Observations are made every week regarding temperature and decomposition levels.

Figure 2. Decomposition of sugarcane trash in a 1 m^2 box

Table 3. Origin and function of each decomposer consortium isolate in the field experiment

No	Isolate code	Origin of the isolate	Isolate function
1	Lemongrass	Lemongrass litter, Semarang, Center of Java	Cellulolytic (bacteria)
2	SPC1	Sugarcane leaf litter PC, Buma Lampung	Cellulolytic (bacteria)
3	SPC3	Sugarcane leaf litter PC, Buma Lampung	Cellulolytic (bacteria)
4	SPC4	Sugarcane leaf litter PC, Buma Lampung	Cellulolytic (bacteria)
5	SPC5	Sugarcane leaf litter PC, Buma Lampung	Cellulolytic (bacteria)
6	SRC1	Sugarcane leaf litter RC, Buma Lampung	Cellulolytic (bacteria)
7	LRC1	Sugarcane leaf litter RC, Buma Lampung	Lignolytic (bacteria)
8	LPC2	Sugarcane leaf litter PC, Buma Lampung	Lignolytic (bacteria)
9	*Trichoderma* sp.	North Sumatra	Cellulolytic (fungi)

Experiment on The Effectiveness of Decomposer Isolates in Petri Dishes. In this experiment, a consortium of cellulolytic, lignolytic bacteria and fungi, as presented in Table 4, was used as decomposers. The media used in this experiment include liquid CMC, liquid methylene blue, and liquid alkali lignin. In a Petri dish (10cm diam), 8g of wet substrate, which has been drained, is added, followed by an analysis of moisture content and pH. Subsequently, 10% of the decomposer with compositions A, B, C, and D (Table 4) is inoculated into the Petri dish, and it is incubated for 5 days. Each decomposer is replicated twice, sealed, then incubated in the dark, and the level of substrate decomposition is tested every week for 8 weeks.

Table 4. Origin of each isolate in the four decomposer consortia

Consortium	Cellulolytic bacteria	Lignolytic bacteria	Lignolytic fungi
A	Lemongrass	LPC1	LGTS1
B	SRC1	LTS2.10^{3}	LG Jolondoro 2
C	STS1.10.4	LBM PC2.10^{-4} (Buma PC)	LGBM PC (Buma PC)
D	STS	LT5.2.10^{-4}	LGT1

Experiment on Trash Composting Optimization. The experiment was conducted in Ciomas, Bogor. The material used was sugarcane trash, amounting to 3 tons for each composting unit, forming piles of sugarcane trash. The first pile of trash was without inoculation (K), while the second (P1) and third (P2) piles were inoculated with decomposers one day after stacking the trash (Figure 3). The inoculum used was 250 ml, diluted 10 times to make 2,500 ml, which was evenly sprayed onto the piles of sugarcane trash according to the treatment, with a moisture content check of 60%. For K, spraying was done using water. On the fourth day, the decomposer was sprayed with 2,500 ml on the trash pile, diluted 10 times, making it 2.5 liters of decomposer per pile (250 ml + 250 ml = 500 ml concentrated inoculum). Molasses was then added at a rate of 0.1%. Subsequently, the three piles were covered with a tarp. After one month, reinoculation was carried out for each pile with 500 ml, diluted 10 times.

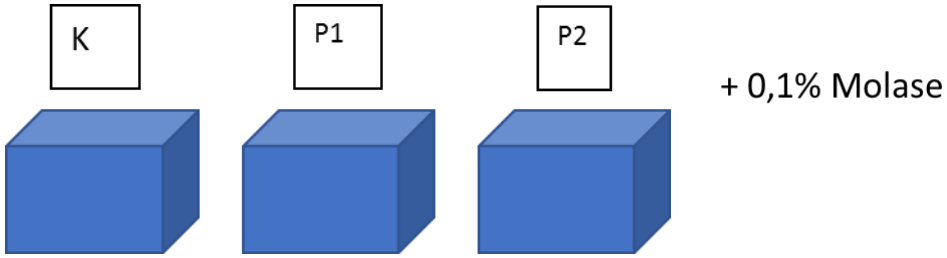


Figure 3. Piles of sugarcane trash undergoing composting using microbial consortium

Table 6. Origin of each isolate in the decomposer consortium in the experiment on trash composting optimization

No	Isolate code	Origin of the isolate	Isolate function
1	Lemongrass	Lemongrass litter, N9	Cellulolytic (bacteria)
2	SPC1	Sugarcane leaf litter PC, Buma Lampung	Cellulolytic (bacteria)
3	SPC3	Sugarcane leaf litter PC, Buma Lampung	Cellulolytic (bacteria)
4	SPC4	Sugarcane leaf litter PC, Buma Lampung	Cellulolytic (bacteria)
5	SPC5	Sugarcane leaf litter PC, Buma Lampung	Cellulolytic (bacteria)
6	SRC1	Sugarcane leaf litter RC, Buma Lampung	Cellulolytic (bacteria)
7	LRC1	Sugarcane leaf litter RC, Buma Lampung	Lignolytic (bacteria)
8	LPC2	Sugarcane leaf litter PC, Buma Lampung	Lignolytic (bacteria)
9	*Trichoderma* sp.	Fungi, North Sumatra	Cellulolytic (fungi)
10	*Omphalina* sp.	Fungi, North Sumatra	Lignolytic (fungi)

RESULT AND DISCUSSION

Assessement effectiveness of Decomposer in Glass Jars (Single Isolate). The sugarcane trash used in this test is in the form of leaves and stems. Physically, the tissue of sugarcane leaves is more fragile compared to those of the stem. Among the tested isolates, the decomposer isolate's ability to degrade the stem was lower than that of the leaves. However, changes in the softening of both sugarcane leaf and stem tissues occurred over time in the control group. For the leaves, this began in the third week, while for the stems, it started in the fifth week. In the first week, for sugarcane leaves, softening had already occurred for isolates Lemongrass, Sugarcane leaf, SRC 2, SRC3, SPC3, SPC4, SPC5, LPC1, LPC2, *Trichoderma* sp., and *Omphalina* sp. Conversely, for sugarcane stems, tissue softening had not yet occurred. Softening of sugarcane stem tissue began in the second week for isolates Lemongrass, SRC2, SPC1, SPC4, SPC5, LRC1, LPC2, *Trichoderma* sp., and *Omphalina* sp. The tested isolates showed variation in their ability to decompose leaf litter, as well as the speed indicated by the variation in the rate of leaf litter softening. Nevertheless, all isolates had the ability to degrade both leaves and stems, whether cellulolytic or lignolytic isolates. In the 6MST observation, the lemongrass isolate demonstrated the highest ability to degrade sugarcane leaves and stems. Theoretically, lignin is located on the outer part of the tissue, followed by cellulose. Therefore, it appears that the lemongrass isolate, isolated using CMC media, also has lignolytic abilities. The isolate with the second-highest ability is SPC5 with scores of 4 and 2 for leaves and stems, respectively.

Table 7. Crumbliness rating (1-5) of leaf and stem after incubation 6 weeks in the jars experiment

Code	1WAI		2WAI		3WAI		4WAI		5WAI		6WAI	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Control	0	0	0	0	1	0	1	0	1	1	2	1
Lemongrass	1	0	1	1	2	1	2	2	3	2	4	3
Sugarcane	1	0	1	0	1	0	1	0	1	1	2	1
SRC 1	0	0	1	0	1	0	1	0	1	1	2	1
SRC2	1	0	1	1	1	0	1	1	1	1	2	1
SRC 3	1	0	1	0	2	1	2	1	2	1	3	2
SPC 1	1	0	1	1	2	1	2	1	3	2	3	2
SPC 2	0	0	1	0	1	0	1	0	1	1	2	1
SPC 3	1	0	1	0	1	1	2	1	2	2	3	2
SPC 4	1	0	1	1	2	1	2	1	3	2	3	2
SPC 5	1	0	1	1	2	1	2	1	2	1	4	2
LRC 1	0	0	1	1	1	1	1	1	2	1	3	2
LPC1	1	0	1	0	1	0	1	0	1	1	2	2
LPC2	1	0	1	1	2	1	2	1	2	2	3	2
Trichoderma sp	1	0	1	1	2	1	2	2	2	2	3	2
Omphalina sp	1	0	1	1	2	1	2	1	2	1	3	1
P.Lillacinus	0	0	1	0	1	1	2	1	2	1	2	1

Note: 0: not crumbly; 1: slightly; 2: moderately crumbly; 3: fairly crumbly; 4: very crumbly and 5: extremely crumbly.

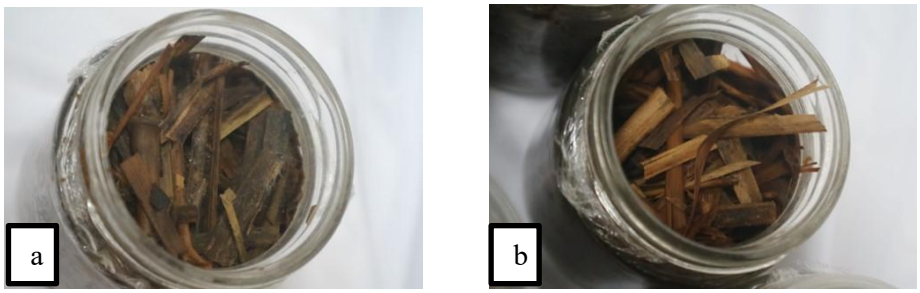


Figure 4. a) control trash 6 WAI b) treated trash 6 WAI

Testing The Effectiveness of Decomposers in Limited Field Conditions (Sacks). The effectiveness testing of the isolates continued using a larger volume of trash, placed in sacks with a weight of approximately 10 kg each. Both the trash in the control group (K) and the inoculated trash showed temperature dynamics. However, the inoculated trash exhibited a higher temperature increase compared to the non-inoculated (K) trash. The peak of the highest temperature occurred in the fifth and sixth weeks, and in the subsequent incubation, there was a decrease in crumbliness. For the control group (K), temperature fluctuations also occurred, with the highest temperature reached in the fifth and sixth weeks. When comparing between the control group (K) and the treatment, the temperature in the inoculated trash was double that of the control group (K). Filamentous fungi have been found to play an active role in the secretion of specific enzymes capable of degrading lignocellulose, while bacterial species can boost the production of hydrolytic enzymes and sugar compounds as nutrients, enhancing the ecological stability of the environment (Vu et al., 2023).

Figure 5. a) Composting of trash using sacks, b) Fungal colonization on inoculated sugarcane trash

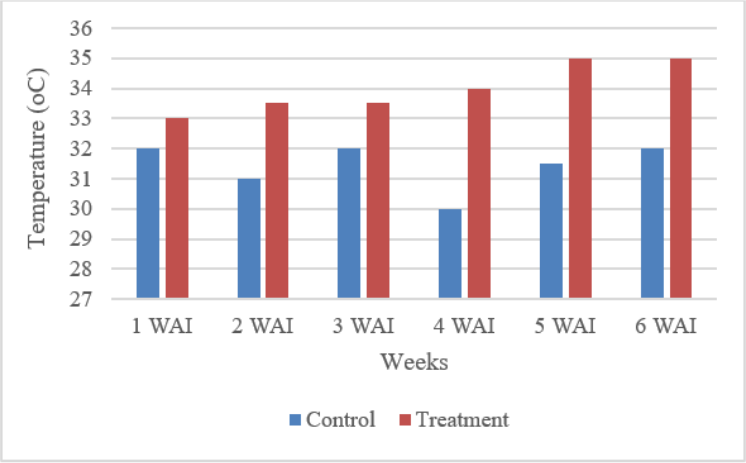
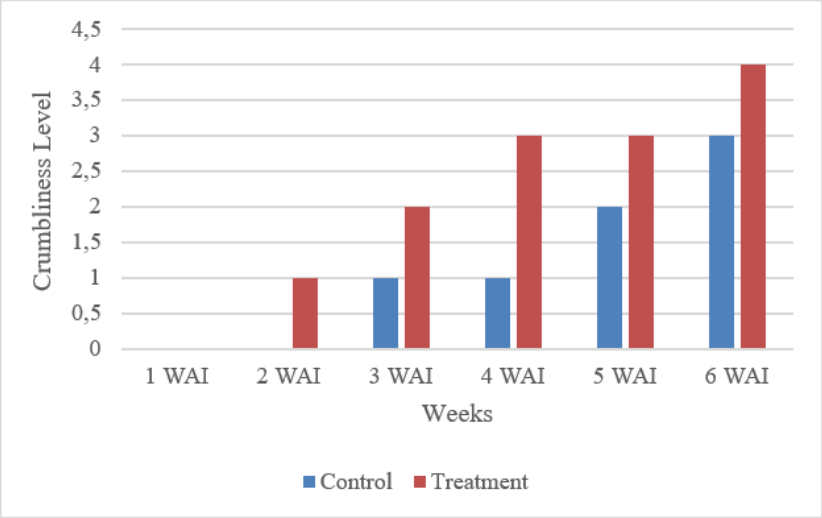


Figure 6. Observation of the temperature of trash up to the age of 6 weeks in the sack experiment.



Note: 0: not crumbly; 1: slightly; 2: moderately crumbly; 3: fairly crumbly; 4: very crumbly and 5: extremely crumbly.

Figure 7. The crumbliness level of the trash up to the age of 6 weeks in the sack experiment.

Testing The Effectiveness of Decomposer Isolates in The Field. Temperature is the result of microbial respiration, which is a reaction to degrade biomass. Observations indicate that the temperature of trash treated with lignocellulolytic decomposer isolates is much higher compared to the untreated (control) trash. The temperature increases with incubation time and subsequently decreases. The highest temperature is reached on the 3rd weeks of incubation and persists for 1 week before declining. Nevertheless, the highest temperature, 34°C, is still within the mesophilic range. Therefore, in this incubation or composting, thermophilic microbes have not yet shown their presence or activity in the decomposition of trash. Combine treatment between steam explosion and consortium microorganism technology could increase lignin degradation efficiency of lignocellulose biomass and can facilitate high value conversion of lignocellulose (Zhang et al., 2023).

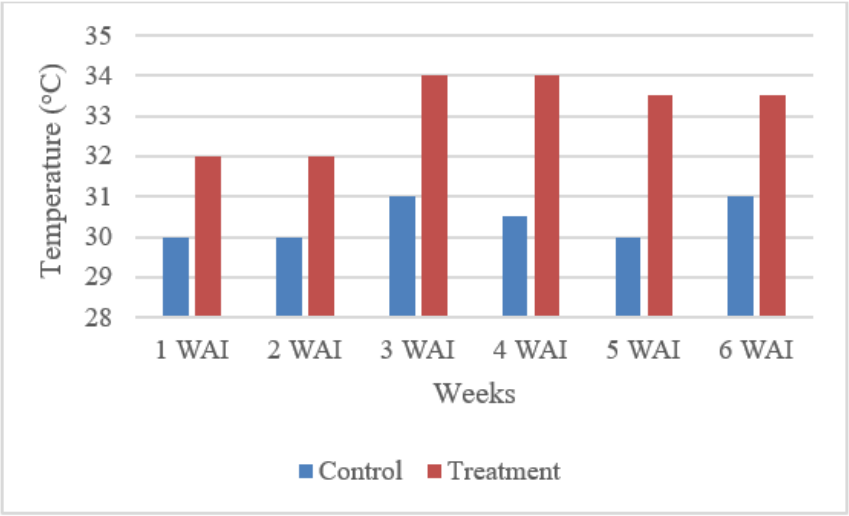
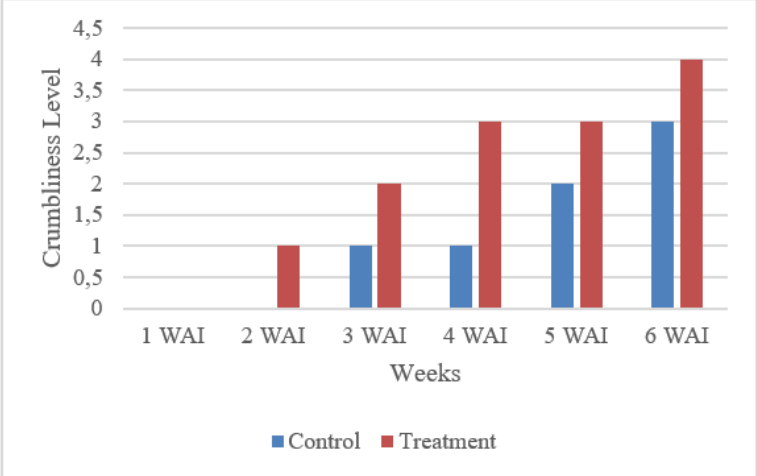


Figure 8. The temperature of trash until the 6-weeks incubation in the Taken plot experiment.



Note: 0: not crumbly; 1: slightly; 2: moderately crumbly; 3: fairly crumbly; 4: very crumbly and 5: extremely crumbly.

Figure 9. The crumbliness level of the trash until the 6-week incubation in the Taken plot experiment.

Experiment on the effectiveness of decomposer isolates in Petri dishes This experiment was conducted to assess the effectiveness of each consortium isolates and to understand the influence of providing a simple carbon source, such as glucose, on the decomposition activity of isolates in the consortium. The consortium contains cellulolytic and lignolytic bacteria as well as fungi. The trash reactions were generally neutral, both in the control trash and in the leaf trash treated with isolates. However, there were slight variations in reactions among the tested decomposer consortia. The addition of sugar did not result in significantly different reactions. Thought Vu et al. (2023) in their research showed that fungi, bacteria, and yeasts that converse the cellulose to sugar showed a relatively high degradation capacity under solid-state conditions due to their synergistic interactions (Vu et al., 2023).

The carbon content (C) was higher than the control, both with the addition of glucose and without glucose. The highest C content was observed when decomposer C was added without glucose, while with glucose, it was when consortium D was added. The high C content with the addition of decomposer consortia indicates suboptimal trash decomposition reactions, and the addition of glucose also does not assist in the decomposition reaction. The nitrogen content (N) increased in the trash treated with decomposer consortia, both without glucose and with decomposer. The calculation of the C/N ratio showed that without the addition of glucose, the addition of decomposer consortia resulted in a lower CN ratio compared to the control. The trash with the lowest CN ratio was the one treated with decomposer consortia B and D. With the addition of glucose, all trash treated with decomposer consortia produced lower glucose levels compared to the control, and the trash with the lowest CN ratio was the one treated with decomposer consortium A.

Table 8. Chemical characteristics of decomposed trash with each decomposer consortium incubated for 8 weeks.

Decomposer	pH (- Glucose)	pH (+ Glucose)	C (- Glucose)	C (+ Glucose)	N (- Glucose)	N (+ Glucose)	C/N Ratio (- Glucose)	C/N Ratio (+ Glucose)
A	7.4	6.9	28.9	33.3	0.54	0.63	53.52	52.86
B	6.9	7.3	**27.5**	30.2	**0.56**	0.52	**49.11**	58.08
C	7.2	7.2	32.4	26.7	0.56	0.49	57.86	54.50
D	7.3	7.1	**28.5**	33.6	**0.57**	0.62	**50.00**	54.19
Control	7.0	7.1	21.6	27.9	0.40	0.47	54.00	59.36

This suggests that, in general, the trash composting reaction is neutral, and the expected decrease in carbon, which should occur with the addition of decomposer consortia, actually increases, both with and without glucose. However, the addition of decomposer consortia increases nitrogen levels, both with and without the addition of glucose. Nevertheless, the addition of decomposer results in a decrease in the CN ratio, especially with the addition of decomposer consortia B and D. Consortium B is effective for crumbliness (without sugar), although the analysis shows high C content.

Observations on crumbliness indicate that the addition of decomposer increases the crumbliness of the trash, but there is variation among decomposer consortia. Nevertheless, there is dynamism in crumbliness. Qualitative observations may explain this variability. However, observations at 6 months post-inoculation (6WAI) show that the addition of decomposer results in trash that is more crumbly compared to the control, and decomposer C produces the highest crumbliness, while the one not significantly different from the control is decomposer consortium A.

Table 9. Crumbliness of decomposed trash with each decomposer consortium up to 6 weeks after inoculation

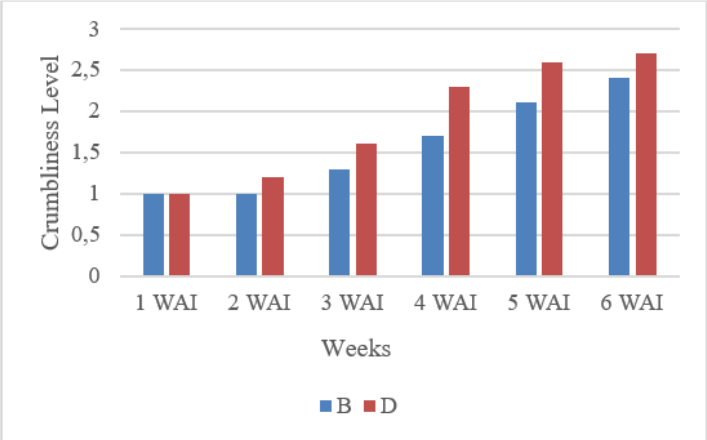
Decomposer	1 WAI -G	1 WAI +G	2 WAI -G	2 WAI +G	3 WAI -G	3 WAI +G	4 WAI -G	4 WAI +G	5 WAI -G	5 WAI +G	6 WAI -G	6 WAI +G
A	1	1.5	1.5	1.5	1	1	1.5	1	1.5	1	1	1
B	2	1.0	2	1.5	1.5	1.5	1.5	1.5	3	2	3	3
C	2	1.5	2	3	3	2.5	2	2	2	2.5	3	3
D	1	2.0	2	3	2	3	1.5	2.5	2	2	2	3
Control	1	1.0	1	1	1	1	1.5	1	1	1	1	1

Note: 0: not crumbly; 1: slightly; 2: moderately crumbly; 3: fairly crumbly; 4: very crumbly and 5: extremely crumbly.

Figure 10. Control trash (left with sugar +; right without sugar -)

Figure 11. Sugarcane trash treated with decomposer consortia A, B, C, and D, a) without sugar (-), b) with sugar (+)

The effectiveness of decomposers in baglogs. The experiment using baglogs shows that the crumbliness level of trash increases with incubation time. However, in the first week, there is no difference between the formulas of decomposer B and D. Nevertheless, after 1 month post-inoculation (1 WAI), decomposer formula D produces higher crumbliness compared to decomposer formula B. In the 2 WAI incubation, the growth of white mycelium is observed, especially with the addition of decomposer D, while for decomposer B, mycelium appears visible in the third week. A significant increase in trash crumbliness is observed in the 4 WAI incubation and continues to increase in the 5 WAI incubation.



Note: 0: not crumbly; 1: slightly; 2: moderately crumbly; 3: fairly crumbly; 4: very crumbly and 5: extremely crumbly.

Figure 12. The influence of consortium decomposer inoculation on the crumbliness level (qualitative) of trash

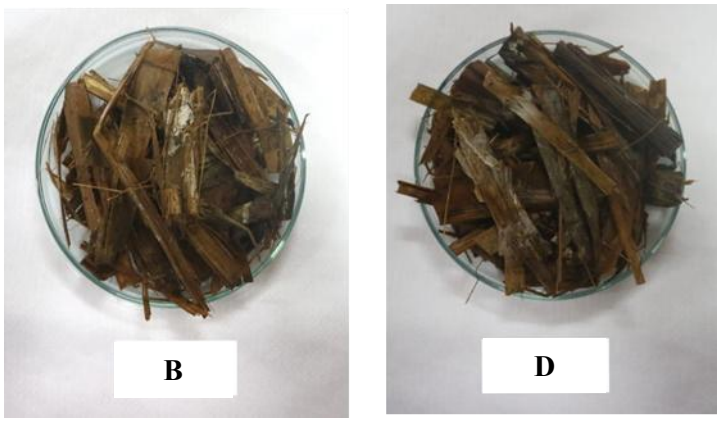


Figure 13. Decomposed trash (left consortium B; right consortium D)

Optimization of Trash Decomposition. Trash composting continues in the field using a pile methode with a hxlxw of 1.5 x 3x1.5 m in size. Temperature observations show an increase at 2 WAI followed by 3 WAI. However, after this period, there is a decrease in the temperature of the trash pile, followed by an increase again at 6 WAI. The temperature measured in trash piles with decomposer consortia is much higher than in those without decomposer. This result indicates that the sugarcane trash is already infested with microbes capable of degrading the trash. Yuan et al., 2021 said that increased temperatures leading to enhanced microbial interactions (Yuan et al., 2021).

Figure 14. Application of decomposer to trash piles using a sprayer and then covered with a tarp

Figure 15. The performance of the trash without inoculation (control, left), inoculated with P1(center), inoculated with P2 (right)

From these results, it appears that thermophilic temperatures have not been reached, although the maximum mesophilic temperature has been achieved. The failure to reach thermophilic temperatures will result in a suboptimal decomposition process, causing the formation of simple compounds, and mineralization has not proceeded optimally.

Crumbliness of trash increases with incubation time. The crumbliness of P1 trash pile increases until 5 WAI and slightly decreases at 6 WAI. Meanwhile, the crumbliness of P2 is highest in the 5th week, and there is no change in the 6th week. The trash pile without decomposer shows crumbliness similar to P2, especially at 6 WAI. Crumbliness is one of the indicators of decomposition occurrence.

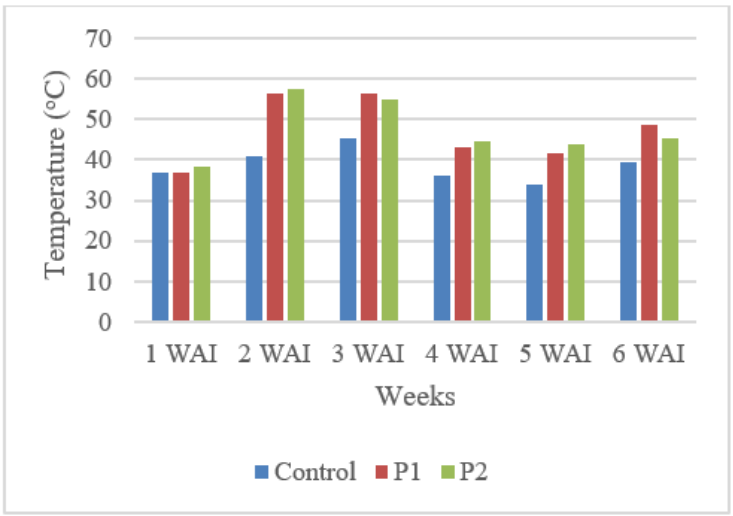
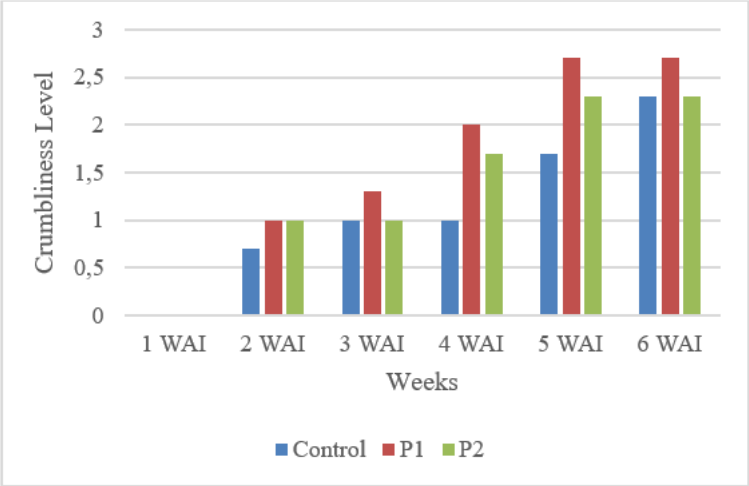


Figure 16. Trash pile temperature for each decomposer until 6 WAI



Note: 0: not crumbly; 1: slightly; 2: moderately crumbly; 3: fairly crumbly; 4: very crumbly and 5: extremely crumbly.

Figure 17. The crumbliness level of trash for each decomposer up to 6 WAI

The N content of the trash is analyzed based on the position of the trash in pile, namely at the bottom, middle, and top. The analysis results show that the highest N content is in the top and middle parts of the trash pile. This indicates that the decomposition process or breakdown of the trash varies. In the top location, there is likely the highest humidity, as well as in the middle part. However, theoretically, the water content at the bottom is expected to be higher than in the middle and top parts. Another factor that may support decomposition occurring better in the top and middle than at the bottom is the difference in density or aeration. The bottom part likely has lower aeration compared to the middle and top parts. Aeration is crucial for aerobic microbes involved in the decomposition of lignocellulosic biomass such as trash as a source of oxygen. Wu et al. (2022) said that composting is a biological and chemical process, and in the presence of oxygen, lignocellulosic biomass is transformed into more stable product (HSs). Aeration influenced the degradation and humification rate by regulating microbial interactions. Interval aeration, featured by 20 min aeration and 10 min pause, could increase microbial interactions and improve composting efficiency (Zhao et al., 2022). Higher aeration rate could increase the alpha diversity (Ge et al., 2020) and slightly alleviate the phytotoxicity (Zhang et al., 2020).

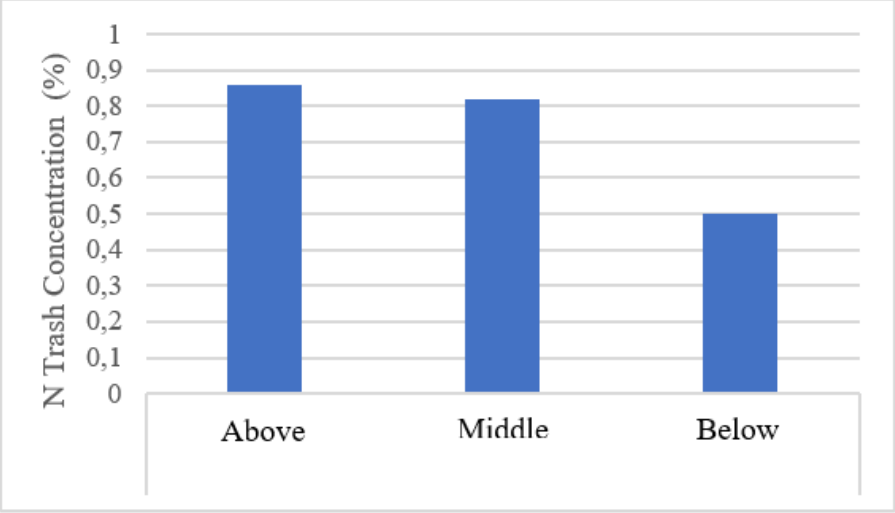


Figure 18. The N content of trash resulting from the decomposition with decomposers P1 and P2 (6 WAI)

The lowest C content is in the bottom part, followed by the top and middle parts. This result indicates that the breakdown of biomass or C is highest at the bottom, followed by the top and middle. Apparently, this result is not in line with the N analysis result. However, from the calculation of the CN ratio, it is shown that the lowest CN ratio is in the trash at the top, followed by the middle and bottom parts.

Inoculation has been promoted as a method to accelerate the composting process, enhance the stabilization and maturity of the final product, promote specific microbial consortia, degrade specific compounds, reduce phytotoxicity, improve pathogen control, and stimulate seed germination (Zainudin et al., 2022; Koker, 2019). However, composting is a highly heterogeneous process due to the variability of the parent organic materials, environmental conditions in which the composting process takes place, and the development of highly competitive native microbial populations (Fan et al., 2017). These factors, especially in open composting systems, may result in a lack of significant microbial inoculation effects, as reported by Yadav et al. (1982) during the composting of plant residues, livestock manure, and wheat straw inoculated with various fungal strains.

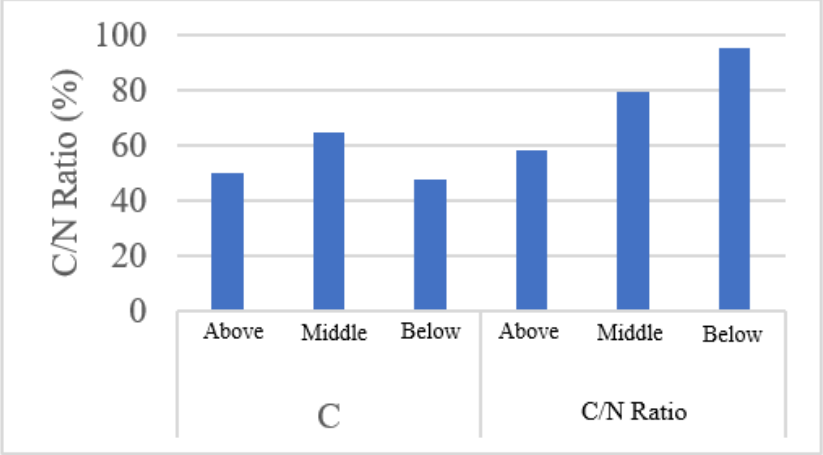


Figure 19. The CN ratio of trash of inoculated pile after 6 weeks after inoculation

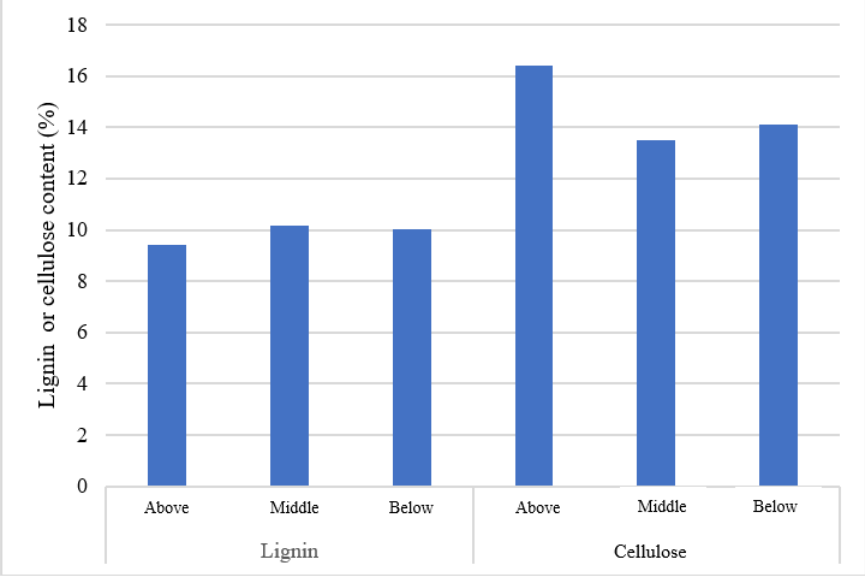


Figure 20. The lignin and cellulose content of trash resulting from the decomposition with P1 and P2 six weeks after inoculation

CONCLUSION

Total nine (9) isolates has been asesss in decomposing sugarcane trash. Consortium isolate could decomposed trash better compared to single isolate. The time of decomposition was 2 months. The characteristics of the decomposed trash was crumb, lower in CN ratio and lignin and cellulose content. Formulation of those concortia isolate have to be done to get optimum decomposition.

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