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Effects of Indigenous Microorganisms on Rice Straw Degradation

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Abstract: The use of indigenous microorganisms (IMO) has been known to improve soil fertility and crop production. This study was carried out to observe the effect of indigenous microorganisms on rice straw composting, consist of different carbon to nitrogen ratio. Composting was carried out for 35 days and was turned manually every week. The results show that carbon and nitrogen ratio decreased during composting period and temperature was observed in compost treatment mixture consist of rice straw, goat manure, rice bran and IMO (T4) compared to rice straw only (T1). Compost treated with IMO showed further decrease of carbon to nitrogen ratio at 9.5 upon maturation state on the 35th day. Analysis of microorganisms showed compost treatment with IMO has higher diversity of microorganisms with 3.16 × 109 CFU g-1 for mesophilic bacteria and 7.9 × 108 CFU g-1 for thermophilic bacteria. In conclusion, compost treated with IMO with combination of goat dung, paddy straw and rice bran contain high diversity of microorganisms in compost systems and thus, undergo early maturation phase of composting process.

Keywords: lignocellulose, *Enterobacteriaceae*, natural farming, indigenous microorganisms

1. Introduction

Malaysia produced an abundance of rice straw waste per year. Currently, the farmers practice on-farm rice straw burning or incorporation into fields by ploughing to manage this waste (MADA, 2010). These practices cause harm to the environment and attract rodents to the fields. Additionally, incorporating rice straw into the field leads to methane production during paddy cultivation due to anaerobic degradation, increasing the greenhouse gas. One of the alternative approaches to managing rice straw effectively is by converting rice straw to compost. The composting process involves the decomposition of organic matter into humus-like material. The approach converts agricultural waste into valuable products as nutrient-rich organic amendments for various agricultural, horticultural, or landscaping applications (Neher et al., 2013).

Microorganisms play an essential role in composting. Using specific microorganism inoculants during the composting process could aid the decomposition rate of raw materials. Thus, understanding the role of microorganisms involved and creating the right conditions for their activity is a key to successful composting. Liu et al. (2011) reported that the added microbiological inoculum facilitates microbial diversity in compost and reduces the maturation time of cow manure compost. Indigenous microorganisms (IMOs) are naturally occurring microorganisms in a specific environment, such as soil, water, or organic matter. These microorganisms are indigenous or native to a particular location and play crucial roles in various biological processes. IMOs are used in organic farming as microbial inoculants to enhance soil fertility, promote plant growth, and suppress diseases. The IMO has been used in compost to facilitate its degradation process. These practices have been introduced through Korean Natural Farming (KNF). This farming approach maximizes the use of on-farm resources, recycles farm waste, and minimizes external inputs while fostering soil health (Wang et al., 2012).

Rice straw has a complex structure comprising cellulose, hemicellulose, and lignin, making it a recalcitrant raw material (Binod et al., 2010). Therefore, the degradation of rice straw without pretreatment would take a much longer

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time. Adding nitrogen sources such as manure would speed up decomposition and give good-quality compost (Bernal et al., 2009). Besides, this combination of carbon and nitrogen raw materials would allow microorganisms involved during composting to have sufficient nutrients for their metabolic activity. An optimum condition of the composting process by adjustment of specific parameters such as C/N ratio, temperature, and moisture has been studied intensively. Application of utilized microorganisms' culture-based method during composting mediate degradation process. Alteration of natural rice straw degradation using microorganism inoculum and a combination of different amounts of rice straw and manure involved microbiological and chemical variables. Therefore, this study investigated the use of IMO as inoculant composting enhancer on different combination rates of rice straw and manure compost treatments.

2. Material and methods

2.1 Composting experiment

The composting experiment was performed in Ladang Organik MARDI, Serdang. Small-scale composting was served in plastic bins using a passive aeration technique. The raw materials used in this study were mainly rice straw, goat manure, and rice bran. Rice straw was obtained from Sungai Burong, Kuala Selangor, goat manure was obtained from MARDI, Serdang and rice bran was obtained from the market. The composition of these materials is shown in Table 1. Composting was carried out for 40 days and was covered to prevent direct sunlight and rain. Treatment was done in triplicates, and samples were collected at three random locations in the bins every 5 days.

Table 1: Different ratio of raw material and C/N value of IMO-composting

Treatment	Mixture	Initial C/N ratio
T1	Rice straw (2.5 kg)	59:1
T2	Rice straw (2.5 kg) + IMO	59:1
T3	Rice straw (2.5 kg) + Goat manure (2.5 kg) + Rice bran (1.5 kg)	25:1
T4	Rice straw (2.5 kg) + Goat manure (2.5 kg) + Rice bran (1.5 kg) + IMO	25:1

2.2 Preparation of IMO

Production of IMO was done based on the principle that environmental microorganisms will attract to rice as their nutrient source. IMO was harvested from the colonisation of microorganisms on rice isolated from the rhizosphere of banana roots (Nurul Ain et al., 2015). Cooked rice was used to allow the growth of microorganisms from this environment. Production of IMO 2 was done after 7 days, where rice containing microorganisms was harvested, and molasses was added as a nutrient source for these microorganisms on a 1:1 basis. White colonies of cotton-like appearance were observed on rice, indicating the successful isolation of IMO collection. IMO 3 was then produced by diluting IMO 2 with water and mixed with 8 kg of rice bran. Rice straw was used to cover IMO 3 for 5 days to allow microorganisms to propagate. The IMO 4 phase was produced by mixing an equal volume of IMO 3 and soil for further usage. This IMO was applied by combining IMO 4 with compost on a 1:2 basis.

2.3 Temperature analysis

Temperature degrees at the center of each pile were recorded using a stem thermometer every day for the first 7 days, followed by every 5 days.

2.4 Chemical analysis

2.4.1 Carbon content analysis

Total carbon was measured using the Walkley and Black Rapid Titration method with some modifications (Walkley and Black, 1934). Compost samples were ground up to pass through a 2 mm mesh sieve, and 100 mg of the sample was placed in a 500 mL Erlenmeyer flask. Ten milliliters of potassium dichromate ($K_2Cr_2O_7$) and 20 mL of concentrated sulfuric acid (H_2SO_4) were added to the sample while stirring it to ensure good mixing of the sample with the reagents (Sato et al., 2014). After a rest of 45 minutes, 200 mL of distilled water was added to the sample. Before titration, three to eight drops of ferroin indicator were added to the mixture. The carbon content of the samples was determined by volumetric titration using ammonium (II) sulfate.

2.4.1 Nitrogen content analysis

Nitrogen contents were measured using the Kjedahl digestion method using 30 mg of ground compost samples added with Kjedahl catalyst in a 100 mL test tube. Samples were mixed with 3 mL H_2SO_4 and placed in a digestion block (Protech, Block Digester BD-40) for 3 hours. Samples were left to cool overnight after the digestion process. Following this process, suspension in the test tube was then transferred to the distillation apparatus. 15 mL of NaOH were added to these suspensions and were distilled for 3 minutes in the distillation chamber (Buchi distillation B-324). 10 mL of boric acid was added to a 200 mL conical flask and placed under the condenser of the distillation chamber prior to the distillation process. Titration was then proceeded to a violet endpoint using sulfuric acid.

2.5 Microbiological analysis

Three points of compost were collected for enumeration of microorganisms analysis. Total aerobic culturable microorganisms were determined using dilution plate count technique. An initial of 5 g compost samples was diluted with 45 mL phosphate sodium buffers and proceeded with serial dilutions until reaching dilution 10^5. About 1 ml of diluents were taken from $1x10^3$, $1x10^4$, and $1x10^5$ dilution factors and transferred into the required media. Mesophilic and thermophilic bacteria were incubated on nutrient agar (NA) at 30 °C and 50 °C for 24 hours. Fungi were incubated on rose bengal chloramphenical agar (RBC) at 30 °C for 7 days. Cultures showing between 30-200 colonies were counted using the colony counter.

2.6 Isolation and identification of IMO

Microorganisms with different morphology were picked and selected for further identification. Selected colony undergo further streak plate technique to ensure the purity of the strains. Identification of the isolated microorganisms were done using API kit (bioMeriux) which composed of API 20 NE, API 20E and API 20C AUX.

2.7 Statistical analysis

All the experiments were conducted using a completely randomized design (CRD) with three replications. Statistical analyses were performed using SPSS version 16. Parameters were analysed using a one-way ANOVA.

3.0 Results and discussion

3.1 Initial characteristics

The initial physicochemical characteristics of raw materials are shown in Table 2. Calculation of the C/N ratio on rice straw showed that it has higher carbon percentage than nitrogen, making it a suitable carbon source. Rice straws are shown to consist of 51.3% carbon and 0.7% nitrogen. In contrast, goat manure consists of 15.5% C and 3.0% N. Goat manures are rich with nitrogen and have a lower C/N ratio of about 5.2 compared to rice straw, which is 73.3. Manure has always been used as one of the options for nitrogen sources (Erickson et al., 2015). Rice bran is used in this study to enhance the composting process as a simple nutrient source for microorganisms. Initial characterization of diverse organisms from raw materials used for composting showed that a few bacteria and fungi were present before the beginning of the composting process (Table 3). IMO collected showed the highest number of microorganisms present, with the most consisting of mesophilic bacteria and fungi at 10.8 Log CFU/g and 9.2 Log CFU/g. This is also stated by Sumathi et al. (2012), which mentioned that the main sources of IMO's are bacteria, fungi, and cyanobacteria. Once the composting process has been started, this microorganism will actively degrade raw materials.

Table 2: Analysis of physicochemical contents of raw material for composting

Raw material	Moisture (%)	C (%)	N (%)	C/N
Rice straw	9.2 ± 1.5	51.3 ± 0.9	0.7 ± 0.3	73.3 ± 2.6
Goat manure	50.0 ± 2.5	15.5 ± 0.8	3.0 ± 0.8	5.2 ± 0.3
Rice bran	12.7 ± 1.1	48.5 ± 1.3	3.4 ± 0.1	14.3 ± 0.9

Table 3: Initial microbiological characteristics of raw materials

Raw material	Mesophilic bacteria (Log CFU/g)	Thermophilic bacteria (Log CFU/g)	Fungi (Log CFU/g)
Rice straw	5.3 ± 0.5	0.7± 0.4	5.8±0.3
Goat manure	5.7± 0.5	1.2± 0.3	5.7±0.5
Rice bran	5.1±0.3	2.4±0.6	5.7±0.5
IMO	10.8±1.2	5.4±2.1	9.2±1.0

3.2 Temperature analysis

Temperature is one of the main parameters that influences decomposition during composting. As reported in our previous study, temperature variation for each treatment depends on the compost mixture (Nurul Ain et al., 2015). Monitoring and maintaining the correct temperature is crucial for optimal decomposition and nutrient release (Martin et al., 2021). To achieve the desired compost temperature, various factors such as proper aeration, moisture levels, and the materials used should be considered.Compost temperature is also related to the C and N ratio of raw materials used during composting.

A lower C:N ratio is associated with higher temperatures, while a higher C:N ratio tends to result in lower temperatures. This is because the microorganisms responsible for composting require carbon as an energy source and nitrogen for their growth and reproduction. Therefore, a lower C:N ratio provides an ample supply of carbon relative to nitrogen, allowing the microorganisms to thrive and generate more heat through their metabolic In this study, higher temperature obtained during composting process indicates active microorganisms' activity. During composting process, the mesophilic phase, where a rapid increase of temperature in the range determines the start of the degradation of organic matter, and its duration varies between 24 and 72 h. The second one is the thermophilic phase, characterized by temperatures between 45 and 70 °C in relation to the metabolic activities of endogenous thermophilic microorganisms which degrade the organic compounds, and it can last from several days to several weeks (Finore et al., 2023). The thermophilic phase inbin composting was shown to occur on day 3. The temperature continued to rise for 6 days, with T3 showing the highest value at 43°C. T1 and T2 offer a range of temperature from 22°C to 31°C, much lesser than T3 and T4. Raut et al. (2008) observed that compost containing 5 kg of raw materials only achieved about 41°C as the highest temperature. Additionally, the used of goat dung in T3 and T4 gives an optimum value of C/N ratio for composting, which provides good conditions for microbial growth.

3.3 Changes in C/N ratio during composting

The value of C/N ratio of different compost treatments is shown in Figure 1. Theoretical initial C/N ratio calculation of compost treatments; T1 and T2 have the value of 59:1. Calculation of the theoretical C/N ratio was based on the method suggested by Mohammad Hariz et al. (2013). However, the compost mixture of T3 and T4 was calculated to meet the optimum C/N ratio for composting at 25:1 (Bernal et al., 2009). Analysis of C/N ratio during early composting, however, showed an actual value which is approximate to the theoretical value where T1 and T2 both have the value of 59:1 and 60:1 while T3 and T4 are at 24.2:1 and 22.2:1. Notably, a high C/N ratio may immobilise the nitrogen in plants instead of mineralising it (Chaves et al., 2005). Therefore, the evaluation of the C/N of the final compost is essential to ensure that its eventual utilisation contributes to positive results.

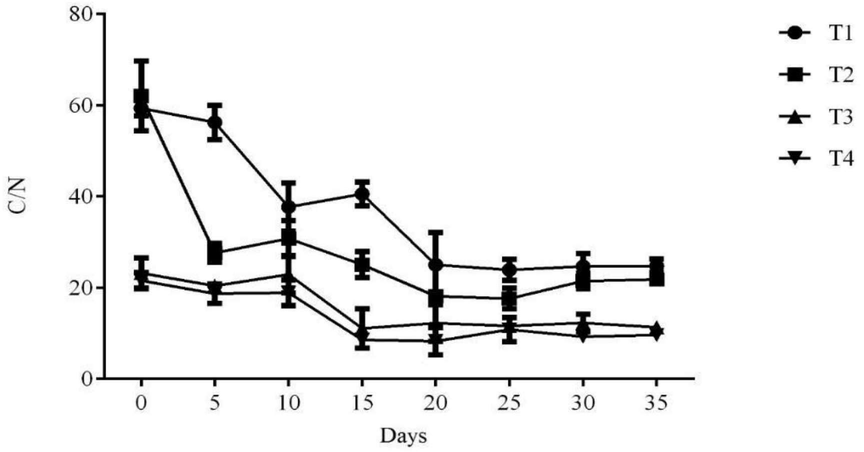

Fig. 1: Changes in the C/N value of treatments with/without IMO. T1 and T2 are natural degradation, which contain only rice straw, while T3 and T4 are composting materials consisting of rice straw and goat manure. IMO was added in treatments T2 and T4 for comparison.

Compost treatments T3 and T4 have much lower C/N ratios than T1 and T2 due to the presence of goat manure. Throughout the composting process, T3 and T4 showed value approaching maturation earlier than T1 and T2. Setting the compost mixture to an optimum C/N fastens the composting process. Li et al. (2008) proved that adding animal manure to rice straw compost lowered the C/N value to an optimum level of 27.8. The value of the T2 C/N ratio dropped rapidly during day 5 to 27.7 compared to T1 at 39.6. A comparison of the C/N ratio between T1 and T2 showed that rice straw treatment with IMO could fasten the degradation activity during composting and reduce the C/N value. In an early study, compost at day 5 shows an increase in temperature for T2, T3, and T4 (Nurul Ain et al., 2015). According to Tchegueni et al. (2013), the drop in C/N value during the early phase of composting are caused by microorganisms activity involved in the degradation of organic matter. Treatment T3 and T4, however, showed no significant differences during day 5. The mixture of rice straw with goat manure and rice bran promotes an optimum environment for the original microorganism present in raw material for degradation. Therefore, adding IMO to this mixture does not give any significant differences.

Compost with a C/N ratio value approaching 10:1 are matured and can be used as a soil conditioner (Islam et al. 2012). At the end of the process, T4 showed mature compost with a C/N value of 9.7. However, the value of the C/N ratio for

T3 is 11.4, T1 is 24.8, and T2 is 21.8. This shows that a combination of rice straw, goat manure, and rice bran with the aid of IMO is the best mixture for composting rice straw. The reduction of the C/N ratio in compost containing IMO is much higher than in compost without IMO. The addition of microorganism inoculum in compost aids in the degradation process. Goyal & Sindhu et al. (2011) also showed that adding inoculum in rice straw composting with cow manure assisted the degradation process and lowered the compost C/N ratio.

3.4 Microbiological analysis

Understanding the diversity of microorganisms in composting is essential for optimizing the composting process and ensuring the production of high-quality compost.The number of microorganism populations changed during composting (Table 4). The reduction of the microorganism population occurred at the end of composting compared to the early phase. The early phase of composting shows a high population number due to the availability of substrate used for microbial growth. Available nutrient during the early composting phase stimulates microorganism growth and initiated self-heating, causing the compost to be in a thermophilic phase (Chandna et al., 2013). Thus, during this composting phase, more thermophilic bacteria were reported (Nurul Ain et al., 2015; Ryckeboer et al., 2003).

Table 4: Microbiological analysis on early and end of the composting process. The reduction of microorganisms was observed until the end of composting.

Treatment	Mesophilic bacteria (Log CFU/g)		Thermophilic bacteria (Log CFU/g)		Fungi (Log CFU/g)	
	Early composting	End of composting	Early composting	End of composting	Early composting	End of composting
T1	8.9±0.2	7.4±1.2	7.7±0.1	6.5±0.9	8.6±0.2	5.5±0.8
T2	9.5±0.2	7.1±0.9	8.2±0.2	6.5±0.7	8.9±0.1	5.5±0.5
T3	9.2±0.4	6.9±0.8	8.0±0.2	6.6±0.6	8.7±0.1	5.6±0.6
T4	9.3±1.2	7.3±1.0	7.6±0.13	6.6±0.6	8.9±0.2	5.8±1.0

The high diversity of bacteria was shown in T2 at 22% and 31% for mesophilic and thermophilic bacteria, respectively (Figure 2). The highest population of thermophilic bacteria was demonstrated in T4, with about 41%. The availability of this microorgaanisms help to further break down the organic matter, contributing to the eventual formation of nutrient-rich compost. Fungi populations are nearly the same for all treatments (25-30%) except for T4, which shows a slightly lower value at 19%. This might be due to the high temperatures recorded by T4 since fungi could not withstand the high thermophilic phase (Ryckeboer et al., 2003). T1 has the lowest number of microorganisms involved in composting. Based on the percentage for the diversity of microorganisms calculated, it was observed that the application of IMO on compost increased the diversity of microorganisms.

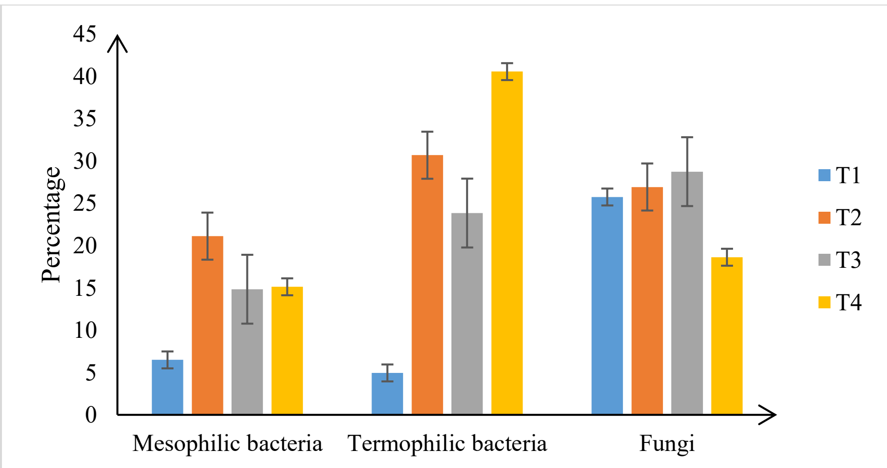

Fig. 2. Percentage of diversity microorganisms; mesophilic, thermophilic, and fungi in each pile of compost treatments.

3.5 Isolation and identification of IMO

Identification of culturable microorganisms present within IMO was done using an API kit. Nineteen species of bacteria and 7 species of fungi were isolated. Among those 19 species of bacteria, 7 were from the Enterobacteriaceae family, and

12 were observed as non-*Enterobacteriaceae*. Two types of API kits were used for bacteria identification: API 20E (Table 5) and API 20NE (Table 6). Identification of bacteria using API kit shows most of the bacteria population were colonised by *Pseudomonas* species. The availability of *Pseudomonas* species is reported to aid in the degradation of organic matter, and it is known as one of the dominant bacteria found in compost (Lin et al. 2013). *Candida* species were the most dominant fungi in composting (Table 7). This fungus aids in the rice straw degradation process by producing enzymes that breakdown lignin, hemicellulose and cellulose(Chen et al., 2019). . The microorganisms identified in compost were similar to those found in soil.

Table 5: Identification of bacteria from non-enterobacteriaceae family using API 20 NE kit

Code	Identification	Index number (API)
NE-1	*Methylobacterium mesophilicum*	0201041
NE-2	*Pseudomonas luteola*	5577610
NE-3	*Pasteurella pneumotropica*	2233100
NE-4	*Photobacterium damselae*	6300004
NE-5	*Pseudomonas luteola*	5577610
NE-6	*Delftia acidovorans*	1004253
NE-7	*Comamonas testosterone*	1030061
NE-8	*Aeromonas hydrophila*	5567744
NE-9	*Pseudomonas luteola*	5577610
NE-10	*Aeromonas caviae*	7777625
NE-11	*Sphingomonas paucimobilis*	3445700
NE-12	*Pseudomonas alcaligenes*	1110001

Table 6: Identification of Enterobacteriaceae family using API 20E kit.

Code	Identification	Index number (API)
E-1	*Pantoea spp 1*	001112045
E-2	*Enterobacter cloacae*	320577345
E-3	*Providencia rettgeri*	221012145
E-4	*Bordetella*	211230041
E-5	*Ewingella americana*	021510145
E-6	*Chryseobacterium indologenes*	025012035
E-7	***Yersinia enterocolitica***	311452223

Table 7: Identification of fungi using API AUX kit

Code	Genus	Identity score (% of similarity
F-1	*Candida utilis*	6000774
F-2	*Candida dubliniensis*	6272134
F-3	*Candida fumata*	6277331
F-4	*Pichia augusta*	6002330
F-5	*Crytococcus albidus*	2145030

4.0 Conclusion

It had been accepted that the initial compost mixture is essential in gaining optimum conditions for the degradation process. Therefore, we observed that our compost mixture, which consisted of rice straw, goat manure, and IMO, encouraged better degradation with an end C/N value of 9.7. Adding goat manure and IMO to rice straw compost provides an optimum environment for the growth of microorganisms, thus increasing the microbial activities in the compost. Due to that, IMO addition is needed to achieve a better degradation process and quality compost.

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Conflict of Interest

The authors declare no conflicts of interest.

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