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The effects of microbial enhancement on the decomposition of rice straw

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Abstract

Rice straw is one of the coproducts generated from rice cultivation. It is managed via on-farm open burning or soil integration during the wet season. However, the straw may not decay completely and hence, not well integrated into the soil. It may eventually affect soil properties during cultivation. The use of microorganisms has been documented to resolve this problem and reduce open field burning. In this study, three treatments were performed to accelerate the decomposition of rice straw. T1 was set as the experimental control with no added microbes, T2 incorporated with *Trichoderma* sp. and T3 involved cellulose degrading bacteria. Rice straw samples were collected weekly for eight weeks for physicochemical, microbiological and sugar-reducing analyses. The results indicated positive trends of C/N ratio reduction for rice straw inoculated with microbes in both seasons. The percentage of moisture content increased, but electrical conductivity declined over the period. Bacterial and fungal growth initially increased and then plateaued after day 14 in season 2. Therefore, rice straw inoculated with *Trichoderma* sp. and cellulose degrader could improve soil organic properties and expedite rice straw decomposition.

Keywords: decomposition, rice straw, soil integration, microbe

Introduction

Rice production is one of the most intensive agricultural activities in the world. Rice is a staple food, particularly in the Asian region and its production promises economic benefits and social stability. It provides a stable income to support the livelihood of many, especially those in rural areas (Zaim et al. 2013).

Rice cultivation activities produce coproducts such as rice straw, rice husk and other milling byproducts mainly wastes generated throughout the production processes. Nonetheless, their reutilisation is limited to certain applications. Rice husk is converted into carbonised charcoal and used as planting media. Rice straw is collected in bales and sold as animal feed or material for field mulching. Meanwhile, milling wastes, such as rice bran and broken rice, have commercial values and are highly sought after for industrial and agricultural uses. Rice bran has been utilised in the food, nutraceutical and pharmaceutical industries (Bodie et al. 2019). Broken rice is generally sold without prior separation or consolidated and ground into flour, regardless of the size (Mukhopadhyay and Siebenmorgen 2017). It is also used as a food additive because of its human nutritional benefits (Kim et al. 2012).

Meanwhile, the issues concerning rice straw are still predominant in field post-harvest management. Field rice straw management depends on the climate during the harvesting period. It is usually managed via field burning in the dry season and soil integration in the wet season. This scenario happens for several reasons, with mechanical and physical factors determining the best practice. It is difficult to apply field burning during the wet season due to uneven soil levels from machinery movements on soft soil and moist straw. Thus, conducting field burning during the dry season is much easier.

Soil integration of straw during the wet season may have several disadvantages. The straw may not properly decompose before soil integration in the pre-season preparation process, affecting the soil properties during

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cultivation. Rice fields are one of the sources of methane (CH_4) emissions (Nunkaew et al. 2015). CH_4 has over 25 times the impact of CO_2 over 100 years in terms of global warming potential (IPCC 2007).

Besides factors such as field water level and climate, rice straw may significantly affect CH_4 emission (Naser et al. 2007). Different methods of rice straw application may affect the degree of emission (Tan et al. 2018). Properly decomposed rice straw has a much lower CH_4 flux in the soil than raw or non-decomposed sources (Yagi and Minami 1990). Likewise, the use of compost increases microbial activity and reduces the CH_4 intensity (Das and Adhya 2014).

A specific combination of microorganisms can improve the decomposition of rice straw. Certain fungi have successful de-lignification capabilities (Chang et al. 2012), besides reducing cellulosic composition, e.g., cellulose, hemicellulose and lignin in rice straw through enzymatic hydrolysis (Taniguchi et al. 2005). This condition is brought about by enzymes, which can work effectively on targeted hemicelluloses, cellulose and lignin within the rice straw structure.

The improvement of rice straw decomposition through microbial technology could reduce CH_4 emissions. It may contribute to the sustainable utilisation of resources and a cleaner environment. It also minimises the cost of rice production by reducing fertiliser costs. Moreover, the integration of straw has other benefits, i.e., improving soil organic carbon (Wang et al. 2015), increasing the availability of nutrients (Mandal et al. 2004) and optimising fertiliser efficiency (Van Asten et al. 2005).

Materials and methods

Plot and experimental design

The experiment was conducted at the glasshouse at MARDI Headquarters, Serdang, Selangor (2°59'04.4"N, 101°42'17.9"E). The rice straw used in the experiment was bought from Tanjong Karang, Selangor. Approximately 7 kg of rice straw was put in a trough measuring 1.44 m^2. The study covered two seasons of 8 weeks each, with three treatments, as shown in *Table 1*.

The rice straw in Treatment 1 (T1) was not mixed with any microbes. Treatment 2 (T2) involved the incorporation of *Trichoderma* sp. obtained from MARDI's Microbial Culture Collection (MMCC), cultured in potato dextrose broth and mixed with 20 g empty and partially filled grain (EPFG), a waste byproduct from the rice mill. After 7 days of incubation at 27 °C, the mixture was scattered over the rice straw in the trough. Treatment 3 (T3) involved the cellulose degrading bacteria as the catalyser for rice straw degradation. The *Bacillus* sp. was isolated from paddy soil, cultured in nutrient broth, and mixed with 20 g of EPFG. Then, the mixture was incubated at 37 °C for 3 days before application on rice straw in the trough. The concentration of the fungi and bacteria was 1´ 10^7 CFU/mL.

Table 1. Treatments for season 1 and 2

	T1	T2	T3
Season 1	Control	Rice straw treated with	Rice straw treated with
Season 2		*Trichoderma* sp	cellulose degrading bacteria

Rice straw samples were collected weekly for 8 weeks (56 days) to analyse the physicochemical and microbiological properties and enzyme activity. Samples were collected in three replicates at three different points and mixed to represent the sample for each treatment.

Physicochemical analysis

Analysis of carbon (C) was measured using the potassium dichromate ($K_2Cr_2O_7$) volumetric method (Nelson and Sommers 1982), while nitrogen (N) was analysed using the Kjeldahl method, according to Bremmer (1996). The CN ratio was calculated from the two results, i.e., carbon over nitrogen content.

Moisture content (MC) was analysed using the MX-50 (AnD) moisture analyser. The samples were incinerated separately at 500°C for 3 hours in a furnace (Carbolite Gero) to determine the ash content (Jakobsen 1995), while the pH of the samples was determined using a pH metre (EC Thermo Electron Corporation Orion 3 Star). The samples were mixed with distilled water at the ratio of 1:10 (w:v ratio) and agitated for 30 min at 200 rpm on a rotary shaker before analysing with the pH meter. Electrical conductivity (EC) was measured using an EC meter (Eutech Instrument PC 700) in µS/cm unit, and the samples were mixed in a ratio of 1:10 (w:v) with distilled water.

Microbiological analysis

The microorganism density during the degradation process was determined using the standard dilution plate count technique, where 5 g of rice straw sample was suspended in 45 mL sterile distilled water. Serial dilutions of 10^4, 10^5 and 10^6 were made, and aliquots of each dilution were spread on either the nutrient agar (NA) or potato dextrose agar (PDA) using the pour plate technique.

Culturable bacteria were incubated on NA at 37 ± 2 °C for 24 hours, while the enumeration of fungi involved incubation on PDA at 27 ± 2 °C for 72 hours.

Enzyme assay

Sugar reducing assay was performed on the rice straw samples. About 5 g of rice straw samples were suspended in 45 mL of sterile distilled water and agitated for 15 minutes. Next, 1 mL aliquots were taken for the assay. In reducing the sugar assay, 3 mL of 3,5-dinitrosalicylic acid (DNS) was added to a test tube containing 1 mL of the sample, followed by the addition of 3 drops of 1 M NaOH. Then, the test tubes were boiled in distilled water at 100 °C for 5 minutes and color change was observed.

The test tubes were immediately cooled down to room temperature at the end of incubation. 20 mL of distilled water was added to each test tube. The samples were subjected to UV-spectrophotometric determination at 540 nm. 3 ml of DNS, 3 drops of NaOH and 20 ml of distilled water was used as blank.

Statistical analysis

Data were collected and subjected to statistical analysis using SAS version 9.4. Differences between treatments were compared using the LSD test at the significance level of $p < 0.05$.

Results and discussion

Total carbon (C), total nitrogen (N) and C/N ratio

Figures 1a and *1b* show that total C is the highest during the early weeks of all treatments and started to decline over time. *Figures 2a* and *2b* show that total N fluctuates over time for seasons 1 and 2. However, statistical analysis revealed no significant difference between treatments of total C and N for both seasons. Likewise, the C/N ratio (*Figures 3a* and *Figure 3b) a*nd percentage reduction (*Figures 4a* and *4b*) also fluctuated over time for seasons 1 and 2. However, the cumulative C/N reduction indicates a significant difference for season 1 (*p-value* = 0.0278) compared to between treatments, and the LSD revealed that T3 gave a significantly higher accumulative C/N reduction. The cumulative percentage reduction analysis approach in *Figures 5a* and *5b* provides an in-depth perspective of microbial application effects.

The results of the C/N ratio show high values at the start of the experiment (day 0) compared to the end (day 56) for all treatments during both seasons. A high C/N ratio in compost indicates the presence of unutilised complex N and C, whereas a complete breakdown of these materials is indicated by a low C/N ratio (Dobermann and Fairhurst 2002). In addition, straw has more soluble organic matter and a higher C/N ratio in the early stage of decomposition, and as it decomposes, the soluble matter and C/N ratio gradually decrease (Jin et al. 2020).

Generally, the mass reduction and nutrient release of rice straw were faster in the early stage of decomposition (0 − 14 days after decomposition) when easily utilised carbohydrates and amines were the preferred substrates for involved decomposers (Guo et al. 2018). Fluctuation in the C/N ratio values in *Figures 3a* and *3b* were also reported by Wang et al. (2020), who highlighted that the reason is due to the different release rates of total nitrogen (TN) and total organic carbon (TOC) in the field which were partly influenced by microorganism activities.

Several studies reported significant results when the treatments included additional factors or substrates that significantly influenced the C/N ratio of straw in the field. These were observed in the study by Lestari et al. (2020), whereby the treatments included increased water contents (50%, 100% and 150%) and microbial addition. The study revealed that the water factor influence was statistically significant than microbial addition. However, although not significant, there were similar positive trends of better reduction in the C/N ratio compared to non-inoculated straw when microorganisms were added at the straw moisture of 50%, which is comparable to the straw moisture of 50% to 80% in this study (*Table 2*).

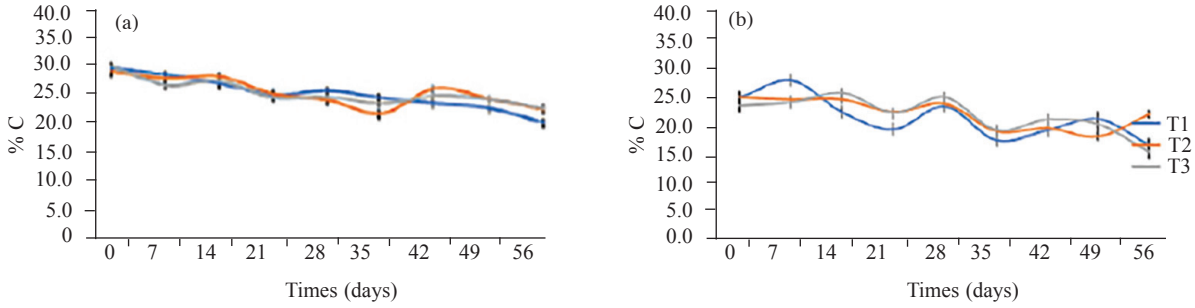

Figure 1. (a) Total C for season 1; (b) Total C for season 2, error bar is the standard error of the mean (SEM)

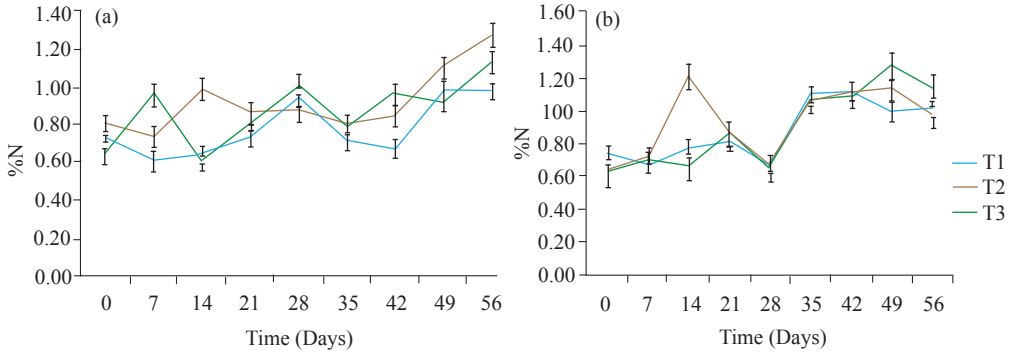

Figure 2. (a) Total N for season 1; (b) Total N for season 2, error bar indicates the standard error of the mean (SEM)

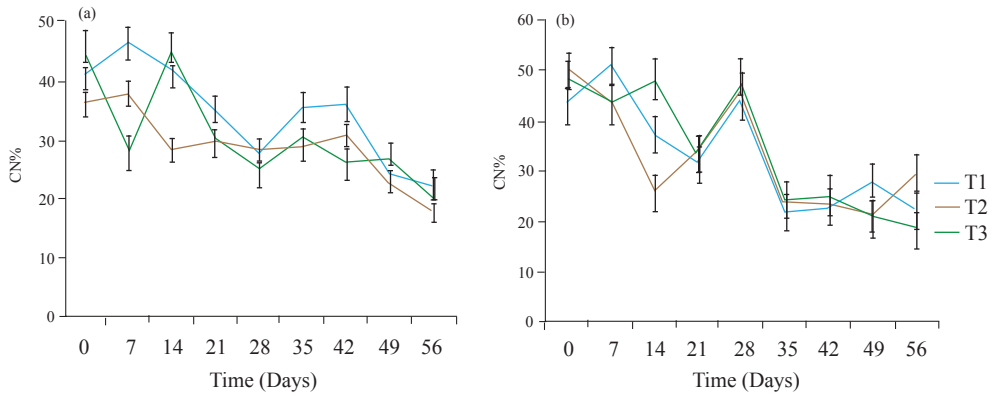

Figure 3. (a) CN ratio for season 1; (b) CN ratio for season 2, error bar is the standard error of the mean (SEM)

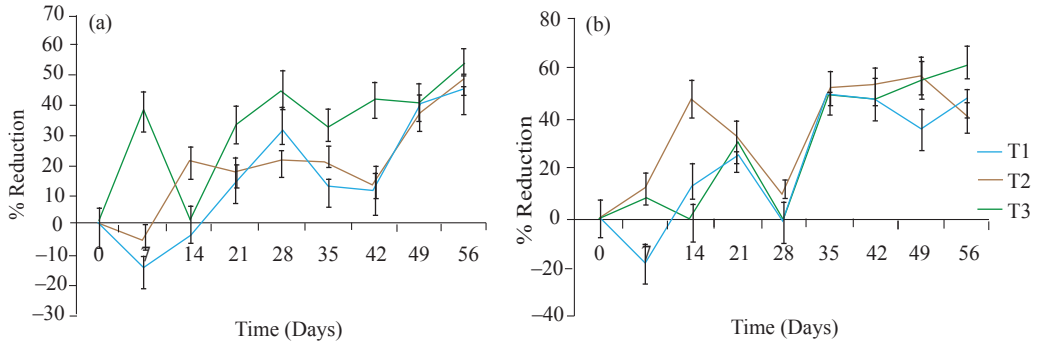

Figure 4. (a) Percentage of CN reduction for season 1; (b) Percentage of CN reduction for season 2, error bar is the standard error of the mean (SEM)

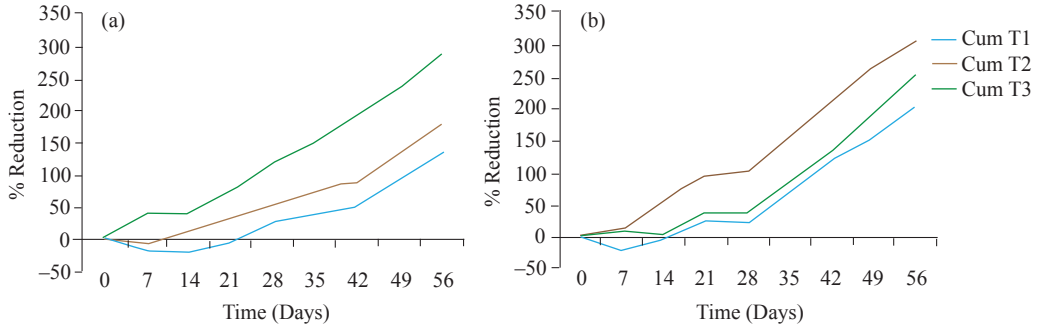

Figure 5. Cumulative data of CN reduction for (a) season 1; (b) season 2

pH, moisture content (MC) and electrical conductivity (EC)

Different microbes used will influence the pH range of the rice straw. Low pH readings are recorded at the start of the experiment (day 0) for all treatments in season 1 (pH range of 6.41 – 6.76) and season 2 (pH range of 5.74 – 5.97). The pH rose gradually for all treatments and T3 recorded the highest pH on day 7 in season 1 (pH 8.12) and T2 on day 7 in season 2 (pH 7.68), (*Table 3*). The pH rose gradually as the decomposition of materials sets in (Karanja et al. 2019). For season 2, the pH generally stabilised at around 7.32 – 7.56 at the end of the experiment (day 56). However, for season 1, the pH for T3 declined from 7.09 – 6.8 and slightly increased to 6.95 on day 56. The acidic condition is due to lactic acid bacteria as one of the components in the cellulose degrading bacterial mixture. According to Li et al. (2013), the pH value during the composting process varied within the range of 4.9 – 8.3, while the optimum pH should be within the range of 7 – 8 when the test on pH dependency of microbial activities was conducted using the liquid medium of proteins and glucose.

The effects of treatments on MC and EC were similar to the pH results. Based on *Tables 2* and *3*, the amount of MC in each treatment fluctuates. The lowest MC levels for control (T1), T2 and T3 in season 1 were recorded on day 0 at 58.82%, 51.08% and 53.18%. Season 2 also recorded the lowest MC levels on day 0 for all treatments at 54.79% (T1), 53.92% (T2), and 61.26% (T3). Meanwhile, the

Table 2. Weekly results for pH, moisture content (MC) and electrical conductivity (EC) for season 1

Days	pH			MC (%)			EC (µS)		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
0	6.76 ± 0.05	6.41 ± 0.11	6.56 ± 0.08	58.82 ± 1.84	51.08 ± 2.65	53.18 ± 2.21	1688.00 ± 138.49	1813.00 ± 137.79	468.89 ± 8.69
7	7.24 ± 0.00	8.11 ± 0.08	8.12 ± 0.09	64.35 ± 1.22	65.28 ± 1.07	60.09 ± 1.44	410.33 ± 3.47	663.00 ± 10.02	488.33 ± 10.85
14	7.11 ± 0.01	7.46 ± 0.01	7.51 ± 0.02	72.06 ± 0.37	73.37 ± 0.17	68.72 ± 0.48	580.00 ± 15.38	581.67 ± 0.98	731.33 ± 37.85
21	7.53 ± 0.04	7.76 ± 0.04	7.48 ± 0.02	81.50 ± 0.68	78.34 ± 0.38	73.77 ± 0.08	296.33 ± 16.14	353.67 ± 24.35	536.33 ± 16.18
28	7.44 ± 0.03	7.64 ± 0.03	7.88 ± 0.07	78.69 ± 0.37	81.54 ± 0.74	77.59 ± 0.50	393.33 ± 5.36	316.67 ± 28.46	546.67 ± 17.33
35	6.67 ± 0.06	7.83 ± 0.05	7.25 ± 0.00	82.10 ± 0.75	78.94 ± 0.45	78.11 ± 0.56	141.07 ± 33.39	545.00 ± 3.09	199.43 ± 21.25
42	7.22 ± 0.00	7.05 ± 0.04	7.09 ± 0.02	80.50 ± 0.57	80.28 ± 0.59	81.22 ± 0.91	148.77 ± 32.54	200.83 ± 41.33	232.60 ± 17.56
49	7.24 ± 0.00	7.02 ± 0.04	6.80 ± 0.05	80.32 ± 0.55	82.76 ± 0.87	81.21 ± 0..91	194.57 ± 27.45	161.80 ± 45.67	160.03 ± 25.63
56	7.55 ± 0.04	7.11 ± 0.03	6.95 ± 0.04	79.83 ± 0.50	82.72 ± 0.87	83.68 ± 1.18	121.97 ± 35.51	520.00 ± 5.87	152.53 ± 26.46

Table 3. Weekly results for pH, moisture content (MC) and electrical conductivity (EC) for season 2

Day	pH			MC (%)			EC (µS)		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
0	5.97±0.15	5.82±0.16	5.74±0.17	54.79±2.38	53.92±2.63	61.26±1.91	1587.00±102.42	2052.25±150.03	1530.75±96.58
7	7.63±0.03	7.68±0.04	7.64±0.04	75.40±0.09	78.70±0.12	78.19±0.03	920.50±28.37	1238.00±59.56	1237.25±63.97
14	7.67±0.04	7.59±0.03	7.58±0.04	77.19±0.11	76.27±0.15	79.90±0.16	843.25±19.79	643.25±6.53	888.50±25.22
21	7.33±0.00	7.29±0.00	7.67±0.05	80.77±0.50	75.27±0.26	79.80±0.15	363.75±33.49	462.00±26.67	593.25±7.58
28	7.61±0.03	7.28±0.00	7.38±0.01	73.11±0.35	77.03±0.06	80.39±0.21	915.75±27.84	547.00±17.22	644.25±1.92
35	7.62±0.03	7.45±0.02	7.47±0.02	79.43±0.36	84.63±0.78	83.95±0.61	288.18±41.89	280.50±46.83	256.00±45.06
42	7.11±0.03	7.48±0.02	7.26±0.00	81.73±0.61	86.25±0.96	80.98±0.28	189.15±52.89	454.75±27.47	312.73±38.75
49	7.64±0.03	7.66±0.04	7.24±0.00	83.73±0.83	82.80±0.58	81.35±0.32	520.25±16.10	490.75±23.47	278.50±42.56
56	7.56±0.02	7.37±0.01	7.32±0.01	79.87±0.40	83.45±0.65	80.26±0.20	358.78±34.04	149.48±61.39	212.38±49.90

highest MC levels for each treatment were recorded on day 35 for T1 (82.1%), day 49 for T2 (82.76%), and day 56 for T3 (83.68%) in season 1. In season 2, the highest MC levels were recorded on day 49 for T1 (83.73%), day 42 for T2 (86.25%) and day 35 for T3 (83.95%).

EC is a climate change mechanism that relates to water absorption by soil. EC readings were higher for T1 and T2 but lower for T3 at the beginning of the experiment in season 1. However, in season 2, the EC readings of all treatments were higher at the beginning of the experiment and decreased until day 21. Overall, on day 56, the EC readings were lower than on day 0 for all treatments in both seasons. Lower EC results show that the level of fertility is maintained and sufficient nutrient is available for the plant, which is paddy in this case (Klaassen 2020).

Microbiological activity

Figure 6 shows the bacterial and fungal population in degraded rice straw over 56 days. In season 1, the bacterial and fungal populations had similar growth patterns between treatments throughout the 56 days, statistically indicating that the treatments had similar effects on bacteria and fungi populations in degraded rice straw. The bacterial growth pattern for the three treatments was similar starting from day 7. However, the growth decreased to 9% after 35 days from the previous 16% on day 14 in T1. Meanwhile, after 35 days, the bacterial growth decreased to 21.15% in T2 and 11.54% in T3. Furthermore, T2 had a higher initial fungal count than T1 and T3, which had similar growth. The fungal count for T1 increased to 16.19% on day 7 and then decreased to 24.22% on day 21. In comparison, T2 and T3 displayed a decline in fungal growth on day 56 by 10.4% and 31.63%. Figure 7 depicts that the bacterial growth for T1 increased by 5.8% in season 2 on day 7 and then declined after day 49. However, T2 maintained consistent bacterial growth throughout the experiment until day 56. Likewise, the growth for T3 increased by 5.6% after 7 days and became constant until day 56. Meanwhile, the fungal growth of T1 and T3 increased by 12.09% and 8.8% on day 7 and remained constant until day 56. However, T2 recorded no significant increase in fungal growth, which was maintained until day 56. Both fungal and bacterial growth plateaued after day 14.

Fungi and bacteria found in cultured rice straws may have a complex microbial community that is necessary for the degradation of rice straw.

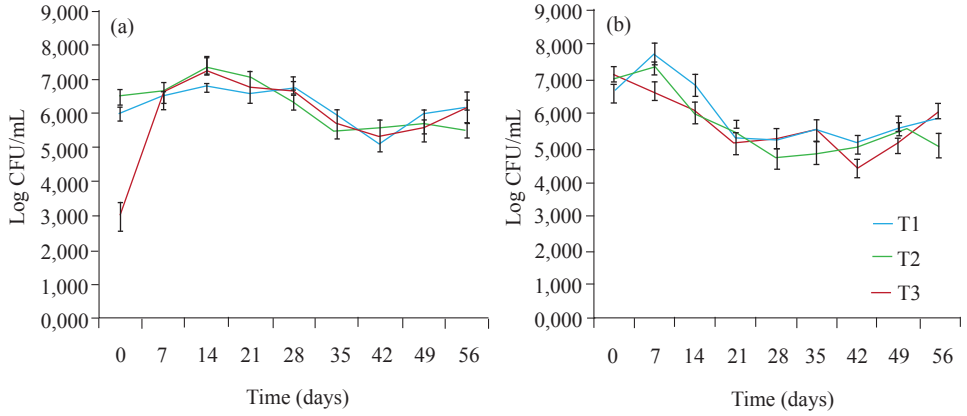

Figure 6. a) Bacteria population of degraded rice straw on nutrient agar plate and b) fungi population of degraded rice straw on potato dextrose agar plate in season 1 where T1 is the control, T2 is rice straw treated with Trichoderma sp., and T3 is rice straw treated with cellulose degrading bacteria. Error bar: standard error of the mean (SEM)

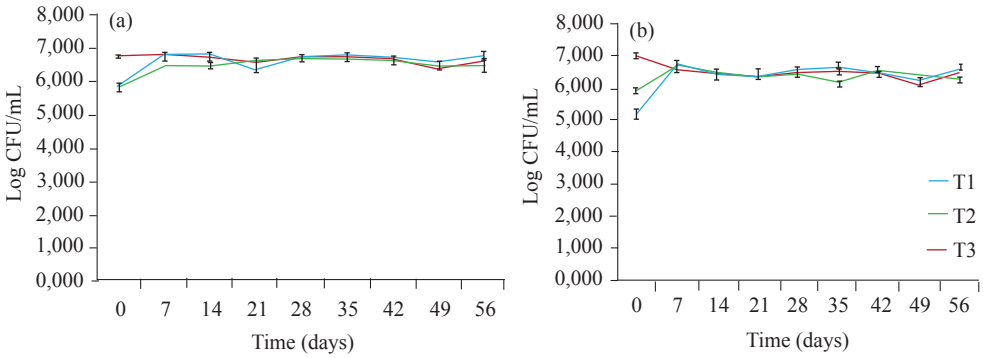

Figure 7. a) Bacteria population of degraded rice straw on nutrient agar plate and b) fungi population of degraded rice straw on potato dextrose agar plate in season 2 where T1 is the control, T2 is rice straw treated with Trichoderma sp. and T3 is rice straw treated with cellulose

Enzyme assay activity

Enzyme assays are conducted to identify and prove the presence and absence of an enzyme in a specimen and to determine the amount of enzyme in samples (Bisswanger 2014). In this study, the sugar-reducing assay was performed to detect the amount of sugar released in the samples. Reducing sugar is any sugar capable of acting as a reducing agent due to its free aldehyde or free ketone groups. All monosaccharides, i.e., galactose, glucose, and fructose, are reducing sugars. This study used the DNS method, i.e., a colorimetric technique involving a redox reaction between 3,5-dinitrosalicylic acid and the reducing sugar present in the samples (Marsden et al. 2007).

Rice straw, lignocellulosic biomass, comprises three components: lignin, cellulose, and hemicelluloses. The fiber organics are made up of cellulose and hemicelluloses, whereas the cell wall is composed of lignin (Klass 1998). The sugar-reducing assay is used to identify the amount of glucose digested during the degradation process. Hypothetically, a high sugar-reducing value indicates rapid degradation of rice straw.

The highest sugar-reducing concentration in season 1 is 0.354 g/L (T2) and 0.782 g/L (T3) for season 2 (*Tables 4* and *5*) on day 56. Statistically, the treatments had no significant effect on DNS in both seasons. However, T3 recorded slight changes where the sugar-reducing activity increased after day 42 in season 2. Meanwhile, the activities for T1 and T2 are similar throughout the period. The reducing sugar concentration for T2 remained constant from day 21 until day 56. The standard error of the mean (SEM) was calculated using the overall standard deviation. Although the treatments were not significant in reducing the sugar released, T2 recorded the highest total reducing sugar from the cumulative calculation (*Figure 8*) throughout the entire experiment for both seasons, indicating that it is a suitable choice for the next large-scale experiment for rapid rice straw degradation.

Table 4. Sugar reducing activity of rice straw in 56 days for season 1

	Sugar reducing concentration (g/L)		
Days	T1	T2	T3
0	0.247	0.330	0.306
7	0.086	0.168	0.159
14	0.209	0.328	0.182
21	0.304	0.284	0.164
28	0.205	0.162	0.215
35	0.197	0.329	0.259
42	0.270	0.256	0.261
49	0.169	0.167	0.129
56	0.333	0.354	0.255
SEM	0.025	0.026	0.020

Table 5. Sugar reducing activity of rice straw in 56 days for season 2

	Sugar reducing concentration (g/L)		
Days	T1	T2	T3
0	0.385	0.693	0.632
7	0.381	0.389	0.341
14	0.626	0.642	0.365
21	0.450	0.522	0.423
28	0.397	0.466	0.711
35	0.496	0.507	0.665
42	0.425	0.522	0.404
49	0.524	0.633	0.473
56	0.401	0.451	0.782
SEM	0.027	0.033	0.055

*SEM is the calculated standard error of the mean using the overall standard deviation

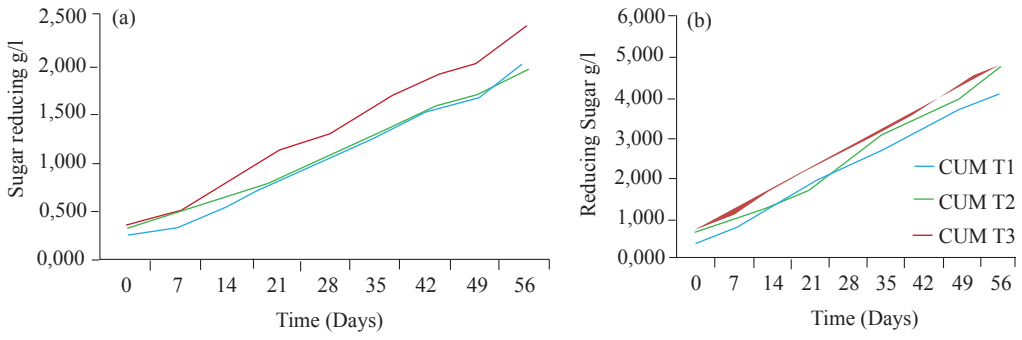

Figure 8. Cumulative DNS data for three treatments for a) season 1 and b) season 2

Conclusion

Based on the study, increasing reducing sugar concentration throughout the experiment, especially for T2 (rice straw treated with *Trichoderma* sp. to break down the cellulose and hemicellulose), helped increase rice straw decomposition. Besides, rice straw treated with the cellulose degrader bacteria (T3) also has a higher reducing sugar concentration than the control treatment (T1) for rice straw decomposition. It indicates that the potential use of microbes can enhance rice straw degradation in soil.

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