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Research Article

Agricultural bio-waste recycling through efficient microbial consortia

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Abstract
In India and other countries, rice straw, a byproduct of rice production, is burned in enormous amounts, which contributes to environmental pollution and climate change by releasing greenhouse gases *viz.,* CO_2, N_2O, CH_4, into the atmosphere. This study aimed to accelerate the degradation of this enormous amount of agricultural biomass via microbial inoculants. Four treatments—rice straw (RS), rice straw plus water (RSW), rice straw plus water plus Pusa decomposer (RSWF), and rice straw plus water plus Tamil Nadu Agricultural University (TNAU) biomineralizer (RSWB) were used in the current investigation. The study's findings demonstrated that rice straw treated with microorganisms decomposed more quickly than RS and RSW treatments. According to EDAX spectra of elemental composition, the carbon content of rice straw in the RS, RSW, RSWF, and RSWB treatments was 33.66%, 29.75%, 13.33%, and 20.65% w/w, respectively. The RSWF treatment of rice straw was found to have the highest nitrogen concentration (0.64% w/w), followed by RSWB (0.61% w/w), RSW (0.45%) w/w, and RS (0.43% w/w). Treatments RSWF and RSWB had lower C/N ratios 20.83, and 33.85, respectively, than that RSW (66.11) and RS (78.28). The RSWF and RSWB treatments' porous, distorted, and rough surface structures provided further evidence that both microbial consortia could decompose rice straw more quickly than the RSW and RS treatments. Therefore, the results of this study imply that rice straw could be added to the soil to improve soil fertility for sustainable crop production rather than being burned.

Keywords: Agricultural bio-waste, Decomposition, Microbial consortia, Recycling, Rice straw

INTRODUCTION

Rice (*Oryza sativa* L.) is the second most important cereal crop cultivated in the world. Globally, in the year 2021-22 about 512.86 million metric tons of milled rice was produced from an area of 166.58 million hectares. With 129 million metric tons of rice production, India contributes about one-fourth of total world rice production (USDA, 2022). Along with rice, about 731 million tonnes of rice straw are produced globally, of which around 126.6 million tonnes are produced in India (Bhattacharyya *et al.,* 2021). This huge amount of rice straw is used for multiple purposes such as animal feed, medium for mushroom production, raw material for pulp and paper industries, production of bio-ethanol, bio-fuels, butanol production, biochar production, and packaging material; moreover, most of the straw is either burnt or left in the field (Moradi *et al.,* 2013; Phitsuwan *et al.,* 2017; Huang *et al.,* 2019; El-Hassanin *et al.,* 2020; Goodman, 2020; Chollakup *et al.,* 2021; Rusdy, 2022). About 60 percent of the total rice straw produced in India is burnt (Bhattacharya *et al.,* 2021). Burning of the rice straw causes environmental pollution across the transboundary by producing smoke and is also responsible for global warming by emitting CO_2, CH_4, and greenhouse gas into the atmosphere (Singh *et al.,* 2021; Junpen *et al.,* 2018). Apart from that, there is a huge loss of nutrients and organic carbon, according to estimation 1 tonne of rice straw burning accounts for a loss of 5.5 kg nitrogen, 2.3 kg phosphorous, 25 kg potassium, and 1.2 kg sulfur and also carbon in the soil (Kedia *et al.,* 2020).

Another nonscientific practice, which is very common in rice-growing countries, including India, is leaving the straw on the surface of the fields. This might be due to non-availability and higher cost of labour, bad weather prevailing during harvest and non-availability of sufficient storage space, which takes longer to decompose. Scientists have reported that the decomposition of rice straw in puddled fields emits potential greenhouse gases CH_4, N_2O and CO_2, the longer the decomposition process, the more the greenhouse emission. The incorporation of rice straw into the soil has positive and negative impacts. The positive impacts are that it improves the physical, chemical, and biological properties of soil. Rice straw incorporation into the soil may reduce the application of chemical fertilizers, as it contains about 80% potassium, 40% nitrogen, and 30% phosphorus, taken up by the rice crop. The negative impacts include, increases C/N ratio of soil and emitting greenhouse gas (Chivenge *et al.,* 2020). The decomposition of rice straw takes longer in normal conditions, which can be accelerated by microbial inoculation. Scientists have used single species and microbial consortia to degrade biomass at an accelerated rate. Yang *et al.* (2004) have reported that microbial consortium has an advantage over a single culture where it helps to grow in agriculture residues that are low in nutrients and protects the culture from contamination. Considering the above, the present study was undertaken with two commercially available microbial consortia, namely Tamil Nadu Agricultural University (TNAU) biomineralizer and Pusa decomposer, to decompose rice straw.

MATERIALS AND METHODS

Description of the experimental site

This study was carried out at Tamil Nadu Rice Research Institute, Aduthurai, located in the Cauvery Delta Zone of Tamil Nadu, India. The Institute lies between 11°00' N and 79° 28'E at 19.5m above mean sea level and receives an average annual rainfall of 1150 – 1250 mm, mainly during the northeast monsoon

Straw preparation

Rice straw was collected from the fields of Tamil Nadu Rice Research Institute, Aduthurai, Tamil Nadu, India. The straw was dried in the air and chopped into small pieces (1–2 cm). Subsequently, it was dried in a hot air oven at 60°C till a constant weight of rice straw was achieved.

Treatment details

The experiment was conducted in pots with 22 cm top diameter, 10 cm bottom diameter, and 20 cm height under glasshouse conditions. Pots were washed in running water and sun-dried. In each pot, 200 g of oven-dried rice straw was taken, moistened with a water sprinkler, and treated with respective decomposers and all the pots except untreated rice straw (S) were kept wet (60% water holding capacity) throughout the experimental duration by spraying the water frequently. The treatment consisted of Rice straw (RS), Rice straw plus water (RSW), Rice straw plus water plus Pusa decomposer (RSWF), and Rice straw plus water plus TNAU biomineralizer (RSWB).

Procurement and preparation of Pusa decomposer culture

Pusa decomposer capsules were procured from the Division of Microbiology, ICAR – Indian Agricultural Research Institute (IARI), Pusa, New Delhi, India (Fig. 1a). For preparing culture, 150 grams of old jaggery was boiled in 5 liters of water and the dirt that appeared on the surface of the boiling water was removed by sieving the solution. The jaggery solution was cooled at room temperature and about 50 g. of chickpea (*Cicer aritinum* L.) flour was mixed with it. After mixing chickpea flour well, four capsules of Pusa decomposer were cut open, poured, and mixed well in jaggery solution with a wooden stick. Then the solution was transferred to a plastic tray, covered with a thin cloth, and placed in a warm place (Fig. 1b). The solution was allowed for one week and after the formation of a layer on the surface of the solution, it was mixed well and applied to the respective treatment. The required quantity of culture was calculated for individual pot at the rate of 5 liters/tones of rice straw.

Procurement and preparation of TNAU biomineralizer culture

A pack of 2 kg TNAU biomineralizer was procured from the Department of Environmental Sciences of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. For treatment imposition, the slurry was made by adding water at the rate of 20 liters per 2 kg biomineralizer and inoculating the respective treatment at the rate of 2 kg/tone of rice straw.

Fig. 1. *(a) Pack containing four capsules of Pusa decomposer, (b) Culture prepared from four capsules of Pusa decomposer*

Scanning electron microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDAX) analysis

Samples were collected on the 30th day of decomposition from all the treated pots, dried in a hot air oven at 60°C till the constant weight was achieved, and kept in airtight packs for further analysis. The SEM-EDAX analysis was performed at Computational Modeling and Nanoscale Processing Unit, National Institute of Food Technology, Entrepreneurship, and Management (NIFTEM), formerly (IIFPT), Thanjavur, Tamil Nadu, India. The surface structure of rice straw was analyzed for all the samples by VEGA 3 TESCAN scanning electron microscope (SEM) and the elemental composition was detected by AMETEK EDAX attached to the scanning electron microscope. Sample imaging was done by scanning electron microscope (SEM) with an acceleration voltage of 3 - 5 kV and at a working distance of 10–17 mm. SEM micrographs and elemental composition were taken from various areas with different magnifications to assess the influence of different treatments on rice straw decomposition.

Chemical analysis

Nitrogen content in rice straw samples was not detected by SEM-EDAX; therefore, it was estimated by the Micro-Kjeldahl method as per the procedure suggested by Association of Official Analytical Chemists (AOAC, 2006). For all the treatments, the nitrogen analysis was performed thrice and the mean value arrived was considered nitrogen content in the respective treatment. The C/N ratio was determined by dividing the carbon content recorded in EDAX analysis by the nitrogen content recorded in the chemical analysis.

RESULTS AND DISCUSSION

SEM analysis

SEM analysis showed noticeable changes in the morphology and surface structure of all rice straw samples under investigation. The surface structure of untreated rice straw (RS) was smooth, intact, and rigid (Fig. 2a). This result was in accordance with Xu *et al.* (2015); Phutela and Sahni (2013), who reported a flat surface and intact, rigid, and compact structure of microbial untreated rice straw. The treatment RSW exhibited damage initiation of the outer parenchyma layer (Fig. 2b). Among all treatments, RSWF treatment exhibited a disrupted and more porous surface structure (Fig. 2c). Similarly Stella and Emmyrafedziawati (2015) also showed disrupted surface structure in SEM micrograph wherein the rice straw was treated with bacterial consortium isolated from the soil. Phutela and Sahni, (2013) reported opened holo-cellulose and separated fibrils, increased external surface area and porosity in rice pretreated with lignocellulolytic fungi *Trichoderma reesei* MTCC 164 and *Coriolus versicolor* MTCC 138. Degradation of lignin and enhanced straw digestibility was reported in *Pleurotus florida* fungi pretreated rice straw (Kaur and Phutela, 2018). Treatment RSWB stands next to RSWF treatment in the ability to decompose rice straw. Rice straw treated with TNAU biomineralizer (RSWB) showed a relatively more rough structure, disappeared outer parenchyma tissue, and exposed inner parenchyma tissue compared to water-treated (RSW) and untreated control rice straw (RS) that showed the ability of the microorganism to penetrate the outer lignin layer (Fig. 2d). This finding was in accordance with Xu *et al.* (2015), wherein disappeared outer parenchyma tissue and exposed inner parenchyma tissue were reported 12 days after microbial inoculation. The findings of this study were also in accordance with Amido *et al.* (2021), who reported the disrupted and extensive perforated surface structure of rice straw treated with *Pleurotus florida*.

EDAX analysis

Elemental compositions of rice straw analyzed by Energy Dispersive X-ray Analysis (EDAX) have been en-

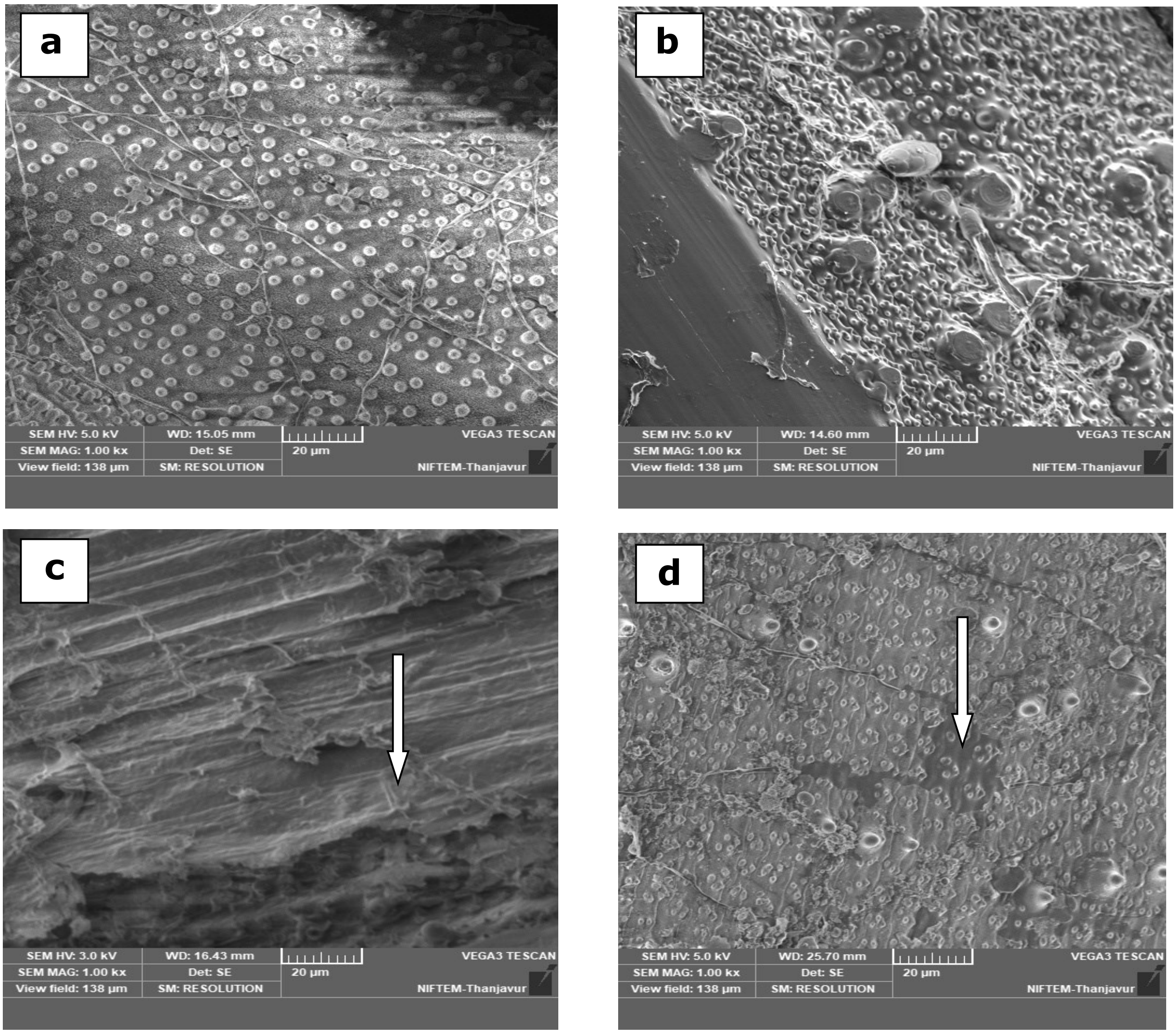

Fig. 2. *SEM micrograph on 30th day of rice straw decomposition, (a) Smooth and organized surface structure of un-treated rice straw (RS), (b) Disappeared outer waxy layer of rice straw + water (RSW) treatment, (c) Distorted, rough, and porous surface structure of rice straw + water + Pusa decomposer (RSWF) treatment and (d) Damaged outer layer and exposed inner layer of rice straw + water + TNAU biomineralizer (RSWB) treatment*

Table 1. Elemental composition of rice straw detected by EDAX on the 30th day of decomposition

S. No.	Elements	Rice straw (RS)		Rice straw + water (RSW)		Rice straw + water + Pusa decomposer (RSWF)		Rice straw + water + TNAU biomineralizer (RSWB)	
		Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %
1	C K	33.66	47.73	29.75	43.94	13.33	21.61	20.65	31.97
2	Ti L	-	-	10.73	3.97	-	-	-	-
3	O K	36.86	39.25	40.06	44.41	46.54	56.65	42.50	49.38
4	Tb M	-	-	2.21	0.25	2.04	0.25	2.55	0.30
5	Al K	-	-	-	-	-	-	0.56	0.38
6	Si K	19.23	11.66	10.85	6.85	29.81	20.67	26.06	17.25
7	Au M	9.04	0.78	6.41	0.58	8.27	0.82	7.68	0.73
8	Cl K	1.22	0.59	-	-	-	-	-	-

listed in Table 1 and presented by various EDAX spectra peaks (Fig. 3). In all rice straw samples, carbon, oxygen, and silica were reported as dominant elements; however, their relative proportion differed in each sample. The above dominant elements were reported in order of 29.75%, 13.33%, and 20.65% carbon; 40.06%, 46.54%, and 42.50%for oxygen; and 10.85%, 29.81%, and 26.06% for silica on a weight basis for treatment RSW, RSWF, and RSWB, respectively against carbon (33.66%), oxygen (36.86%) and silica (19.23%) weight basis in untreated rice straw (RS). There were about 3.91%, 20.33%, and 13.01% reductions in carbon content in treatments RSW, RSWF, and RSWB**,** respectively, compared to untreated control RS. At the same time, the oxygen content was found to have increased by 3.2%, 9.68%, and 5.64% in RSW, RSWF, and RSWB treatments respectively. The silica content was reduced by 8.38% in RSW treatment, whereas it was reported to have increased by 10.58% and 6.83% in RSWF and RSWB treatments**,** respectively compared to untreated control (RS).

Rice straw comprises mainly three lignocellulosic biopolymers: cellulose, hemicelluloses, lignin, and a considerable amount of silica (Sindhu *et al.,* 2011; Phitsuwan *et al.,* 2017). Carbon and oxygen are the main structural elements in these lignocellulosic components of rice straw (Boundzanga *et al.,* 2020). Microorganisms use carbon as the main source of their energy to build up cells (Al-Dhabaan, 2021; Dash *et al.,* 2021). The reduction in carbon content in microbial-inoculated RSWF and RSWB rice straw might be due to microorganisms' utilization of carbon as an energy source. Jusoh *et al.* (2013) reported similar results, in which microorganism-treated rice straw contained 36% total organic carbon compared to untreated control (49%). The results of the present study were also in accordance with the findings of Dash *et al.* (2021), who reported 31.8 to 36.1% carbon content in rice straw treated with bacterial and fungal consortia compared to untreated control rice straw with 45.6 to 52.6% carbon content on 30th day of decomposition in a pot experiment.

Nitrogen content and C/N ratio

Chemical analysis of rice straw samples revealed the increased N content and narrowed C/N ratio in microbial-treated rice straw samples on the 30th day of decomposition (Table 2). The highest N content of 0.64% w/w was reported in treatment RSWF and was followed by RSWB (0.61%) w/w, RSW (0.45%) w/w and RS control (0.43%) w/w. Similarly, the lowest C/N ratio of 20.83 and 33.85 was reported in treatment RSWF and RSWB, respectively, followed by RSW (66.11), where-

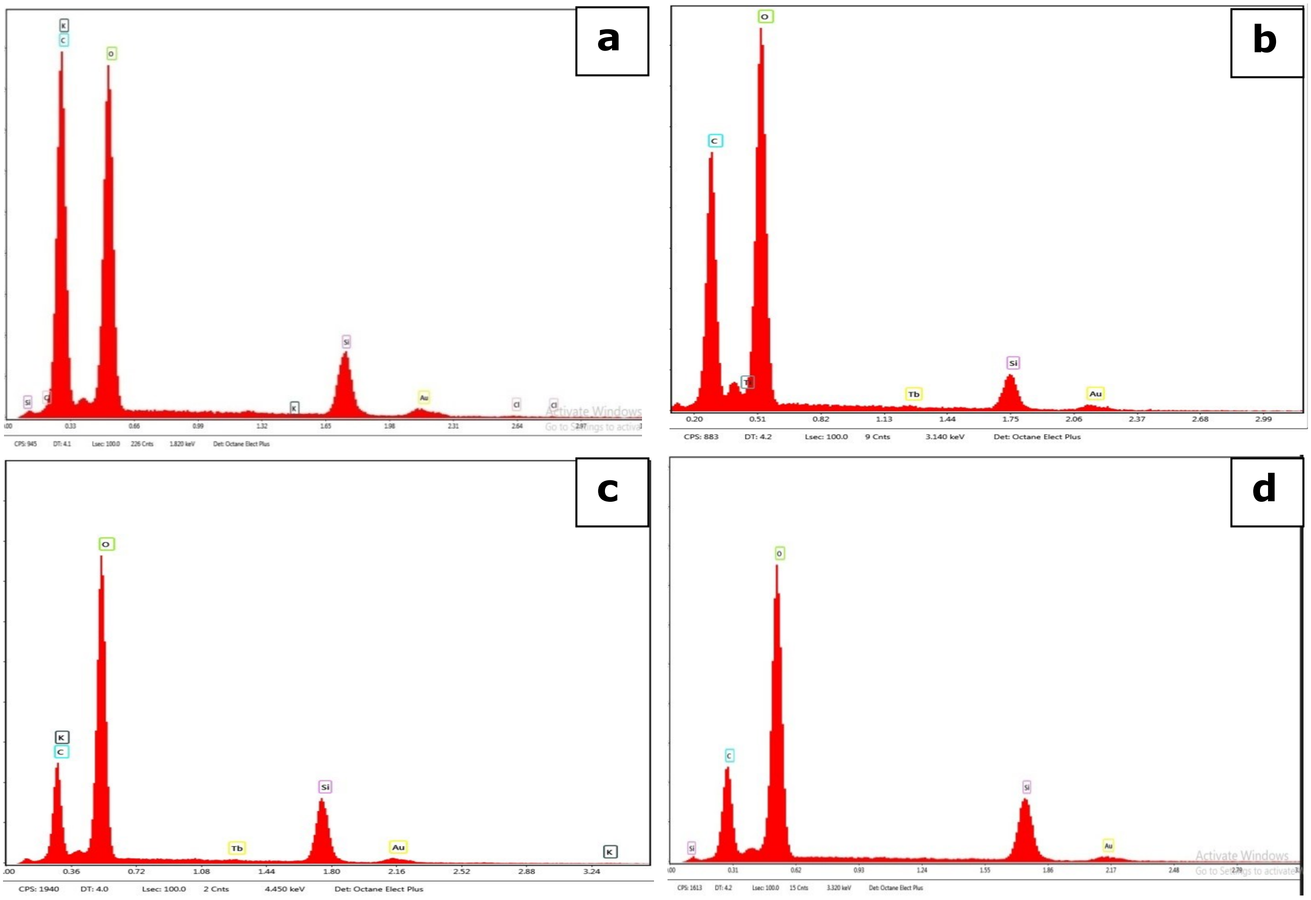

Fig. 3. *EDAX spectra of (a) Rice straw (RS), (b) Rice straw + water (RSW), (c) Rice straw + water + Pusa decomposer culture (RSWF), and (d) Rice straw + water + TNAU biomineralizer (RSWB)*

Table 2. Nitrogen content and C/N ratio of rice straw on the 30th day of decomposition

S. No.	Parameters	Rice straw (S)	Rice straw + water (SW)	Rice straw + water + Pusa decomposer (SF)	Rice straw + water + TNAU biomineralizer (SB)
1	N	0.43	0.45	0.64	0.61
2	C/N ratio#	78.28	66.11	20.83	33.85

Ratio of total carbon reported in EDAX analysis to total nitrogen content

as untreated rice straw (RS) reported the widest C/N ratio of 78.28.

The C/N ratio of biomass is an important determining parameter for its degradation (Sharma *et al.,* 2014; Goyal and Sindhu, 2011). Goyal and Sindhu (2011) reported an increased nitrogen content from 0.71 to 1.38% and decreased C/N ratio from 73.7 to 27.5 of composted rice straw in 30 days, inoculated with the fungal consortia containing three fungi, namely *Aspergillus awamori, Paecilomyces fusisporus and Trichoderma viride*. Aye *et al.* (2016) also reported increased nitrogen content (1.52 %) in effective microorganisms-treated rice straw compared to 0.92 % N content in untreated rice straw. In the present study, the decrease in the C/N ratio of microorganism-treated RSWF and RSWB rice straw might be due to the consumption of carbon for their energy. This result was in confirmation by the finding of Jusoh *et al.* (2013), who reported decreased total organic carbon (TOC) and C/N ratio in rice straw treated with commercially available effective microorganism (EM) consortia which contain lactic acid bacteria, yeast, and phototrophic bacteria. Al-Dhabaan (2021) reported that the rice straw treated with *Streptomyces Tendae* recorded decreased C/N ratio from an initial 30.4 to 17.2 within 42 days of the decomposition process.

Conclusion

In the present study, the two microbial consortia, namely Pusa decomposer and TNAU biomineralizer accelerated the decomposition process of rice straw. SEM micrograph and EDAX spectra of the elemental composition of microbial-treated rice straw showed lower carbon content and porous, rough, and disturbed surface structure. Rice straws treated with microorganisms also reported a higher nitrogen content and lower C/N ratio. Therefore, it can be concluded that the degradation of rice via both microbial consortia is a better alternative to straw burning. Future research may be focused on the collective effect of both the microbial consortia on the degradation of rice straw biomass.

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Conflict of interest

The authors declare that they have no conflict of interest.

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