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EFFICIENCY OF MICROBIAL CONSORTIUM IN DEGRADING RICE STRAW UNDER SOLID STATE FERMENTATION

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Rice (*Oryza sativa* L.) is one of the most widely cultivated and consumed crops globally. India holds a significant position in global rice production, contributing approximately 19.5% to 24.5% of the total worldwide output. In terms of crop residue, the rice straw-to-grain ratio is approximately 1:1.4 (IPCC, 1996). Rice straw is primarily composed of cellulose, hemicellulose, lignin, and a considerable amount of silica (Rashad & Hussien, 2013). Traditionally, farmers have resorted to the practice of on-field burning of rice straw as a cost-effective means of preparing fields for subsequent crops. However, the incomplete combustion of rice straw during this process leads to a range of environmental concerns, including air quality degradation, soil nutrient depletion, and the emission of greenhouse gases (Rosmiza *et al.*, 2014). These practices not only pose risks to environmental sustainability but also compromise soil fertility and contribute to climate change. Therefore, there is an urgent need to develop more sustainable and environmentally friendly methods for managing rice straw that do not compromise human health or ecological integrity.

Lignocellulosic materials, such as rice straw, which are considered second-generation feedstocks, have not yet proven to be cost-effective for bioethanol production. This is primarily due to the crystalline structure of cellulose fibrils, which are encased by hemicellulose and a lignin "seal," making it difficult for degrading enzymes to access the cellulosic core (Gong *et al.*, 1999). To improve enzymatic digestibility, lignocellulosic biomass typically undergoes various physicochemical pretreatment methods. However, these approaches are not only costly but also generate inhibitors that hinder both the hydrolysis and fermentation processes. Consequently, there is a critical need to develop alternative strategies that circumvent the limitations of conventional physicochemical pretreatment methods, thereby enhancing the efficiency and cost-effectiveness of bioethanol production.

Solid-state fermentation (SSF) refers to the growth of microorganisms on moist solid substrates in the absence, or near absence, of free water (Lonsane *et al.*, 1985). This method has been widely employed for the production of enzymes (Cen & Xia, 1999). Paddy straw, primarily composed of cellulose (36%), hemicellulose (24%), and lignin (15%), presents a valuable yet challenging substrate for microbial degradation, as it can be broken down by microorganisms producing lignocellulolytic enzymes. Several microorganisms, including *Candida tropicalis*, *Lactobacillus* spp., *Streptomyces globisporus*, *Phanerochaete chrysosporium*, and the Pusa compost inoculum, have demonstrated effective and rapid decomposition of paddy straw (Sharma *et al.*, 2014). In addition, a microbial consortium consisting of *Aspergillus* spp., *Bacillus* spp., and *Streptomyces* spp. has also been shown to effectively degrade paddy straw, as reported by Shrivastava *et al.* (2018). These findings highlight the potential of utilizing microbial degradation through SSF as a promising strategy for the valorization of paddy straw, transforming it into valuable products such as bioethanol or other bio-based chemicals.

Biological delignification, utilizing fungi, bacteria, actinobacteria, and the lignolytic enzymes they produce, has recently gained significant attention as an environmentally sustainable alternative to traditional chemical methods. This approach offers the advantage of improving enzymatic hydrolysis and fermentation processes without generating harmful chemical inhibitors. The use of microbial consortia, rather than individual microbial cultures, is particularly advantageous due to the possible synergistic interactions between the different metabolic pathways of the strains involved. Such synergies can enhance the efficiency of the delignification process, accelerating lignin degradation more effectively than the action of single microbial species (Kato *et al.*, 2005).

For the solid-state fermentation (SSF) process, a microbial consortium was employed, consisting of two isolated bacterial cultures, one actinobacterial culture, one fungal culture, and three standard strains previously isolated in our laboratory (Kaur & Katyal, 2020), namely *Bacillus* sp., *Pseudomonas* sp., and *Delftia* sp. The consortium was applied to both untreated rice straw and rice straw pretreated with 5% urea, with the aim of evaluating its effectiveness in degrading rice straw under SSF conditions.

The rice straw (variety PR121) used in this study was sourced from the Agronomy field of Punjab

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Agricultural University (PAU), Ludhiana. The straw was manually cut into 3-4 cm pieces to facilitate processing. Additionally, a paddy straw-based compost sample was collected from the Mushroom Research Complex at PAU Ludhiana for the isolation of lignocellulolytic microbes.

Serially diluted samples of paddy straw-based compost were pour-plated on Paddy Straw Agar (PSA) medium, with the following composition (g/100 mL): Potassium dihydrogen phosphate (KH_2PO_4) - 0.1, Ammonium sulfate ($(NH_4)_2SO_4$) - 0.5, Magnesium sulfate ($MgSO_4$) - 0.5, Yeast extract - 0.1, paddy straw - 5, and agar - 2. The plates were then incubated at 35±2°C for 2-3 days to allow the growth of bacterial colonies and for 3-5 days to allow fungal colonies to develop. The plates were regularly monitored for the appearance of bacterial and fungal colonies, which were subsequently sub-cultured to obtain pure isolates. These pure cultures were preserved at 4°C on PSA slants for further use.

The bacterial and fungal isolates were qualitatively assessed for their lignocellulolytic potential using minimal media broth composed of 0.1% KH_2PO_4, 0.5% $(NH_4)_2SO_4$, 0.5% $MgSO_4$, and 0.1% yeast extract. Various carbon sources (substrates) were incorporated into this medium to investigate the enzymatic activities of the isolates. For cellulase activity, the medium was supplemented with 10% Carboxymethyl Cellulose (CMC) as the substrate. Similarly, xylan was added for the assessment of hemicellulase activity, while laccase activity was evaluated using guaiacol and Remazol Brilliant Blue as substrates. Qualitative enzyme activity was determined using the agar plate assay method (Okino *et al.*, 2000; Mtui and Masalu, 2008; Pointing, 2001; Machado *et al.*, 2005). Prior to autoclaving at 121°C for 20 minutes, the medium was solidified with 2-2.5% (w/v) agar. Depending on the specific enzyme being tested, the corresponding dye was added: 1% Congo Red for cellulase, 0.15% iodine for hemicellulase, and 0.01% Remazol Brilliant Blue for laccase. Enzyme activity was visually assessed by the formation of clear zones around the microbial colonies, with the potency index calculated as the ratio of the clearance zone diameter (mm^2) to the colony size (mm^2) on the respective agar plates.

Based on the results of the qualitative analysis, two bacterial isolates (B4 and B6), an actinobacterium (A1), and a fungal isolate (F1), along with three standard bacterial cultures (*Bacillus*, *Delftia*, and *Pseudomonas*), were selected as potential lignocellulolytic cultures. Following a compatibility test, a microbial consortium comprising these potential isolates and the standard bacterial cultures was established for the quantitative analysis of enzyme activity. The production of extracellular lignocellulolytic enzymes was evaluated under solid-state fermentation conditions, using untreated and urea-pretreated rice straw as the substrate. Enzyme production was carried out in 500 mL flasks containing 25 g of rice straw, moistened with 100 mL of minimal medium, and sterilized at 121°C for 20 minutes. Each flask was inoculated with a 1% (v/v) inoculum of the developed consortium and incubated at 30 ± 2°C in a BOD incubator. Enzyme activity was monitored every seventh day over a 30-day period to determine the optimal production time. After incubation, the residue was filtered through muslin cloth, and the filtrate was centrifuged at 10,000 rpm for 10 minutes. The resulting supernatant was stored at 4°C in a sterilized glass bottle and used as the crude enzyme extract for the assessment of lignocellulolytic activity using standard enzymatic assays. For cellulase activity, the endoglucanase (EC 3.2.1.4) and exoglucanase (EC 3.2.1.91) activities were measured according to the method described by Mandels (1975), while β-glucosidase (EC 3.2.1.21) activity was determined following the procedure outlined by Toyama and Ogawa (1977). One unit of cellulase activity was defined as the amount of glucose (in µmol) produced per minute per milliliter of crude enzyme extract.

For hemicellulase activity, xylanase (EC 3.2.1.8) was quantified following the method described by Singh *et al.* (2000). One unit of xylanase activity was defined as the amount of xylose (in µmol) released per minute per milliliter of crude enzyme extract. Laccase (EC 1.10.3.2) activity was measured using guaiacol as a substrate, as outlined by Desai *et al.* (2011). One unit of laccase activity was defined as an increase in optical density (OD) of 0.001 per minute under the assay conditions. Manganese peroxidase (MnP, EC 1.11.1.13) activity was determined by measuring guaiacol oxidation in the presence of $MnSO_4$, following the procedure of Paszczynski *et al.* (1988). One unit of MnP activity was defined as the amount of enzyme required to oxidize 1 µmol of guaiacol per minute under the assay conditions. One unit of Laccase enzyme was defined as an increase in optical density of 0.001 in 1 min.

Samples of rice straw were analyzed for Acid Detergent Fibre (ADF) and Neutral Detergent Fibre (NDF) to assess the extent of biodegradation during microbial treatment. On day 28, the cellulose, hemicellulose, and lignin contents of the rice straw were determined using proximate analysis as described by Van Soest (1963). ADF consists primarily of cellulose and lignin, while NDF includes cellulose, hemicellulose, and lignin. Therefore, the difference between NDF and ADF values provides an estimate of the hemicellulose content in the rice straw. In addition, the total solids, volatile solids, ash content, and total organic carbon

were measured for both untreated and pretreated rice straw, following the methodology outlined by the AOAC (2000). To further investigate the effects of microbial treatment and urea pretreatment, rice straw treated solely with the microbial consortium, as well as rice straw pretreated with 5% urea followed by microbial consortium treatment, were subjected to scanning electron microscopy (SEM).

The results of triplicate enzyme and proximate analysis data were analyzed using CPCS1 software (Cheema and Singh, 1990). Post-hoc comparisons were conducted using Tukey's Test to evaluate differences between measurement means at a 5% significance level ($P \leq 0.05$).

A total of six bacterial isolates (B1 to B6), one actinobacterium (A1), and one fungal isolate (F1) were obtained from mushroom compost, while three standard cultures, *Bacillus* sp., *Pseudomonas* sp., and *Delftia* sp., were already available in the laboratory. These cultures were subjected to preliminary qualitative screening for enzyme activity, which led to the selection of A1, B5, B6, F1, and the three standard cultures based on their higher potency indices on media supplemented with various substrates and indicator dyes. The potency index for hemicellulase activity was highest in isolates B5 and B6, with values of 5.26 and 1.82, respectively. The actinobacterium A1 and fungal isolate F1 exhibited notable cellulase activity, with potency indices of 1.45 and 2.00, respectively. Among the standard cultures, *Bacillus* sp. showed the highest activity for Remazol Brilliant Blue (RBB) decolorization, *Pseudomonas* sp. exhibited the greatest xylanase activity, and *Delftia* sp. demonstrated superior cellulase activity, with potency indices of 2.95, 5.89, and 1.33, respectively. The use of dyes and coloured markers in this study enabled the visual detection of lignocellulolytic activity, offering a simple method for enzyme screening that does not require quantitative measurements (Machado *et al.*, 2005). Several studies have demonstrated that the size of the clearance zone correlates with enzyme activity (Mandhulika *et al.*, 1993; Jahangeer *et al.*, 2005). For instance, an earlier study evaluated the lignolytic potential of 125 basidiomycetes isolated from tropical ecosystems by correlating the intensity of RBB decolorization with enzyme production (Machado *et al.*, 2005). The zones of clearance produced by different isolates for cellulose, hemicellulose, and lignin degradation are shown in Plates 1 to 3.

The prepared consortium was inoculated to observe the production of lignocellulolytic enzymes under solid-state fermentation of rice straw and pretreated rice straw. Fungal isolates have been shown to grow more quickly and produce more biomass on solid substrates than in submerged conditions. The results presented in Table 1 revealed that maximum activity was observed on the 21st day, with endoglucanase activity reaching 2.77 U/ml, which decreased to 1.35 U/ml by the 28th day. Maximum exoglucanase activity was also recorded on the 21st day at 7.54 U/ml, while β-glucosidase activity

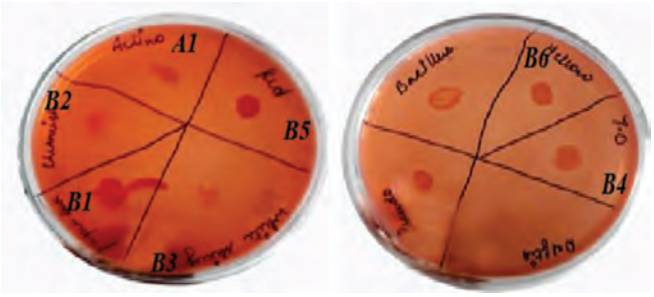

Plate 1. Qualitative estimation for cellulase activity of lignocellulolytic cultures

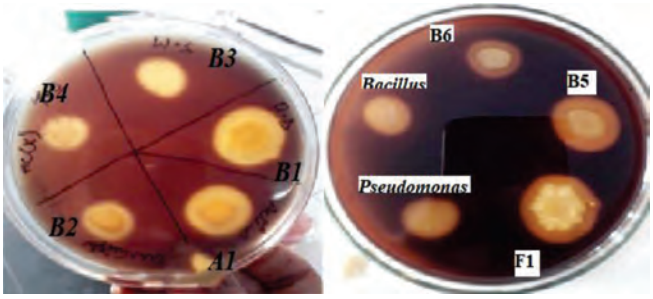

Plate 2. Qualitative estimation for hemicellulase activity of lignocellulolytic cultures

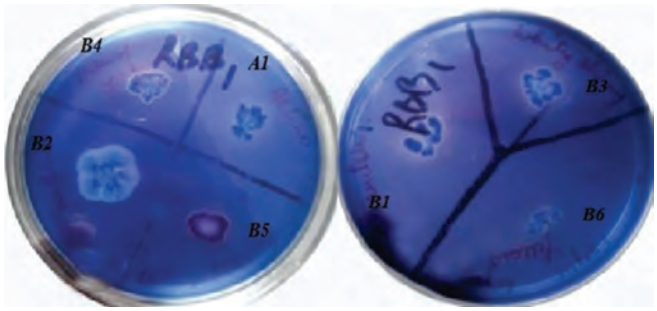

Plate 3. Qualitative estimation for laccase activity of lignocellulolytic cultures

Table 1. Quantitative estimation of lignocellulosic enzymes on untreated rice straw (Mean±SE)

Days	Endoglucanase (U/ml)	Exoglucanase (U/ml)	β-glucosidase (U/ml)	Xylanase (U/ml)	Laccasse (U/ml)	MnP (U/ml)
0	0.39^{e}±0.05	5.19^{d}±0.10	6.73^{e}±0.01	49.27^{e}±0.08	2.80^{d}±0.10	4.20^{e}±0.08
7	1.51^{c}±0.02	5.97^{c}±0.06	11.27^{d}±0.29	53.94^{d}±0.03	3.40^{c}±0.08	5.39^{c}±0.21
14	2.27^{b}±0.07	6.48^{b}±0.08	15.05^{c}±0.05	66.25^{b}±0.13	3.98^{b}±0.05	5.98^{b}±0.15
21	2.77^{a}±0.03	7.54^{a}±0.15	18.32^{a}±0.09	77.73^{a}±0.03	5.40^{a}±0.04	6.20^{a}±0.09
28	1.35^{d}±0.08	5.93^{c}±0.21	16.63^{b}±0.05	57.34^{c}±0.05	2.60^{e}±0.12	4.80^{d}±0.24

Within a column, means with the same letter did not differ significantly after Tukey's HSD test ($p \leq 0.05$), a>b>c

was observed at 18.32 U/ml. The hemicellulolytic enzyme xylanase exhibited its highest activity of 77.73 U/ml on the 21st day, after which the enzyme activity declined to 57.34 U/ml by the 28th day.

In the qualitative estimation, it was observed that the microbes were inefficient in showing activity for MnP, as no clearance zone was produced on plates containing guaiacol as the substrate. However, in the quantitative analysis, Table 1 indicates that MnP activity was higher compared to laccase. The maximum MnP activity recorded was 6.20 U/ml, while laccase activity was 5.40 U/ml on the 21st day. Although MnP activity on the 14th day (5.98 U/ml) was not significantly different from the activity on the 21st day, enzyme activities decreased on the 28th day to 4.80 U/ml for MnP and 2.60 U/ml for laccase, respectively. Zhu *et al.* (2011) found that *Trametes versicolor* exhibited the highest CMCase activity of 4.68 U/g corn stover on the 21st day of incubation under solid-state fermentation (SSF) under optimum conditions. The carbon source and microorganisms play a crucial role in SSF, and rice straw has been shown to be a suitable carbon source for the production of hemicellulolytic enzymes by microbial consortia in SSF. Cellulolytic enzymes produced by *Inonotus obliquus* have been used for the saccharification of rice straw (Xu *et al.*, 2018), and the lignocellulolytic enzymes of *Auricularia auricula* have been utilized for the hydrolysis of corn stalks (Lu *et al.*, 2022). Additionally, lignocellulolytic enzymes produced by a hyper-producing mutant of *Aspergillus allahabadii*, using mild-alkali deacetylated paddy straw as a substrate, have been applied *in situ* for the efficient bioconversion of rice straw into fermentable sugars and biogas (Sharma *et al.*, 2022). Furthermore, Gao *et al.* (2023) reported significant lignocellulolytic activity, with maximum CMCase, FPase, and xylanase production of 237.04 U/g, 54.8 U/g, and 987.33 U/g, respectively, by *Chaetomium globosum* using paddy straw as a substrate.

Figure 1 represents the biochemical components of untreated paddy straw degraded by microbial consortia over a 28-day period. It was observed that the initial content of total solids, volatile solids, and total organic carbon was 93.5%, 87.5%, and 48.6%, respectively. After microbial decomposition for 28 days, these values decreased to 78.6%, 85.39%, and 47.44%, respectively. Meanwhile, the ash content increased from 12.5% to 14.6%. The percentage decline in total solids, volatile solids, and total organic carbon was 15.93%, 2.41%, and 2.38%, respectively, while the increase in ash content was 16.8%. Earlier studies have also reported that among five fungal strains, *Pleurotus ostreatus* (T3) reduced the cellulose content from 33.9% to 26.4% and hemicellulose content from 24% to 12%, with the maximum dry matter reduction (Salem *et al.*, 2020).

During the microbial degradation of rice straw, the lignocellulosic constituents such as lignin, hemicellulose, and cellulose showed a decline from the first to the fourth week. The periodic examination of these constituents, presented in Fig. 2, revealed a non-significant but numeric decline in the ADF content of untreated paddy straw upon application of microbial consortia, as compared to the control. The initial values of cellulose, hemicellulose, and lignin on day 0 were 33.8%, 26.4%, and 16.5%, respectively. During microbial decomposition with the selected consortium, the cellulose content decreased from 33.8% to 24.4%. Similarly, hemicellulose content significantly decreased

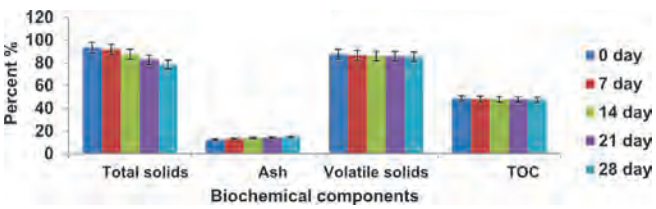

Fig. 1. Biochemical components of untreated paddy straw during solid state fermentation

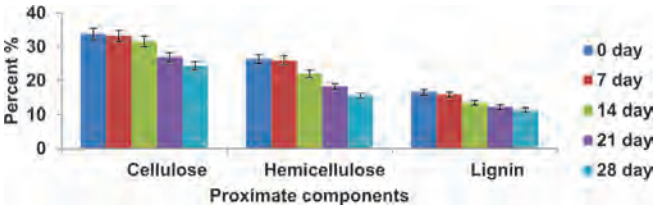

Fig. 2. Proximate composition of untreated paddy straw during solid state fermentation

from 26.4% to 15.5%. The maximum decrease was observed in lignin, which dropped from an initial value of 16.5% to 11.4% after 28 days of incubation. It can be inferred that the microbial consortium led to a 27.81% decrease in cellulose, a 41.28% decrease in hemicellulose, and a 30.91% decrease in lignin after 28 days of incubation. Fig. 2 illustrates the proximate components of untreated rice straw under solid-state conditions. Zhang *et al.* (2020) also reported the degradation effect of cellulose-degrading microbes on paddy straw, identifying *Penicillium oxalicum* as responsible for 45.72% degradation.

Urea (5%) pre-treatment led to a significant reduction in the lignocellulosic content of rice straw, thereby facilitating microbial action that further decreased the lignocellulosic content. This is reflected in the increased activity of lignocellulolytic enzymes. In the urea-pretreated rice straw, results from Table 2 showed that maximum enzyme activity was observed on the 14th day. Specifically, endoglucanase activity was 2.50 U/ml on day 14, decreasing to 1.76 U/ml by the 28th day. Maximum exoglucanase activity was recorded at 7.47 U/ml on day 14, then decreased to 5.84 U/ml by day 28. Similarly, β-glucosidase activity peaked at 7.32 U/ml on day 14, dropping to 5.84 U/ml by the 28th day.

Similarly, xylanase activity was highest on the 14th day, reaching 44.59 U/ml, and decreased to 38.16 U/ml by the 28th day. MnP activity was higher (3.60 U/ml) than laccase activity (3.20 U/ml) on the 14th day. However, by the 28th day, laccase activity (2.20 U/ml) surpassed that of MnP (1.99 U/ml). Additionally, an alkali-treated birch sawdust medium resulted in higher degradation rates of cellulose, hemicellulose, and lignin (39.24%, 51.00%, and 31.3%, respectively) after 11 days of submerged fermentation by *Inonotus obliquus* (Zhang *et al.*, 2022).

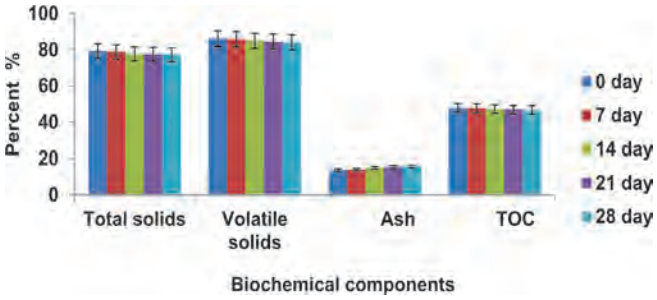

Fig. 3. Biochemical components of pretreated paddy straw during solid state fermentation

The degradation of straw was enhanced by the urea pre-treatment from the beginning of fermentation. The periodic examination of the proximate analysis, presented in Table 3, showed a decrease in cellulose, hemicellulose, and lignin content by 37.7%, 38.8%, and 25%, respectively, compared to their initial values at the start of the experiment, after 28 days.

The periodic changes in the biochemical components of paddy straw pretreated with 5% urea, followed by the application of microbial consortia, are shown in Fig. 3. The initial content of total solids, volatile solids, and total organic carbon in the control was 79.34%, 86.15%, and 47.85%, respectively. After 28 days, these values decreased to 77.26%, 84.23%, and 46.79%. In contrast, the ash content increased from 13.85% to 15.77% over the 28-day period.

Table 2. Quantitative estimation of lignocellulolytic potential on pretreated rice straw (Mean±SE)

Days	Endoglucanase (U/ml)	Exoglucanase (U/ml)	β-glucosidase (U/ml)	Xylanase (U/ml)	Laccasse (U/ml)	MnP (U/ml)
0	1.45^{e} ± 0.04	5.84^{d} ± 0.04	4.65^{e} ± 0.05	28.30^{e} ± 0.03	0.99^{d} ± 0.13	1.97^{c} ± 0.01
7	1.55^{d} ± 0.08	6.23^{c} ± 0.05	5.05^{d} ± 0.13	36.01^{d} ± 0.05	2.97^{b} ± 0.01	3.40^{a} ± 0.28
14	2.50^{a} ± 0.09	7.47^{a} ± 0.07	7.32^{a} ± 0.01	44.59^{a} ± 0.04	3.20^{a} ± 0.08	3.60^{a} ± 0.02
21	1.88^{b} ± 0.01	7.03^{b} ± 0.05	6.73^{b} ± 0.17	40.73^{b} ± 0.02	2.40^{c} ± 0.15	2.40^{b} ± 0.18
28	1.76^{c} ± 0.06	5.84^{d} ± 0.01	5.84^{c} ± 0.07	38.16^{c} ± 0.12	2.20^{c} ± 0.14	1.99^{c} ± 0.09

Within a column, means with the same letter did not differ significantly after Tukey's HSD test ($p \leq 0.05$), a>b>c

Table 3. Proximate composition of pretreated paddy straw

Days	Cellulose (%)	Hemicellulose (%)	Lignin (%)
0	21.20^{a} ± 0.03	21.60^{a} ± 0.16	9.60^{a} ± 0.09
7	20.40^{b} ± 0.07	20.40^{b} ± 0.17	9.20^{ab} ± 0.36
14	18.20^{c} ± 0.10	18.20^{c} ± 0.02	8.70^{b} ± 0.22
21	16.80^{d} ± 0.24	16.80^{d} ± 0.10	7.60^{c} ± 0.24
28	13.20^{e} ± 0.08	13.20^{e} ± 0.23	7.20^{d} ± 0.10

Values of the experiment are means of three replications ± standard error.
Means with the same letter down the column are not significantly different based on Tukey's HSD test (p ≤ 0.05), a>b>c.

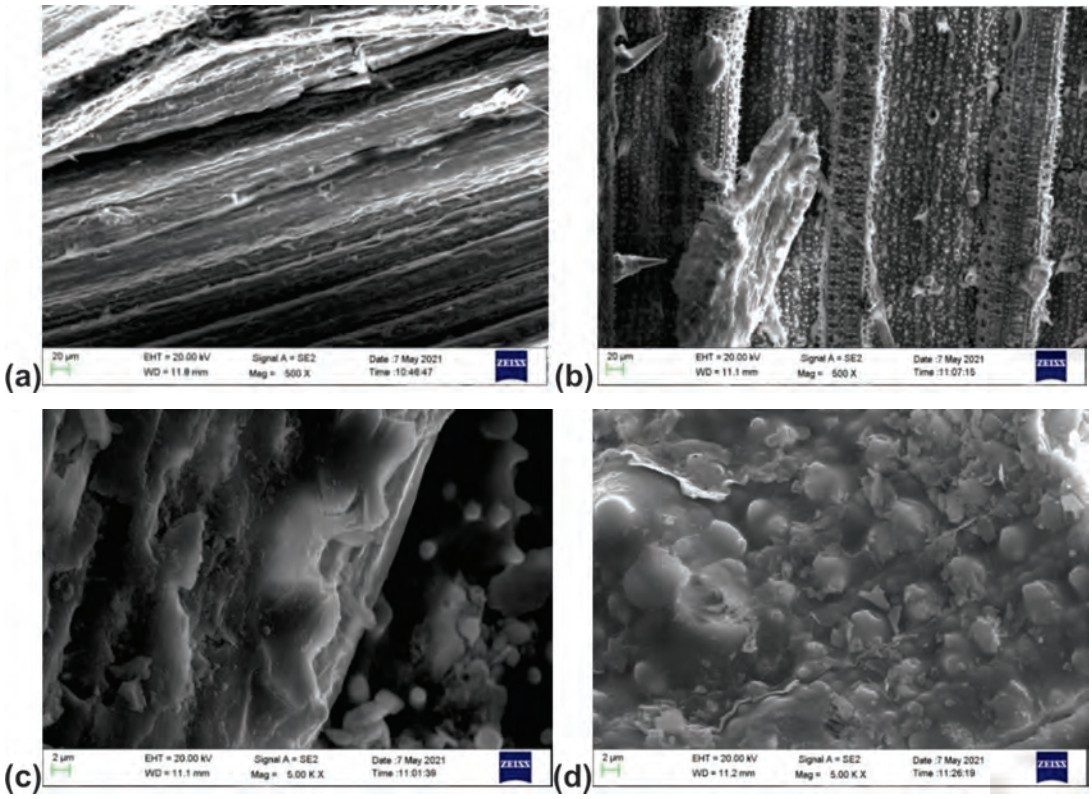

Fig. 4. Scanning electron micrographs of untreated paddy straw before and after biological treatment (a) longitudinal section of untreated paddy straw (control) at 20µm (b) latitudinal section of untreated paddy straw (control) at 20 µm (c) latitudinal section of treated paddy straw with microbial consortia for 28 days at 2 µm (d) longitudinal section of treated paddy straw with microbial consortia for 28 days at 2 µm.

Figures 4(a) and 4(b) represent the untreated rice straw sample at day 0, which served as the control for the experiment. It is observed that the structure of cellulose, hemicellulose, and lignin remains intact in the control sample. Figures 4(c) and 4(d) represent the rice straw sample after 28 days of solid-state fermentation using the selected microbial consortium. These figures reveal significant sloughing off of cellulose, hemicellulose, and lignin. However, the microbial treatment was ineffective in degrading the silica, and silica granules are still present in the sample. Kurniati *et al.* (2016) also analyzed the surface modification of rice straw after degradation by α-L-arabinofuranosidase and reported that enzymatic hydrolysis caused damage to the surface structure of rice straw.

To observe changes in the cell wall architecture due to microbial degradation, scanning electron microscopy (SEM) was performed. After pretreating the rice straw with 5% urea, the sample was examined under SEM. It was observed that the trichomes were intact and embedded. However, after fermentation, the bonds between the trichomes were broken, and the trichome structure was opened. Silica granules appeared to be intact even after 28 days of fermentation (Figure 5), indicating that the microbes in the consortium played a significant role in the degradation of cellulose, hemicellulose, and lignin, but were unable to degrade silica. This finding contrasts with a previous study by Phutela and Sahni (2013), in which treatment of rice straw with *Trichoderma reesei* and *Coriolus versicolor* resulted in an increased surface area and pore size of the treated rice straw, due to the solubilization of silica components.

According to the current study, the use of microbial input can aid in the decomposition of rice straw. Employing a microbial consortium for lignocellulosic delignification is an environmentally favourable approach. In this study, a consortium of seven isolates was used to treat rice straw, resulting in not only high enzyme production but also a significant reduction in cellulose, hemicellulose, and lignin, as demonstrated by solid-state fermentation. The identified microbial consortia show promise for delignification of rice straw and other lignocellulosic biomasses. While earlier studies have reported significant silica reduction using *Phanerochaete* species (Gill *et al.*, 2016), no such activity was observed in the present consortium. To enhance silica degradation, *Phanerochaete* species could be incorporated into the consortium to improve rice straw decomposition, making it more scalable for

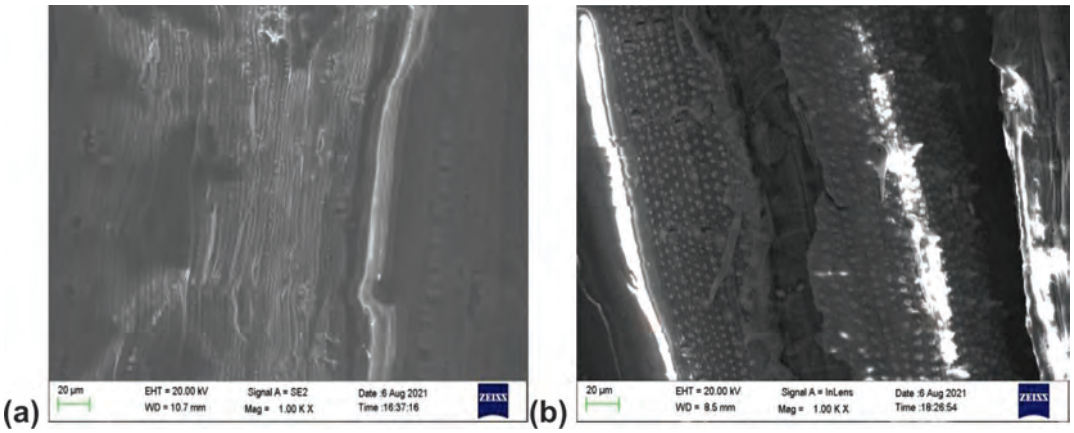

Fig. 5. Scanning electron micrographs of pre-treated paddy straw before and after biological treatment (a) longitudinal section of pre-treated paddy straw (control) at a scale of 20 µm, (b) longitudinal section of pre-treated paddy straw treated with microbial consortium for 28 days at a scale of 20 µm

field applications, where large quantities of biomass are generated. For successful field-scale applications, the selected consortium must be able to multiply efficiently on inexpensive agricultural by-products, enabling mass production for large-scale use. Therefore, further research is needed to assess the degradation potential of these microbes, as well as their ability to break down waste biomass in the field under specific environmental conditions.

Authors' contribution:

Conceptualization and designing of the research work (PK); Execution of lab experiments and data collection (N); Analysis of data and interpretation (PK, N); Preparation of manuscript (PK, N).

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