**Enhancing Composting Efficiency: Thermophilic Lignocellulolytic Microbes for Bioconversion of Rice and Wheat Straw**

**ABSTRACT**

Effective management of large amounts of crop residue from agricultural farms is crucial. Composting is a viable strategy for rice and wheat straw management, but traditional methods are inefficient due to the recalcitrant nature of the straw. This study aimed to identify and utilize thermophilic lignocellulolytic bacteria for efficient bioconversion of rice and wheat straw into compost. Samples were collected from hot springs in Madhya Pradesh and Chhattisgarh, leading to the isolation of 101 bacterial strains, of which 20 showed significant cellulolytic and lignolytic activity. Seven key isolates (five bacteria and one fungus) were further characterized and applied to compost rice and wheat straw in controlled conditions. The composts treated with these bacteria showed improved nutrient content and enzymatic properties compared to untreated compost. Specifically, in wheat straw, nitrogen (0.98%), phosphorus (1.49%), and potassium (1.37%) were higher than in the control (N: 0.69%, P: 0.99%, K: 0.86%). Similarly, in paddy straw, treated compost had higher N (1.17%), P (0.55%), and K (2.10%) compared to the control (N: 0.87%, P: 0.28%, K: 1.78%). The results demonstrated that these microbial isolates enhanced the composting process, improving compost maturity and nutrient content. This study recommends the formulation of these thermophilic lignocellulolytic bacteria for efficient crop residue composting.By reducing the reliance on straw burning, this microbial approach offers an eco-friendly alternative for sustainable agriculture and climate change mitigation.

**KEYWORDS: Hot springs,** **Composting, Soil health, Thermophiles**

**INTRODUCTION**

Agriculture, especially in developing nations, faces multiple challenges that threaten food production, including land degradation, high input costs, limited farming resources, and climate change (Pimentel and Burgess, 2013). Additionally, the global energy and resource crisis has intensified the need for sustainable solutions, prompting efforts to develop renewable resources and explore cost-effective raw materials for energy and resource production (Chovau et al., 2013; Ma et al., 2020). Various biomass wastes, such as food waste, sewage sludge, livestock manure, and straw, are now being utilized to produce value-added products like biogas, biofertilizers, bioethanol, and biodiesel (Ma et al., 2017; Li et al., 2019; Awasthi et al., 2019).

Among these, straw is a particularly abundant and promising resource for energy recovery. Global straw production has surged with agricultural expansion, reaching approximately 7 billion tons annually (Sun et al., 2018; Ma et al., 2020). Rice, corn, and wheat straw account for nearly 90% of total straw production, with paddy straw alone contributing 800 to 1,000 million tons per year, mostly from Asia (Singh et al., 2022; Srivastava et al., 2023). India produces around 107 million metric tons (MMT) of wheat, generating about 182 MMT of wheat straw annually (Yumnam et al., 2023). Traditionally, straw is disposed of by *in situ* burning, which causes severe environmental and health hazards, including air pollution, soil degradation, and greenhouse gas emissions, contributing to climate change. Therefore, traditional straw management methods are unsustainable and require eco-friendly alternatives.

Composting presents a viable solution by transforming straw into nutrient-rich organic matter for crop cultivation. Effective composting relies on microorganisms capable of degrading lignocellulose under high temperatures, improving the process efficiency and yielding thermostable biomass-degrading enzymes for biorefinery applications. Understanding the thermophilic microbial community involved in composting is crucial for optimizing decomposition and enhancing compost quality (Moreno et al., 2021). Thermotolerant microorganisms, with their stable and heat-resistant enzymes, offer advantages for industrial processes over thermolabile counterparts (Bhalla et al., 2013; Hemati et al., 2018).

This study focuses on the selection and utilization of thermophilic bacterial strains for composting of rice and wheat straw. By investigating the microbial dynamics during composting, this research aims to enhance the efficiency of modern composting techniques and contribute to sustainable agricultural waste management.

**2.MATERIALS AND METHODS**

***2.1. Sampling and Isolation Procedures***: Soil sediment, water and mat samples were collected from natural hot water springs Choti Anhoni (22˚38‘42‘‘E,78˚21‘26‘‘N) and Badi Anhoni (22˚35‘3‘‘E,78˚36‘16‘‘N) hot water spring, Chindwara district of M.P., and Tatapani hot water spring, Chhattisgarh of Central India. Samples from all the three places were collected in the sterile plastic containers i.e. plastic bottles and plastic bags, disinfected with ethanol and labelled properly. All the samples were placed in the dry ice at place and after that these are immediately brought to laboratory were stored at -20˚C temperature for analysis for furthure use. For isolation of bacteria and fungi, three replications of 10-4and 10 -5dilutions were taken. Bacteria were isolated by pouring these diluted samples on Nutrient Agar (NA) media under sterile conditions.

***2.3. Screening of Potential Isolates***: Bacterial isolates of hot water springs have been screened out for thermotolerance. For this after serial dilution cultures were grown on NA Media at temperatures 30, 45, 50, 60, 70 0C for 2-4 days*.* ***Liquid medium for further analysis*** M9 minimal media is a highly-referenced microbial growth medium used for the cultivation of E. coli. This buffered minimal microbial medium contains only salts and nitrogen, so it is traditionally supplemented with glucose, amino acids and vitamins as needed. following media composition were prepared .KH2PO4-15 g/l, Na2HPO4⋅7H2O-64 g/l, NaCl-2.5 g/l, NH4Cl-5.0 g/l and 1% CMC (Carboxymethylcellulose sodium salt).

**2.3 Production of extracellular enzymes**

***2.3.1 Qualitative assay***

**Cellulase Production**

Cellulase activity of Anhoni and Tattapani hot water springs has been determined by nutrient agar medium amended with carboxymethylcellulose as the sole carbon source (kasana et.,2008). On following media, bacterial cultures were surface inoculated and then Congo red and NaCl were used to detect cellulolytic activity. For cellulase activity, a mineral–salt agar media containing was prepared with following composition (Pandey et al., 2013), Ammonium Tartarate (C4H12N2O6)- 5g, Potassium dihydrogen phosphate (KH2PO4)-1g,MgSO4.7H2O-0.5g,CaCl2.H2O-0.001g,Yeastextract-0.1g,Agar-20g,Carboxymethyl cellulose (medium)-1.5% and 2% agar. Cultures were incubated at 60˚C temperature. After 48 hours, staining was done with Grams Iodine/Red congo+NaCl and Cellulolytic activity has been detected by calculating Hydrolyzing capacity index (HCI) Hydrolysing Capacity Index (HCI) = Diameter of clear zone/ Bacterial colony diameter.

**Lignocellulase Production**

For lignocellulase activity of thermophilic bacteria, following media composition were prepared, KH2Po4-1g, C4H12N2O6-0.5g, MgSO4.7H2O-0.5g, CaCl2.2H2O-0.01g, Yeast Extract-0.01g, CuSO4.5H2O-0.001g, Fe2(SO4)3-0.001g, MnSO4.H2O-0.001g, Lignin-0.25%, Agar-20g (Huang et al.,2013; Patel et al.,2017). Media were poured into sterilized plates and streaked with bacterial inoculation. Plates were placed in incubator at 60˚C temperature. After 5 days flood it with 1% aq. solution of FeCl3+K3[Fe(CN)3]. Presence of zone formation around the growing colony was considered as positive.

***2.3.2 Quantitative assay*:** The isolates were inoculated into M9 liquid medium for cellulase assay and incubated for 2, 3 and 5 days for bacteria and fungi. The inoculum was centrifuged at 10,000 rpm for 10 min at 4˚C. The pellets were discarded and the supernatant portion assumed to contain the extracellular cellulolytic crude enzymes that were used for estimation of enzyme production. **The enzyme activities of all resultant supernatants were estimated. Laccase, lignin peroxidases, manganese-peroxidase, CMCase, and β-glucosidase activities were analyzed of all selected isolates.**

**Laccase** Laccase (Lac) activity was determined by oxidation of 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) method (Bourbonnais and Paice 1990, Zhang et al.,2020). Assays were performed in a 3 mL mixture containing 2.7 mL 50 mM sodium acetate buffer (pH 5.0), 15 mM ABTS 200 μL, and suitably diluted crude enzyme 100 μL. The oxidation of ABTS was performed at room temperature by monitoring spectrophotometrically the change in absorbance at 420 nm. One unit of enzyme activity is defined as the amount of enzyme required to oxidize 1 μmoL ABTS/ min using an ɛ420 value for oxidized ABTS of molar absorption coefficients 36,000 M-1cm-1.

**CMCase:** CMCase activity of cellulase was measured by DNS (3,5 dinitrosalicylic acid) method through the amount of reducing sugarsliberated during hydrolysis (Berghem *et al.,*1976, Zhang et al.,2021). CMCase reaction mixture containing 1 mL of appropriately diluted enzyme.3 mL of 1% CMC in 50 mm sodium citrate buffer (pH 4.8) was incubated at 50° C for 30 min. DNS was added to the solution to stop the reaction. The treated samples were boiled for 10 min, cooled in water for color stabilization and the optical density was measured at 540 nm. One unit of endo-β-1,4-glucanase activity was defined as the amount of enzyme that could hydrolyze CMC and release 1 µmol of glucose within 1 min of reaction.

**β-glucosidase:** The β-D-glucosidase activity was assayed with modified method of (Korotkova et al., 2009) A 20μl aliquot of the acquired enzyme was mixed with 180 μL of 5 mM pNPG ( p-nitrophenyl-β-D-glucopyranoside) substrate in 50 mM sodium acetate buffer (pH 7.0). the mixture was incubated at 50° C for 10 min. The reaction was arrested by adding 100µl of 0.5 M sodium carbonate which was followed by the release of p- nitrophenol from pNPG, the absorbance was measured at 405nm optical density using UV-Spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme required to release 1 micromole of p-nitrophenol per minute.

**Lignin peroxidase (LiP):** Lignin peroxidase (LiP) activity was measured as described by (Tien and Kirk 1984, Zhang et al.,2020). Lignin peroxidase activity was determined at room temperature in a 3 mL reaction mixture containing 2.24 mL 50 mM sodium tartrate buffer (pH 2.5), 10 mM veratryl alcohol 600 μL, suitably diluted crude enzyme 100 μL, and activator 20 mM H2O2 60 μL. The reaction is initiated by the addition of H2O2 and the increase in absorbance measured at 310 nm.

**Manganese peroxidases (MnP):** MnP activity was measured as described previously with slight modification (Wariishi et al.,1992, Zhang et al.,2020).Briefly, 100 µL of culture medium was incubated with 900 µL of 50 mM sodium malonate buffer containing 1 mM MnSO4. The reaction was initiated by adding hydrogen peroxide to a final concentration of 0.1 mM followed by incubation at 35℃ for 30 min. The complex form of Mn3+-malonate was qualified at 270 nm (ε270 = 11.59/mM/cm). One unit enzyme was defined as amount of required enzyme to produce 1 µmol product per minute under the experimental conditions.

**xylanase activity:** Xylanase in the sample hydrolyzes the substrate, beech Xylan, and the amount of released reducing carbohydrate is determined spectrophotometrically using dinitrosalicylic acid (Baily, M.J. and Poulanen, K. 1989) Pipette 1.8 ml of substrate solution into 25 ml screw cap tubes, add 200 µl of suitably diluted enzyme solution to the test tube and mix with a vortex mixer. Continue enzyme addition at sufficient interval to each tube except enzyme blanks. Incubate at 50°C for 5 minutes. After exactly 5 minutes, add 3.0 ml of DNS reagent to each tubes and vortex. Remove the tubes from the water bath and place in a boiling water bath. After boiling for exactly 5 minutes remove the tubes and cool in cold water to room temperature. Read the absorbances in a 1- cm cuvette at 540 nm using air to set the spectrophotometer to zero. Correct the value of each enzyme test by subtracting the reading of the respective enzyme blank.

**On the basis of qualitative and quantitative screening of the bacterial and fungal isolates, six efficient bacterial and one fungal strain were selected for further work.**

***2.5. Selection and Identification of Isolates***: According to the result of extracellular enzyme activity assays, the promising strains were selected for colony morphology (Tankeshwar,2013), compatibility test (Santiago et al.,2017) microscopic visualizations and molecular identification by extracting microbial DNA.

**Composting process**

**Experimental Design**: The experiment was laid out with completely randomized block design (CRBD), experiments were conducted with two different straw viz., wheat straw and paddy straw. For each straw, 5 treatments were set with 3 replications; totalling 15 samples per straw type. The treatment consists of different combinations viz., wheat /paddy straw (control)(T1); wheat /paddy straw + TLC bacteria (T2); wheat /paddy straw + excel decomposer(T3); wheat/paddy straw + Pusa decomposer(T4); wheat/paddy straw + waste decomposer(T5). The container were filled with 150 g of straw in each container. At the end of the composting period (30 days), compost was collected and used for further analysis.

***Compost Analysis:***

The plastic container were turned regularly every week to provide aeration. Samples were collected at 30 days during the composting process. Non inoculated substrates served as control. For organic matter and organic carbon determination, ash was determined in a muffle furnace at 5500C for 5 h. Organic matter was calculated as the difference between ash and dry weight as a percentage (Tiquia and Tam, 1998). From values of organic matter, the percentage of organic carbon was calculated as described by Haug (1993). The pH was directly measured in the water extracted sample 1:5 w/v using a glass electrode pH meter. Electrical conductivity measurements were run in 1:5 w/v compost water extracts using EC meter. Total nitrogen was determined as described by Chapman and Parker (1963). C/N Ratio was calculated using values of the organic carbon and total nitrogen. Phosphorus (%) of samples was determined calorimetrically according to the methods described by Snell and Snell (1967). Total potassium was determined in the digested solution by flame photometer (Jackson, 1967). The cellulose and lignin content were analyzed using acid detergent fibre (ADF) method (Van Soest et al.,1991).

**Statistical Analysis** The collected data were subjected to one-way analysis of variance (ANOVA) to determine any significant variations (at the *P ≤ 0.05* level) between the treatment data (Roy *et al*., 2024a). Duncan's multiple range test (DMRT) was used to determine the mean ± standard error (SE) of the treatment data, following the method described by Duncan in 1955. The statistical analysis was performed using SPSS software (version 20.0, SPSS Inc., Chicago, IL, USA) (Roy *et al*., 2024b).

**RESULTS AND DISCUSSIONS**

***Isolation of bacteria*** Among the samples of soil, water and mat collected from hot water springs. The colony forming units are found maximum in mat samples followed by soil samples and water samples. Higher CFU (colony forming unit) in mat samples and soil samples. These results are in consensus with Arya et al. (2015). Isolation and purification procedures were carried out with both water and soil samples from the hot spring of Soldhar, Himachal Pradesh. The temperature and pH of the sampling site was 90°C and 7.5, respectively. A total of 50 and 57 CFUs were isolated from soil and water samples, respectively, collected from the hot spring under study. maybe due to different types of substances released by the algae present in the HWS (hot water spring) and different types of compounds released from the roots such as nucleotides, flavonoids, enzymes, organic acids, vitamins, flavonoids, hormones etc. These substances present in the samples may increase microbial activity in these areas.

**Preliminary Screening of Lignocellulolytic producer isolates.** The present study is an effort towards an exploration potential thermophilic microbes for utilization in composting rice straw and wheat straw. While in our explorations, we have isolated and purified 101 different isolates from various samples. All the isolated strains were subjected to Qualitatively assay for preliminary screening. Based on the formation of a clear visible zone around the colony on the solid media supplemented with the suitable specific indicators which demonstrated that isolates have ligninolytic or cellulolytic activity. From the results of the preliminary screening process, 20 isolates showed a visually positive result in the Gram's iodine and aqueous solution of FeCl3 and K3[Fe (CN)6] decolorization. The diameter of the halo zone around the colony was used to assay for the degree of cellulose degradation (Teather and Wood 1982). Cleavage of lignin is important in composting rice straw as lignin protects cellulose and hemicelluloses from biodegradation. Therefore, selection of bacterial isolates with ligninolytic ability was crucial in the rapid composting of rice straw. The involvement of bacteria in the bioconversion of lignocellulosic substrates is well documented (Yang et al.,2002; Gilbert et al., 2008). With the findings of Zainudin et al. (2013) who reported indigenous lignocellulolytic bacteria enhanced composting of oil palm empty fruit bunch in 40 days compared to conventional oil palm empty fruit bunch composting, which took 90 days.

***Extracellular Enzyme Profiling***. Different types of hydrolytic and oxidative enzymes were detected in cultures for lignocellulolytic enzyme production. The selected isolates were evaluated for extracellular enzyme profiling including laccase, LiP (Lignin peroxidase), MnP (Manganese peroxidases), CMCase (carboxymethyl cellulase), Xylanase, and β-D-glucosidase activities in the liquid culture medium. Secretion of the extracellular enzyme plays a vital role in the lignocellulosic decaying process of biomass depolymerization and/or functionalization. The isolates exhibited different levels of enzyme activity, and the enzyme activity profile of bacteria and fungi was, respectively, presented in Fig. 1.

The bacterial isolate CAS-5 exhibited the highest CMCase activity, measuring at 0.474 µM min-1. Following closely, the bacterial isolate BA-33(2) displayed the second-highest CMCase activity, recording an activity of 0.367 µM min-1. Similar study have shown by Potprommanee et al. (2017). In their study, they isolated a thermophilic cellulase-producing bacterium from a hot spring, which they identified as Geobacillus sp. HTA426. This strain was found to efficiently produce cellulase when grown on alkali-treated sugarcane bagasse, with a CMCase activity of 103.67 U/mL. It also worked well with rice straw (74.70 U/mL) and water hyacinth (51.10 U/mL) as carbon sources. This means that this strain has potential for applications involving the conversion of plant-based biomass into valuable products. This confirms that the bacteria that isolated from the hotspring have high CMCase activity. and the highest β-glucosidase activity was observed in the bacterial isolate CAS-5, with an enzymatic activity of 0.415 µM min-1.in the second position, the bacterial isolate BA-33(2) demonstrated a β-glucosidase activity of 0.352 µM min-1. According to Jurado et al. (2014), β-glucosidase is one of the main enzymes that regulate the carbon cycle and its activity is an indication of the presence of organic matter easily usable by microorganisms as energy source. Though the similar finding have also been reported by Singh *et al*. (2019) they isolated 11 cellulose degrading bacterial strains were from water and soil samples of hot springs in the Chumathang village, Leh and Ladakh region, India. This isolated strains were identified as *Bacillus subtilis, Bacillus aryabhattai, Bacillus stratosphericus, Bacillus altitudinis, and Brevibacterium frigoritolerans.* all the strains were evaluated for the total cellulase, endoglucanase, exoglucanase, and β-glucosidase enzyme activities. The effect of cellulase activities of bacterial strains were evaluated ranged up to 6.06 and 0.72 mg ml−1 glucose by agro-residues of sugarcane bagasse and wheat straw. this isolates is almost similar to one this study, perhaps this happened because the microbes that are isolated from the hotwater springs have good cellulolytic enzyme activities. On the other hand, bacterial isolates B-17 and CAM-1 exhibited the highest xylanase activities, measuring at 93.750 M min-1 and 81.250 M min-1, respectively. The entire three major cellulolytic enzyme activities were recorded maximum in the bacterial isolates CAS-5, BA -33(2), CAM-1. These findings were concordant with Harnvoravongchai et al. (2020) they studied a microorganism called *Thermoanaerobacterium sp.* strain R63.this microbe exhibited remarkable hydrolytic properties with the highest cellulase and xylanase activities. displayed an ability to break down tough plant materials and various sugars. These cellulase activity levels reflect the ability of the isolates to be actively involved in the saccharification process of the delignified or cellulose substrates (Nakajima et al.,2018)

The bacterial isolate BA-36 displayed the highest laccase activity at 29.17 µM min-1. The second-highest laccase activity was recorded in the bacterial isolate TAW-B-10, with an enzymatic activity of 20.00 µM min-1. The results are also well corroborated the findings of Zhang et al. (2021) In their study, they identified Pleurotus spp. as microorganisms with high laccase activity. This enzyme is crucial for breaking down lignin, a major component of plant cell walls. The study suggests that these laccase-producing microbe can effectively pretreat lignocellulosic agricultural wastes. By breaking down lignin, these microorganisms make cellulose and hemicellulose more accessible for fermentation. and the highest lignin peroxidase activities were observed in bacterial isolates TAM-B-1 and BA-33(1), both registering an enzymatic activity of 6.452 M min-1. In the third position, bacterial isolate BA-36 exhibited a lignin peroxidase activity of 4.301 M min-1. These findings were concordant with those of past studies (Pangallo et al., 2009; Bugg et al., 2011; Wang et al., 2013). Bacteria cleave lignin in lignocellulosic materials through a diffusible chemical process by utilizing lignin peroxidase activity and Phenol oxidases (Jing et al., 2009). while the bacterial isolate TAM-B-23 demonstrated the highest manganese peroxidase activity at 0.933 M min-1. Following closely, the bacterial isolate BA-17 exhibited a manganese peroxidase activity of 0.889 M min-1. These results are in close conformity of the results observed by Huy *et al. (*2017) they isolated a potential MnP producing fungal strain isolated from a forest area. The strain produced MnP under fermentation separately using rice straw and wood chips as the carbon source. Highest MnP activity on rice straw medium was 1.76 U/mL and 1.91 U/mL on wood chips medium. the enzyme MnP are the important peroxidases, which play an important role during the initial stages of lignin degradation (Datta et al.2017). TAM-B-23 presents the best MnP activity of which is a potential source for MnP production and phenolic compound oxidization. Lignolytic enzymes produced by some potential microbial isolates can also be a source of rapid biodegradation module for large-scale and effective lignin degradation (Fenga et al.,2011)

This data reveals significant variations in the enzymatic activities among different bacterial isolates, suggesting their potential utility in composting rice straw and wheat straw.

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Description automatically generated

**Fig. 1: Lignocellulolytic enzyme activity profile of bacteria and fungi**

**Molecular Identification of Microbial Strains**

The lignocellulolytic bacteria selected for use in composting the wheat and paddy straw in this study included 6 bacterial and 1 fungal isolates. Results from various identification techniques used showed that the appearance of most of the bacterial colonies varied from shiny to dull. Several colonies however had very outstanding colony colours including cream, white. most of the colonies were, flat, whitish in colour and irregular in shape. It was also observed that several colonies were Smooth while others rough and crusty appearance. Arya et al. (2015) have also reported the morphological features of thermophilic bacteria isolated from Soldhar hot spring, white-colored bacterial colonies dominated the yellow and creamish isolates from Soldhar hot spring. All the isolates from water samples exhibited serrate margins, all the 11 bacterial isolates unanimously exhibited flat elevation and circular form. Molecular identification was done by DNA extraction method. Most of the bacterial isolates belonged to Genus Bacillus while most of the fungi were mainly in Genus Trichoderma. Bacillus spp. has been well documented in the composting of lignocellulosic materials (He et al., 2013). B. subtilis is thermo-tolerant and can maintain a high population by sporulating during composting (He et al., 2013; McDonald et al., 1998)**.** And apart from TLC-B-6, all the cultures were determined to be compatible with one another in all possible combinations and permutations. Results from compatibility test 5 bacterial and one fungal isolates are selected for further analysis. Isolates are said to be compatible if there is no zone of inhibition in the streak area of the two isolates (Santiago *et al.,* 2017).

**Physico-chemical Characteristics of Compost** Characteristics of compost sample at the 30 days (matured compost samples) are shown in Tables (1) and (2).

In wheat straw, the results presented in Table 1. Treatment T2 displayed the highest total phosphorus content (1.49%) In comparison the other treatment, the lowest value (0.99 %) was recorded in control (T1) Significant difference in total phosphorus content in compost was observed. Similar findings in paddy straw, it was observed that Treatment T2 had the highest recorded total phosphorus content (0.55%) as compare to the other treatment. Conversely, the control treatment, referred to as T1, displayed a notably lower total phosphorus content of 0.28%. It might be due to the microbial inoculants significantly (P < 0.05) increased total N, total P and total K contents, implying that microbial inoculants are essential for speeding up the composting process and improving compost quality (Nigussie *et al*. 2021). The results from this study indicate that both Pusa decomposer (T4) and waste decomposer (T5) were effective in facilitating the decomposition of rice and wheat straw. The highest total phosphorus was recorded in paddy straw + waste decomposer + effective microorganisms + soil was identified as the best treatment, followed by waste decomposer + effective microorganisms + paddy straw as in comparison to control-paddy straw alone. This result was in confirmation by the finding of Yumnam *et al.* (2023). The study by Zhang et al. (2021) also revealed that the total phosphorus content of rice straw composting increased during the thermophilic phase of composting and then decreased during the maturation phase. Manu *et al*. (2023) concluded that paddy straw incorporation + Pusa decomposer @25 litre/ha + urea @10 kg/ha (S7) treatment significantly enhanced the nutrient uptake, crop productivity and soil nitrogen status in the rice–wheat cropping system (RWCS) under aerobic as well as transplanted rice and thus proved better in situ rice residue management option.

In wheat straw, treatment T2 recorded maximum amount (1.37%) of total potassium content as compare to other treatment, lowest value (0.86%) was recorded in control(T1). In paddy straw, treatment T2 recorded the highest total potassium content value (2.10%) as compare to (T1, T3, T4, T5), while the control treatment(T1) had the lowest value (1.78%). A similar study found that adding a microbial inoculant to rice straw compost increased the compost's potassium content depending on the compost's maturity (Wang et al., 2019). Both pusa decomposer (T4) and waste decomposer (T5) have shown a constructive interaction with rice and wheat straw, leading to an increase in total potassium (K) content. This result suggests that these decomposers or treatments may enhance the availability or uptake of potassium in the soil. from a similar study conducted by Yumnam et al.,2023. The finding that the treatment paddy straw + waste decomposer + effective microorganisms + soil had the highest total potassium content compared to the control is significant. Gurung,2023 concluded that in their study the application of waste decomposers can bring about significant enhancements in agricultural outcomes, both in terms of physical characteristics and nutritional value.

In wheat straw, treatment T2 recorded maximum amount (0.98 %) of total nitrogen content as compare to other treatment and lowest value (0.69%) was recorded in control treatment. Similarly in paddy straw, the study revealed that treatment T2 exhibited the highest total nitrogen content, quantified at 1.17% followed by T3 (1.07%). Conversely, the control treatment displayed the lowest total nitrogen content, which was determined to be 0.82%. Total nitrogen content increased perhaps due to the anabolism of cell structure, synthesis of enzymes, and hormones in microorganisms, and an increase of nitrogen concentration due to the volatilization of organic matter. These findings were consistent with the results of Veeken et al. (2001) and Raviv et al. (1999) who stated that total nitrogen content increased during bioconversion of sludge, lignocellulosic materials, and chicken manure. Result from this study also find that pusa decomposer (T4) and waste decomposer (T5) have also positive interaction with the rice and wheat straw, it is also increase the total nitrogen content. A similar study found that the highest N content of 0.64% w/w was reported in treatment RSWF (rice straw plus water plus Pusa decomposer) and was followed by rice straw plus water plus Tamil Nadu Agricultural University (TNAU) biomineralizer (RSWB) (0.61%) w/w, rice straw plus water (RSW) (0.45%) w/w and rice straw (RS) control (0.43%) w/w (Meena, S. K. et al.,2023).The highest nitrogen content was recorded in waste decomposer + effective microorganisms + paddy straw and paddy straw + waste decomposer + effective microorganisms + soil was on par with 24.46 % and 26.04 % as compared to paddy straw alone in an experiment on composting paddy straw (Yumnam et al., 2023).

One of the often-used parameters to assess the rate of decomposition in the composting process is the C:N ratio, since it can reflect the maturity of the compost. In wheat straw, table (2) demonstrates the reduction in the C/N (Carbon-to-Nitrogen) ratio across various treatments as a result of organic matter mineralization. The lowest values of C/N ratio 23.29 for T2 as compared to other treatment. A C/N ratio of less than 20 is considered as mature and can be used without any restriction. similarly in paddy straw, treatment T2 showed the most significant reduction, resulting in the lowest C:N ratio recorded at 15.02 as compared to other treatment. During composting, carbon content dropped due to catabolism and nitrogen content increased due to anabolism in microbial cells and the volatilization effect of organic matter. These results were in line with the findings of Eiland et al. (2001) who stated that C/N ratio dropped during composting of Miscanthus straw. Jurado et al. (2014) also reported that B. licheniformis BT575, and *B. smithii* AT907 mineralized high C/N ratio and enhanced the composting of lignocellulosic materials. A C/N ratio of less than or equal to 20 is considered a mature compost if the initial value ranges from 25 to 30 (Heerden et al., 2002; El Fels et al., 2014). C:N ratio of resultant composts of present study also indicated that pusa decomposer (T4) also show the decrease in the C/N ratio. the decrease in the C/N ratio of microorganism-treated RSWF (rice straw plus water plus Pusa decomposer) might be due to the consumption of carbon for their energy. This result was in confirmation by the finding of Jusoh et al., 2013 and Wang et al., 2022.

The highest values of lignin: cellulose ratio 1.79 for T2 and lowest value (0.82) was recorded in control treatment. The standard method for determining the biomass composition is lignocellulose ratio content, by chemical method; in paddy straw, the highest values of lignin:cellulose ratio 1.63 for T2 and lowest value (0.75) was recorded in control treatment. However, after easily degraded compounds were exhausted, the lignocellulose component were subjected to degradation, resulting in a rapid rise in cellulose and lignin degrading ratios. inoculations with PTCMA (psychrotrophic-thermophilic complex microbial agent) improved lignocellulose-degrading microbial activity, resulting in more complete degradation of cellulose and lignin (Zeng et al., 2009; Zhu et al., 2021) perhaps due to abundant water-soluble carbon fractions in raw materials that can be used by microorganisms (Awasthi et al., 2014)

These research findings offer valuable insights into nutrient content dynamics and decomposition processes associated with paddy and wheat straw composting. Specifically, Treatment T2 exhibited higher levels of essential nutrients, including phosphorus, potassium, and nitrogen. The reduction in the C:N ratio across all treatments indicates the breakdown of organic matter during the composting.

**Table 1. Effect of decomposition of wheat and paddy straw with thermophilic ligno-cellulolytic bacteria on total nitrogen (%). total phosphorus (%), total potassium (%)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | | **Total N %** | **Total P %** | **Total K %** |
| **Wheat Straw** | | | | |
| T1 | **Control** | 0.69 ±0.01d | 0.99±0.00d | 0.86±0.01e |
| T2 | **TLC Bacteria** | 0.98 ±0.01a | 1.49 ±0.06a | 1.37±0.01a |
| T3 | **Excel Decomposer** | 0.95 ±0.01b | 1.34±0.00b | 1.28±0.02b |
| T4 | **Pusa Decomposer** | 0.95 ±0.01b | 1.20±0.05c | 1.09±0.01c |
| T5 | **Waste Decomposer** | 0.75 ±0.01c | 1.00±0.01d | 0.92±0.06d |
| **Paddy Straw** | | | | |
| T1 | **Control** | 0.82±0.01d | 0.28±0.00d | 1.78±0.07d |
| T2 | **TLC Bacteria** | 1.17±0.05 a | 0.55±0.04 a | 2.10±0.00a |
| T3 | **Excel Decomposer** | 1.07±0.02b | 0.45±0.03 b | 2.04±0.01b |
| T4 | **Pusa Decomposer** | 1.01±0.01c | 0.34±0.02c | 1.94±0.01c |
| T5 | **Waste Decomposer** | 0.82±0.01d | 0.29±0.01d | 1.78±0.01d |

The different *letters* following the data (mean ± SE) indicate significant (at the *P ≤ 0.05* level) differences based on Duncan’s multiple range test (DMRT) (Duncan, 1955).

**Table 2. Effect of decomposition of wheat and paddy straw with thermophilic ligno-cellulolytic bacteria on C/N and Lignin: Cellulose**

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment** | | **C : N** | **Lignin:Cellulose** |
| **Wheat Straw** | | | |
| T1 | **Control** | 58.9 ± 0.9 a | 0.82± 0.04 e |
| T2 | **TLC Bacteria** | 23.29 ± 0.6 e | 1.79 ± 0.02 a |
| T3 | **Excel Decomposer** | 26.79 ± 2.1d | 1.65 ± 0.00 b |
| T4 | **Pusa Decomposer** | 30.56 ± 0.7 c | 1.43 ± 0.07 c |
| T5 | **Waste Decomposer** | 46.29 ±01.2 b | 1 ± 0.04 d |
| **Paddy Straw** | | | |
| T1 | **Control** | 48.05± 2.5 a | 0.75± 0.02 e |
| T2 | **TLC Bacteria** | 15.02±2.0 d | 1.63± 0.04a |
| T3 | **Excel Decomposer** | 19.65±0.5 c | 1.54± 0.00 b |
| T4 | **Pusa Decomposer** | 22.39±2.0c | 1.33± 0.09 c |
| T5 | **Waste Decomposer** | 40.42±3.2 b | 0.92± 0.00 d |

The different *letters* following the data (mean ± SE) indicate significant (at the *P ≤ 0.05* level) differences based on Duncan’s multiple range test (DMRT) (Duncan, 1955).

**Conclusions**

In a study lignocellulolytic bacteria isolated from hot water springs, The study found that out of 101 isolates, five specific isolates were found to have optimal potential for degrading rice and wheat straw, facilitating rapid composting. Particularly strains TLC-B-1, TLC-B-2, TLC-B-3, TLC-B-4, and TLC-B-5, along with TLC-F-1 were identified as highly effective. When applied to rice and wheat straw, these specific microbial strains enhance compost quality. This eco-friendly approach to managing straw waste not only supports sustainable agriculture but also contributes to biodiversity preservation and mitigates the environmental impact associated with harmful practices. The research indicates that traditional methods like burning of straw are not environmental friendly or sustainable. Instead, using these natural microbial communities in composting processes represents a promising solution, suggesting a more eco-friendly approach to managing straw waste and providing a potential solution for sustainable agriculture. These research findings offer valuable insights into nutrient content dynamics and decomposition processes associated with paddy and wheat straw composting.

**Disclaimer (Artificial intelligence)**

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1. ChatGPT used for rephrasing the sentence only.

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