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Research Article

Biodegradation of Rice, Wheat and Corn Straw Powder by Cellulase Producing *Bacillus* species Under Controlled Conditions

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Abstract

The thermophilic bacteria (AMB-T-6S) isolated from hot spring of Tatapani, Balrampur district was selected for their specific extracellular enzyme screening response. The medium of experiment conducted on submerged and solid-state form of straw specially wheat, rice and corn straw. The all straw powder (rice, wheat, corn) was compared with control separately, each treatment replicate with three replication and follow the Completely Randomized Design (CRD) model. The isolate was selected for further studies and their growth performance of rice straw, wheat straw and corn straw separately with Carboxymethyl Cellulose (CMC) broth on submerge condition were examined at various time intervals after incubation at 45 ^{0}C.This growth response found significant with different straw. The degraded power form showed higher levels of cellulose and lignin degradation in the FTIR readings when compared to control rice straw powder, wheat straw powder and corn straw powder, where all exhibited C=C aromatic skeletal vibration. After the analysis of the organic content (OC, N, P, K and C:N ratio) Among the all treatment comparatively lowest amount of organic carbon content was determined at 30-day T_2 (rice straw with inoculumn) (68.67%) followed to T_4 (wheat straw) (69.00%) compare to uninoculated straw. The maximum nitrogen content was high in T_6 (Corn straw with inoculum) (0.58%), followed by T_4 (Wheat straw with inoculum) (0.53%). Phosphorus and potassium both contents were significantly high in T_6 (Corn Straw with inoculum) (0.59%&1.27%) followed by T_5 (Corn straw) (0.55%&1.17%). status of compost material found maximum C: N ratio was significantly high in T_3 (Wheat Straw) (37.01) followed by T_1 (Rice Straw), (32.88) & the minimum C: N ratio recorded was in T_6 (Corn Straw with inoculum) (25.61).

Key word: Bacteria, corn straw, decomposition, rice straw, straw, Thermophilic, wheat straw

1. Introduction

Governments are passing laws in support of more environmentally friendly waste management plans based on the circular economy idea as a result of growing public awareness of this issue (Gontard *et al.*, 2018). Composting is the process by which biodegradable materials break down by microbiological, physiological and physicochemical means. It is commonly known that microbial communities, which include bacteria, actinomycetes and fungi are essential to the composting process. However the range of composting materials and nutrient additions used during the composting process may affect the microbial diversity. Consequently, research on the variety of microorganisms present during the composting of various agricultural byproducts, such as rice bran, wheat bran, and rice husk, in addition to grass clippings and bulking agents, is required. The variety of culturable bacteria during the composting of agricultural byproducts has been attempted to be evaluated here. According to Federici *et al.* (2011) and Zang *et al.* (2017), composting is regarded as an appropriate technology for disposing of solid organic wastes because it involves the quick succession of specialized microbial populations that secrete different enzymes, causing the organic wastes to transform into a complex mixture rich in humus. Microorganisms and their related enzymes have a crucial role in the bio decomposition of organic matter (OM), lignocellulose, and the production of humic substance (HS) and dissolved organic matter (DOM) (Karadag *et al.,* 2013; Ren *et al.,* 2016).

Microbial derived enzyme has been extensively used in waste management, biofuel, food, paper, detergent, medicinal and pharmaceutical industries, pulp, feeds, starch, textile and used as biocatalysts, biotransformation and biodegradation due to their extreme stability in elevated high temperatures (Kumar *et al.,* 2019). Bacterial -amylase, protease and cellulase enzymes are the most enzymes used in biotechnology processes (Baltaci *et al.,* 2017). The amazing degradation skills of microorganisms have made them ability applicants for decomposing agricultural residues into compost (Suyal*et al.* 2021). Various microorganisms had been stated as speedy decomposers, bio degraders, and bio converters of non-beneficial products (Gautam *et al.* 2012; Singh *et al.* 2021).Microbes are diagnosed to immobilize mineralized vitamins into molecular biomass and launch it returned to soil after microbe decomposition and accordingly influencebio-availability of vitamins to plants (Tomer *et al.* 2017; Kumar *et al.* 2018; Sahu *et al.* 2018; Negi *et al.* 2020). The aim of this study was to isolated straw decomposing thermophilic bacterial strain (AMB-T-6S) use for decomposition and evaluate their ability to produce cellulose degrading enzymes. Accordingly, know-how the dynamic evolution of microbial groups for the duration of the compost humification method might be useful for a success and most beneficial usage of inoculation all through composting. Bioconversion of agricultural waste into compost is accomplished via way of means of diverse microbial communities.

2. Materials and Methods

2.1 Bacterial Strains used in Study

One bacterial strains *Bacillus flexus* AMB-T-6S was obtained from the previous study (Sahu *et al.* 2025), which was originally isolated and identified from the soil sediment collected from the Tatapani hot spring, located in the Balrampur district of Chhattisgarh, India (23.41° N latitude and 83.39° E longitude). This isolates was already reported for its isolated strains exhibited significant extracellular enzyme activities, including amylase, cellulase, lipase, protease, and xylanase (Sahu *et al.,* 2025).

2.2 Bio-degradation or Decomposition Assay

2.2.1. Submerged medium (Broth)

This experiment was conducted submerged condition with 500 mL conical flask; insure separately cut the sterile condition of rice, wheat and corn straw. All plant

Table 1: Treatment details on the effect of submerged condition of available straw.

S. No.	Treatment	Details
1.	T1	Control (Minimal broth without CMC)
2.	T2	Minimal broth with CMC
3.	T3	Minimal broth without CMC + AMB-T-6S
4.	T4	Minimal broth with CMC + AMB-T-6S
5.	T5	Minimal broth with rice straw
6.	T6	Minimal broth with rice straw + AMB-T-6S
7.	T7	Minimal broth with wheat straw
8.	T8	Minimal broth with wheat straw + AMB-T-6S
9.	T9	Minimal broth with corn straw
10.	T10	Minimal broth with corn straw + AMB-T-6S

material collected from the Institution farm of IGKV, Raipur. This experiment conducted on controlled condition of environment at used shaking incubator at 45°C temperature. A 15 days long experiment was setup in 500 mL conical flask with following treatment (table 1). CMC and/or rice straw, wheat straw and corn straw were used as a sole carbon source. All treatments were performed in triplicate with the completely randomized design (CRD) model. All the flasks were kept at 45 °C with shaking for 15 days. Observations were taken properly with specific time intervals of the day (0, 1, 2, 3, 5, 7, 9, 11, 13, 15 Day after incubation). Microbial growth was recorded by optical density at 600nm though UV-Vis spectrophotometer (aliquout 3 mL was taken from each treatment to record O.D. at 600 nm.

2.2.2 Dry state (Powder form)

The agro-waste from major cereal crops i.e. rice, wheat and corn were converted into powdered form using a kitchen mixture grinder machine. A 30 days experiment was setup with 500g of each powdered rice straw (RS), with bacterial inoculate AMB-T-6S (RSI), wheat straw (WS) with inoculate (WSI), corn straw (CS) with inoculate (CSI). Powdered RS, WS and CS were used as a control in which no inoculant was added during composting. The concentration 1×10^{8} CFU/mL of strain was mixed to keep 30% moisture whereas sterile water was added in control and all treatments were replicated thrice and completely randomized design (CRD) model was used (Table 2). This experiment was conducted one month (30 day) and temperature (45 ^{0}C) was provided by the incubator.

Table 2: Treatment details on the effect of bacterial inoculation on powder form of straw.

S. No.	Treatment	Details
1.	T1	Rice straw powder (control)
2.	T2	Rice straw powder + Bacterial isolate (AMB-T-6S)
3.	T3	Wheat straw powder (control)
4.	T4	Wheat straw powder + Bacterial isolate (AMB-T-6S)
5.	T5	Corn straw powder (control)
6.	T6	Corn straw powder + Bacterial isolate (AMB-T-6S)

2.3 Sample Analysis

2.3.1 Fourier Transform Infrared Spectroscopy (FTIR) analysis of degraded power

To assess the chemical alterations in both treated and untreated lignocellulosic substrates, Fourier Transform Infrared (FTIR) spectroscopy was performed using a Spectrum One FTIR system (Agilent Cary 630). Prior to analysis, all solid samples were oven-dried at 60 °C for three days. For FTIR measurements, the samples were finely ground, mixed with potassium bromide (KBr), and pressed into thin, transparent discs to ensure compliance with the Beer–Lambert Law. Spectral data were collected in the range of 400 to 4000 cm^{-1} (Guo *et al.,* 2008). The samples were analysis by Sophisticated Analytical Instrument Facility at CSIR-Central Drug Research Institute (SAIF, CSIR-CDRI) Lucknow, Uttar Pradesh, India.

2.3.2 Analysis of Nutrient status on compost material

For estimating total organic carbon (OC), N, P, K content & C:N ratio estimation the methodology was adopted from previously published report by Sahu *et al.* (2025).

2.4 Statistical analysis

The analysis was laid out in a Completely Randomized Design (CRD). The data obtained from various experiments under this study was analyzed by the method of analysis of variance as described by Gomez and Gomez (1984). The NARS, Indian Agricultural Statistics Research Institute, New Delhi, India (http:/www.iasri.res.in/sscnars) online statistical computation server has been used to statistically evaluate information.

3. Result and Discussion

3.1 Submerged medium (Broth)

Based on the preliminary screening on Carboxymethyl Cellulose (CMC) agar, the bacterial isolate AMB-T-6S exhibited the highest cellulolytic efficiency (**Table 3**) among all tested thermophilic isolates, as evidenced by a large clear hydrolysis zone and a high hydrolytic capacity index. Owing to its strong cellulase- and ligninase-producing ability, it was selected for detailed evaluation of lignocellulose degradation under submerged fermentation. Similar approaches have been recommended by Sahu *et al.* (2023), who emphasized that the selection of thermophilic lignocellulolytic bacteria based on enzymatic zone diameter and growth kinetics ensures efficient degradation of agricultural residues.

Table 3: Quantitative assessment of Cellulase enzyme production by AMB-T-6S strain.

Sample name	Clear Zone A (mm)	Colony B (mm)	Ratio (A/B) (mm)	Cellulolytic Index (mm)	Cellulase Activity (U/mL)
AMB-T-6S	36	11	3.3	2.27	2.980

The growth performance of AMB-T-6S was further evaluated in submerged broth containing RS, WS, and CS supplemented with CMC at 45 °C. Among the treatments, the maximum bacterial growth was recorded in T_2 (Rice straw + inoculum) with an optical density of 0.989 (at 600nm) on the 15^{th} day of incubation, followed by CS and WS inoculated broths (Fig. 1). The control treatments exhibited comparatively lower turbidity and absorbance, confirming active microbial proliferation in inoculated samples. The distinct turbidity observed in the inoculated broths (Fig. 2) and corresponding spectrophotometric readings at 600 nm demonstrated rapid substrate utilization and biomass formation by the isolate. The superior performance of AMB-T-6S compared to other isolates can be attributed to its thermotolerant nature, ability to produce a broad range of lignocellulolytic enzymes and efficient colonization on complex carbon substrates. Hence, AMB-T-6S was selected as the most potent strain for further biodegradation and FTIR analysis. To evaluate the decomposition of different straw types, samples were processed into dry powder form. Visual changes in color and physical texture were observed across treatment combinations from day 0 to day 30 after inoculation (Fig. 3), with inoculated samples showing more pronounced alterations compared to their respective controls. These physical transformations indicated active microbial degradation. Subsequently, the samples were subjected to FTIR analysis to investigate the chemical changes associated with the biodegradation process.

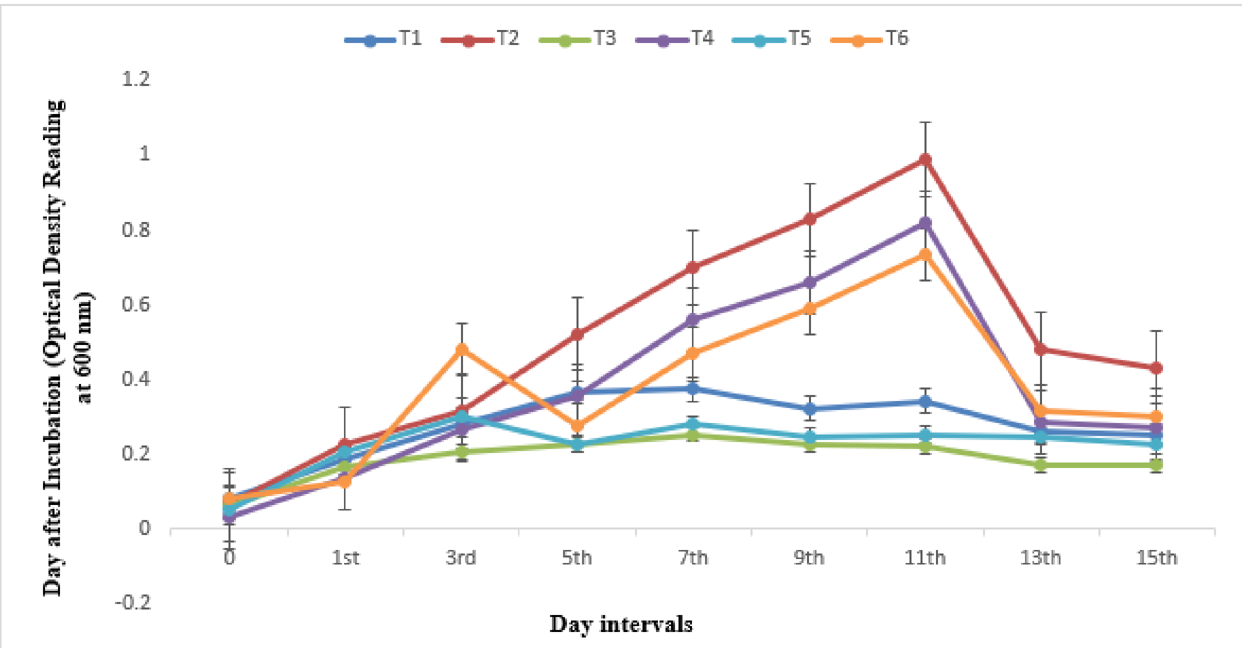

Fig 1: Microbial growth curve on broth medium

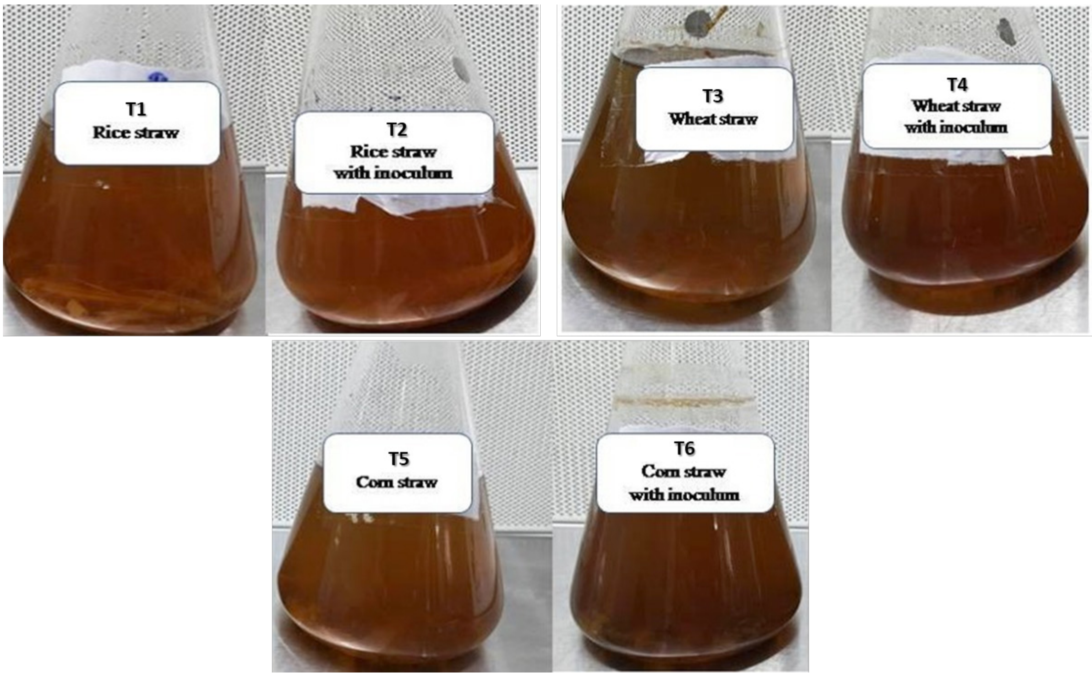

Figure 2: Performance during straw decomposed by isolate in broth form of thermophilic bacteria Different (AMB-T-6S).

Fig 3: Performance during straw decomposed by isolate in powder form of thermophilic bacteria (AMB-T-6S). Where is RS (rice straw), RSI (rice straw with inoculate), WS (wheat straw), WSI (wheat straw with inoculate), CS (corn straw) and CSI (Corn straw with inoculate).

3.2 Fourier Transform Infrared (FTIR) Spectral Analysis

FTIR spectroscopy was performed to assess structural alterations in ligno-cellulosic components of paddy, wheat, and corn straw samples degraded by the bacterial isolate AMB-T-6S over 30 days of incubation. The comparison of FTIR spectra between untreated and treated straw powders is presented in Figures 4–6.

The FTIR spectra of paddy straw powder (control- T_1) exhibited strong absorption peaks corresponding to cellulose and lignin components. The characteristic lignin band appeared at 1621.71 cm^{-1}, while deformation bands of cellulose and lignin were observed around 770.24 cm^{-1}. In the degraded paddy straw powder inoculated with AMB-T-6S (T_2), noticeable shifts were observed in the lignin-associated region, showing new peaks at 1733.6 cm^{-1} and 1637.6 cm^{-1}, indicating disruption in the aromatic skeletal vibrations (C=C) and breakdown of crystal structure in lignin. Similarly, deformation bands shifted to 984.89, 832.2 and 765.86 cm^{-1}, corresponding to C–O stretching and C–H in-plane and out-of-phase ring vibrations. These shifts suggest cleavage of cellulose linkages and partial depolymerization of lignin. Such spectral changes signify active degradation of ligno-cellulosic structures by *AMB-T-6S*, leading to reduced crystallinity and enhanced biodegradability of rice straw. The findings suggest that *AMB-T-6S* actively degrades lignin and cellulose. Notably, the observed spectral shifts and loss of crystallinity align with the structural changes reported by Patel *et al.* (2025) during the simultaneous saccharification and fermentation (SSF) of paddy straw using *Zymomonas mobilis* in combination with lignocellulolytic microorganisms.

The control wheat straw (T_3) spectrum showed characteristic cellulose bands around 4000–3500 cm^{-1} for O–H stretching and at 2416.78 cm^{-1} for C–H stretching and C=O stretching, typical of cellulose and hemicellulose. Upon degradation with AMB-T-6S (T_4), the spectrum revealed peaks at 3851.34 and 3742.31 cm^{-1}, indicating alterations in the O–H stretching vibrations and disruption in cellulose hydrogen bonding. The peak shift from 2416.78 cm^{-1} to 2922.27 cm^{-1} indicates modification of aliphatic C–H and C=O groups. In the lignin region (2000–1500 cm^{-1}), the appearance of peaks at 1732.92 cm^{-1} and 1639.36 cm^{-1} reflects deformation of the aromatic C=C bonds, signifying lignin oxidation or breakdown. Additionally, new peaks at 949.28, 834.15, and 757.05 cm^{-1} correspond to C–O stretching and C–H bending vibrations, confirming structural modifications in cellulose and lignin. These changes confirm that AMB-T-6S promoted significant structural rearrangement in the ligno-cellulosic matrix, leading to higher enzyme accessibility findings consistent with the microbial lignin degradation patterns reported by Sun *et al.* (2016).

FTIR analysis of corn straw includes absorption peaks stretching vibration of amino acid; 3010–2960 cm—1 band indicates symmetrical and antisymmetric stretching vibration peaks of –CH3, –CH2, and –CH in carbohydrate, lignin, and aliphatic compounds (Sahu *et al.,* 2025). The untreated corn straw (T_5) exhibited strong absorption at 3389.84 cm^{-1}, characteristic of O–H stretching vibrations in cellulose. The bacterial-treated sample (T_6) displayed peaks at 3851.85 cm^{-1} and 3738.53 cm^{-1}, suggesting alteration of hydroxyl groups and disruption of hydrogen-bonded cellulose structures. Significant changes were also noted in the low-frequency region (1000–450 cm^{-1}), where peaks shifted from 756.07 cm^{-1} (control) to 984.83, 832.83, and 769.85 cm^{-1} in the treated straw. These correspond to C–O stretching and C–H deformation, indicating cleavage of ether and ester linkages within the ligno-cellulosic matrix. The appearance of additional aromatic peaks in the 2000–1500 cm^{-1} region reflects lignin oxidation and partial depolymerization.

Across all straw types, the FTIR spectra of bacterial-treated samples exhibited clear shifts in characteristic peaks corresponding to cellulose (O–H and C–O groups) and lignin (aromatic C=C and C=O groups). These alterations confirm the enzymatic action of AMB-T-6S, likely involving cellulases, ligninases, and oxidative enzymes

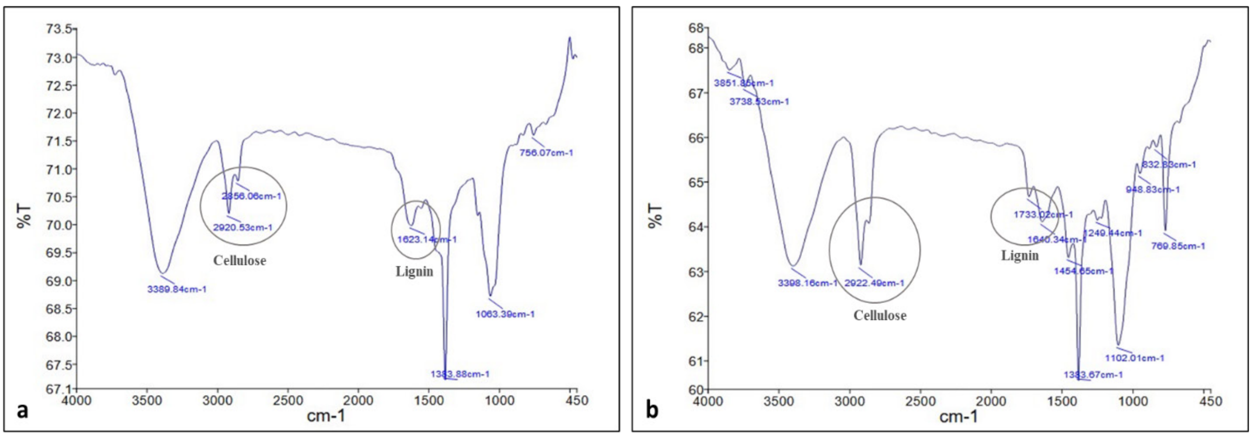

Fig 4: Comparison FTIR spectrum (a) powderd Rice straw (T_1) and (b) powderd Rice straw +AMB-T-6S (T_2) at 30 days after incubation.

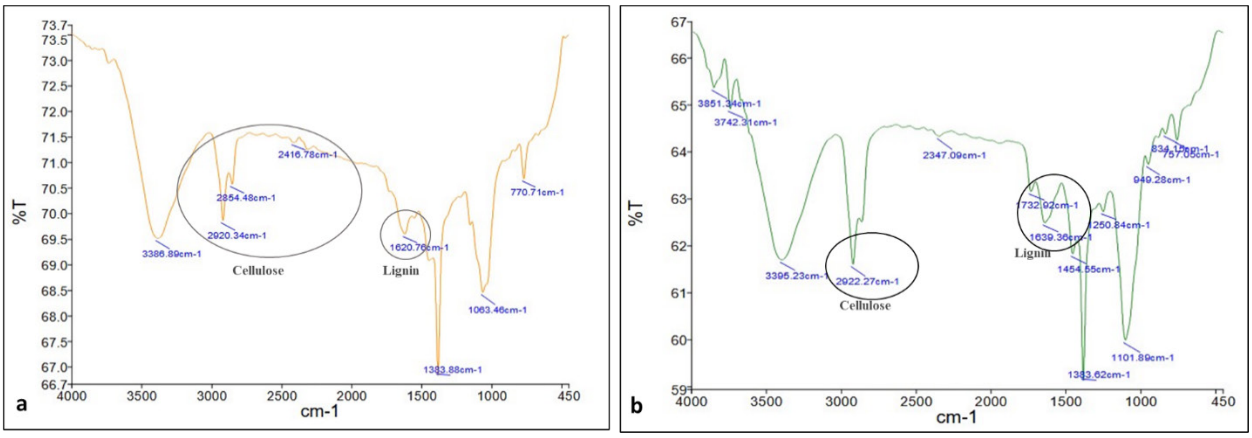

Fig 5: Comparison FTIR spectrum (a) powdered Wheat straw (T_3) and (b) powdered Wheat straw + AMB-T-6S (T_4) at 30 days after incubation.

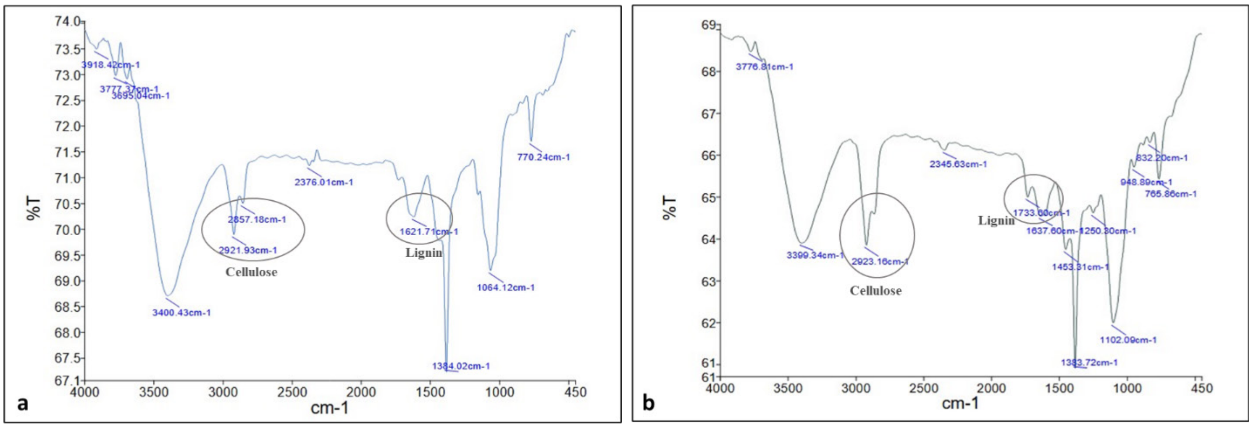

Fig 6: Comparison FTIR spectrum (a) powdered Corn straw (T_5) and (b) powdered Corn straw + AMB-T-6S (T_6) at 30 days after incubation.

capable of breaking β-1,4-glycosidic bonds and aromatic ether linkages. The decrease in band intensity and peak shifts toward lower wavenumbers imply degradation and reduced crystallinity of cellulose and lignin components. The microbial degradation causes rearrangement of functional groups and reduction of lignin aromaticity (Kumar & Sharma, 2017). Thus, FTIR results substantiate the potential of *AMB-T-6S* in effective bioconversion of straw residues, enhancing their suitability for composting or bioenergy applications.

3.3 Total OC, total N, P, K content and C: N ratio, of compost powder

The biochemical analysis of degraded straw revealed that inoculation with the thermophilic bacterial isolate *AMB-T-6S* significantly improved nutrient mineralization and organic matter decomposition. The observed decreases in organic carbon and C:N ratio, along with increases in nitrogen, phosphorus, and potassium, demonstrate the isolate's strong lignocellulolytic potential (Fig. 7). These findings suggest that AMB-T-6S can be effectively employed for rapid straw composting, enhancing the nutrient profile and reducing environmental pollution from agricultural residues.

3.3.1 Available Total Organic Carbon (OC)

The reduction in total organic carbon during the biodegradation process was mainly due to microbial oxidation of organic matter, leading to the release of energy and carbon dioxide as metabolic end products. The highest reduction in OC was observed in the inoculated treatments compared to the uninoculated controls, indicating effective microbial decomposition of straw. Among all treatments, the lowest organic carbon content was recorded at 30 days in T_2 (Rice straw with inoculum) (68.67%), followed by T_4 (Wheat straw with inoculum) (69.00%). The reduction in OC suggests efficient utilization of carbon compounds by the bacterial isolate AMB-T-6S for energy generation and biomass synthesis. The reduction in the OC content is due to the activity of lignocellulolytic microorganisms reflecting microbial conversion of cellulose and lignin into simpler metabolites and CO_2 (Zhu *et al.,* 2021).

3.3.2 Total Nitrogen (N)

Nitrogen content significantly increased in the inoculated treatments compared to the uninoculated controls. The maximum total nitrogen was recorded in T_6 (Corn straw with inoculum) (0.58%), followed by T_4 (Wheat straw with inoculum) (0.53%). In contrast, the lowest nitrogen content was observed in T_3 (Wheat straw control) (0.43%), followed by T_5 (Corn straw control) (0.48%). This enhancement of nitrogen in inoculated samples could be attributed to microbial proliferation and the synthesis of proteinaceous cellular components during straw decomposition. The bacterial isolates being lignocellulolytic and thermophilic, likely contributed to the enrichment of available nitrogen through nitrogen fixation and ammonification processes (Bang *et al.,* 2024).

3.3.3 Total Phosphorus (P)

Phosphorus levels increased significantly in the inoculated treatments compared to controls. The maximum total P content was recorded in T_6 (Corn straw with inoculum) (0.59%), followed by T_5 (Corn straw control) (0.55%). The

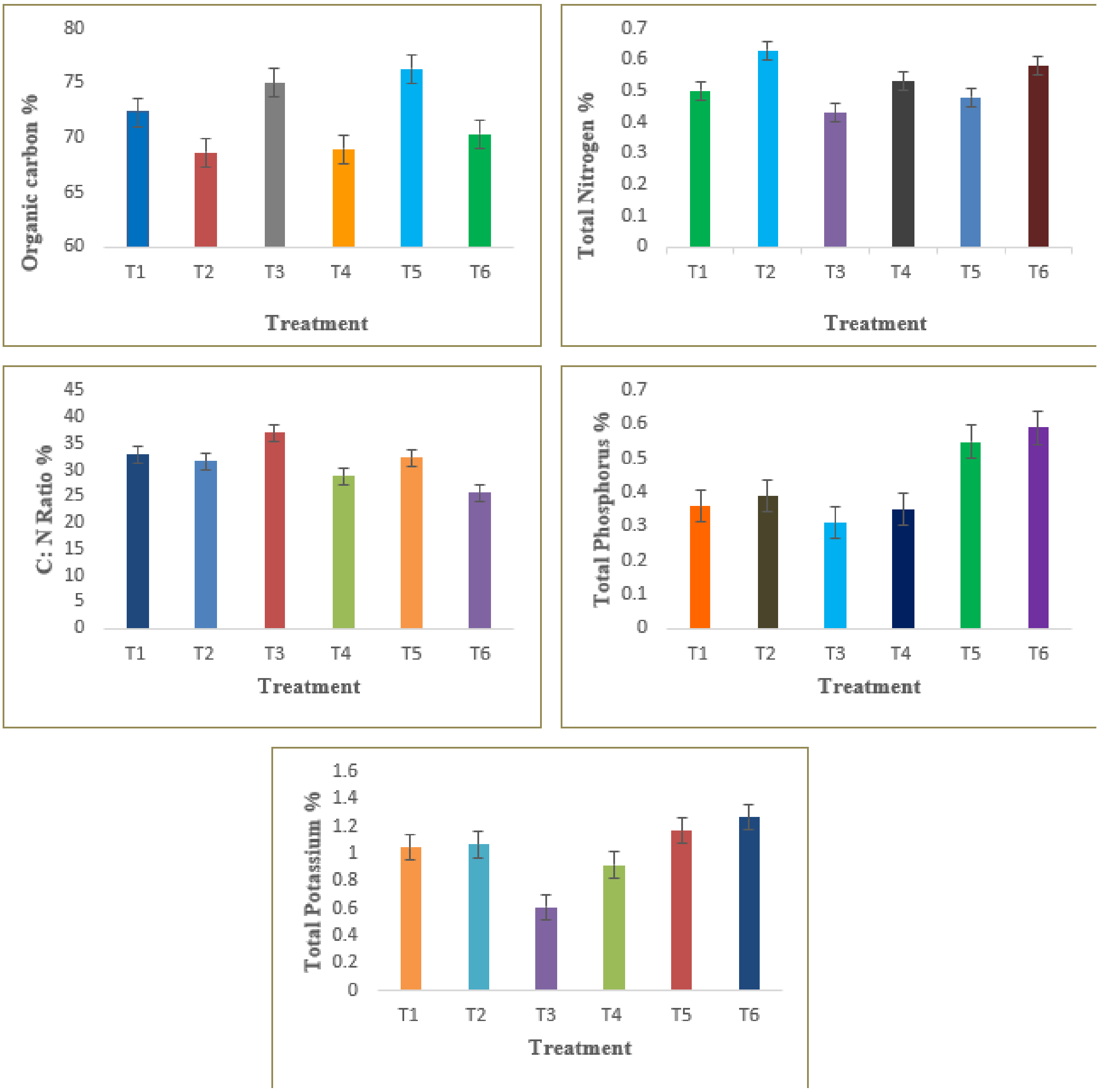

Fig 7: Available total organic carbon, total nitrogen, total phosphorus, potassium and C:N ratio estimation

minimum P content was found in T_3 (Wheat straw control) (0.31%), followed by T_4 (Wheat straw with inoculum) (0.35%). This increase in P content upon microbial inoculation may be due to the solubilization of inorganic phosphates during biodegradation and the release of phosphorus from organic complexes through microbial phosphatase activity (Zhang *et al.,* 2021).

3.3.4 Total Potassium (K)

A significant increase in K content was also recorded in the inoculated treatments. The highest K concentration was found in T_6 (Corn straw with inoculum) (1.27%), followed by T_5 (Corn straw control) (1.17%). The lowest K content was observed in T_3 (Wheat straw control) (0.61%), followed by T4 (Wheat straw with inoculum) (0.92%). The increase in K levels can be attributed to the mineralization of plant cell walls and the release of bound potassium during microbial degradation (Sharma *et al.,* 2024; Huo *et al.,* 2023). The enzymatic activity of *AMB-T-6S* may have enhanced the solubilization of K compounds, improving its availability in the degraded substrate.

3.3.5 Carbon-to-Nitrogen (C: N) Ratio

The C:N ratio is an important indicator of compost maturity and the balance between carbon and nitrogen during biodegradation. The maximum C:N ratio was recorded in T_3 (Wheat straw control) (37.01), followed by T_1 (Rice straw control) (32.88). The lowest C:N ratio was found in T_6 (Corn straw with inoculum) (25.61), followed by T2 (Rice straw with inoculum) (31.64). A lower C:N ratio observed in the inoculated samples indicates accelerated decomposition and enhanced stabilization of organic matter. Microbial degradation facilitated the reduction of carbon content while simultaneously increasing nitrogen availability, resulting in a decreased overall C:N ratio. This decline in the C:N ratio during microbial treatment reflects efficient mineralization and compost maturation, thereby rendering the substrate more suitable for agricultural application (Xie *et al.,* 2022).

4. Conclusion

A thermophilic bacterium, isolate *Bacillus flexus* (AMB-T-6S) from the Tatapani hot spring, effectively degraded the lignocellulosic components of rice, wheat, and corn straw. Among these substrates, corn straw exhibited the highest enzymatic degradation efficiency when inoculated with AMB-T-6S. FTIR analysis confirmed this, revealing cellulose and lignin breakdown. These findings establish AMB-T-6S as a potent cellulolytic agent for converting agricultural waste into nutrient-rich compost, with corn straw being the optimal substrate for both microbial degradation.

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Data Availability

The data supporting the findings in the manuscript is available from the corresponding author on a reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

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Authors' contributions

All the authors equally contributed in the preparation of the manuscript.

Ethical Approval

The article doesn't contain any study involving ethical approval.

Use of Generative AI or AI-assisted technologies

Authors declare that generative AI or AI-assisted technologies have not been used in the preparation of this manuscript

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