Nature Environment and Pollution Technology
An International Quarterly Scientific Journal
p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454
Vol. 23 No. 2 pp. 761-773 2024
Original Research Paper
https://doi.org/10.46488/NEPT.2024.v23i02.013
Open Access Journal

Biodegradation of Cellulosic Wastes and Deinking of Colored Paper with Isolated Novel Cellulolytic Bacteria

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 16-11-2023
Revised: 19-12-2023
Accepted: 27-12-2023

Key Words:
Cellulases
Bioethanol
Bacillus paramycoides
Deinking
Endoglucanases

ABSTRACT

Biofuels are the cheapest source of energy, and the continuous decline of traditional sources of energy with the increasing population leads to looking for alternatives to reduce the consumption of traditional sources of energy. Bioethanol production from lignocellulosic wastes and cellulosic wastes is not a new approach for fuel production but a cheap and accessible way for the production of fuel. *Bacillus* is one of the major species that can act as a source of diversified enzymes. In this study, it was emphasized on screening and isolation of a novel, characterization, and best catalytic action on both celluloses and proteins in the presence of different carbon and nitrogen sources. It was observed the effective catalytic breakdown of cellulose with the crude enzyme to glucose allowed fur for fermentation with *Saccharomyces,* ultimately leading to the generation of alcohol. The study aims to isolate the microbes that can produce cellulases and enzymes and could be used for biodegradation to produce ethanol in the reaction. The maximum enzyme activity was achieved at 3.112 UI with optimized pH and temperature, and the maximum conversion of sugars into alcohol was about 70% in the newspaper, cartons, colored paper, and disposable paper cups. An essential observation was the decolorization of the origami craft paper within 24 hours. The study was involved in enhancing the maximum Enzyme activity of cellulases from different cellulosic raw materials. Hence, it was achieved by JCB strain, optimization of pH, temperature, and acids for the biodegradation. The presence of peaks at 3200 and 2900 was a confirmation of ethanol bonds in the biodegradation reaction mixtures.

INTRODUCTION

The increasing demand for fuel and dependency on fossil fuels leads to the search for alternatives for energy (Sun et al. 2022). Within the global energy industry, fossil fuels stand out as a pivotal non-renewable resource. They played a crucial role in driving the Industrial Revolution and fostering technical, social, and economic progress. However, the excessive reliance on these non-renewable fuels in industrial and transportation processes has led to environmental pollution. The combustion of fossil fuels releases greenhouse gases, contributing to climate change and global warming. Moreover, the rapid depletion of fossil fuel reservoirs due to over-exploitation emphasizes the urgent need to explore alternative, renewable energy sources to address future energy challenges. In response to this dilemma, lignocellulosic biofuels emerge as a promising alternative energy source capable of replacing fossil fuels. Lignocellulosic biofuels, derived from flexible ligninolytic microbes, offer a sustainable solution by converting lignocellulosic biomass (LCB) and biological waste into energy fuels. This approach presents a significant opportunity to mitigate the pressing issue of environmental pollution caused by fossil fuel use. By harnessing the potential of lignocellulosic biofuels, we can pave the way for a more sustainable and eco-friendly energy future (Rath et al. 2022). Bioethanol has piqued the interest of researchers in recent decades due to its versatility in applications such as hydrogen generation, pharmaceutics, and fuel. Cellulosic ethanol is routinely produced from lignocellulosic biomass via pre-treatment, enzymatic saccharification, and fermentation. However, the refractory nature of lignocellulosic biomass inhibits enzyme accessibility, making conversion into fermentable sugars and bioethanol more challenging. Many straw biomass resources in China have been squandered and cannot be efficiently utilized (Xian et al. 2022). Renewable biofuels such as biodiesel, bioethanol, and biobutanol are long-term solutions to the depletion of fossil fuels. Plant biomass is a sustainable feedstock for their production, which is degraded to sugars with the help of microbe-derived

enzymes, followed by microbial conversion of those sugars to biofuels. Given their global need, more attempts have been made to produce them on a big scale, which is ultimately leading to breakthrough research in biomass energy (Sartaj et al. 2022). The bulk of the world's energy demand is fulfilled by fast-depleting fossil fuels, which are also environmentally harmful. With rising global energy consumption, the price of fossil fuels significantly rises every quarter. As a result, there is an urgent need for an alternative that not only meets energy demand but is also clean and renewable. Various renewable feedstocks, mainly lignocellulosic biomass, are fermented by microorganisms to create biofuel and other bio-based platform chemicals (Sahoo et al. 2023). In countries with a large agricultural population, like India and other Asian nations, improper handling of biowaste can seriously contaminate the environment. The most obvious, economical, and ecologically friendly method will be microbial waste degradation, as most biowaste can be readily broken down by many microbial communities and successfully converted into less toxic or hazardous forms. Certain bacteria target plant-based celluloses, which are the primary structural element of most plant cells, by producing cellulases. Several energy-dense derivatives are produced by this process, including glucose, cellobiohydrolase, and others, which may be used to make bioethanol. Enzymes called cellulases hydrolyze glucose's polymeric structure, which is found in the majority of waste products derived from plants (Bose & Sarwan 2023). the shift to biomass valorization by biorefineries, including the composition of the biomass (cellulose, hemicellulose, and lignin if it is lignocellulosic material; humidity level, distribution of triglycerides and fatty acids, concentration of sugars if wet wastes), the classification of biorefineries (from first to fourth generation), the target products that can be produced (ethanol, biodiesel, methane, hydrogen), and the process technologies (biochemical, chemical, or thermochemical) used for biomass transformation (Giuliano 2023). The deinking process of the cellulosic wastes remains in the sewage treatment, deinking of the colored paper can lead to the eco-friendly approach to the wastewater treatment. Similarly, during the deinking process of the recycling paper, chemical methods are used for deinking the colored paper. Therefore, using cellulolytic bacteria is helpful as a cost-effective and nontoxic method for deinking the colored paper. Several examples of effective protease-producing Bacilli strains in the bacterial community are *B. amyloliquifaciens, B. subtilis,* and *B. licheniformis*, besides other bacterial species known as *Staphylococcus, Pseudomonas, Serratia, Alcaligenes, Vibrio, Brevibacterium, Flavobacterium and Halobacterium* (Sarwan & Bose 2022). The main aim of this study is to isolate and characterize novel bacterial strains from soil and to determine their ability to produce cellulase and protease enzymes and their potential for degradation of various bio-waste (Sarwan & Bose 2021, 2022, Sarwan et al. 2021, Sharma et al. 2023) Therefore using the novel cellulolytic bacteria for the degradation of cellulosic wastes and deinking of colored paper can be used as a cheap and nature-friendly approach for the degradation and energy production.

MATERIALS AND METHODS

Sample Collection for the Isolation of the Cellulases-Producing Microorganisms

Different soil samples were collected with gloves and kept in sterilized polybags. Collected soil samples were brought into the lab. The soil samples were serially diluted in the (v/w) 1g in 99 mL of distilled water. For the isolation of cellulolytic microbes, CMC media was prepared by using 1% CMC, 0.01g $MnSO_2$, 0.01g $MgSO_4$, 0.01g K_2HPO_4, 0.01g KH_2PO_4 in total volume of 100 mL in Erlenmeyer flask (Darwesh et al. 2020). Media constituents were mixed thoroughly, pH was maintained from 6.7-7.2, and plates were incubated for 48 h at 37°C. Pure cultures were obtained with the streaking method.

Visualization of Cellulase Enzyme Activity with Congo Red Assay

The plates were made with the mentioned CMC agar and floored with Congo red solution after the incubation of 48 h of the cultures. Congo red was used as aqueous Congo red of 1% in 100 mL of distilled water and treated for 15 minutes on the plates. After the treatment of Congo red to the plates, the plates were treated with NaCl solution for 10 minutes. Clear zones on the plates were showing cellulolytic activity in isolated microbe (Saleh et al. 2023).

Enzymatic Assay of Cellulases by Microbes

Subculturing bacteria in the Luria broth culture medium with cellulose and 50 mM of citrate buffer at pH 5 was used for the enzymatic test, which was then incubated for 48 h at 140 rpm in a shaking incubator. After centrifuging for 15 min at 40°C at 10,000 ×g, the supernatant was recovered. After treating the 0.2 mL supernatant with CMC, phosphate buffer was added to bring the pH down to 6.5. Using the 3,5-dinitric salicylic acid (DNS) technique, the total reducing sugars were determined and compared to the glucose standard curve (Islam 2019, Shanmugapriya et al. 2012). One unit of enzyme activity denoted as U/mL, can be used to further describe the enzyme activity of microbial enzymes. The amount of enzyme involved in this activity is measured in units of 1 u mol of reducing sugar released per minute (Asem

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et al. 2017, Zeng et al. 2016). Using the Lowry method and a colorimetric technique with Folin reagent, determine the free amino acid content of a given sample (Islam et al. 2019, Mothe & Sultanpuram 2016, Singhania et al. 2018). One mol of reducing sugar is released each minute by the amount of enzyme involved in this enzyme activity. The equation used to determine enzyme activity.

$$\text{Enzyme Activity (U/mL)} = \frac{\mu\text{g of glucose released} \times 1000}{\text{Mol. of glucose} \times \text{incubation time}}$$

Determination of Different Cellulases in Isolated Microbes

Exoglucanases: For determination of the presence of exoglucanase activity in the microbial cells, the cells were treated with a solution of 1% avicel dissolved in 50 mM of citrate or phosphate buffer of the total volume of 400 µL, and pH was adjusted 5 -5.5. 0.5 mL of crude enzyme mixture was incubated at 50°C for 1 h in a water bath. For termination of the reaction, the mixture was instantly kept in an ice bath. The reaction mixture was centrifuged at 10000 rpm for 10 min at 40°C. After collecting the 0.5 mL of supernatant added 0.5 mL of sulphuric acid and 1% phenol in it. The presence of free-reducing sugar was determined with the DNS method on a UV-Vis Spectrophotometer at 450nm and compared with the glucose standard curve(K & Sarwan, 2023b)

Endoglucanases: For determination of the endoglucanase activity in the isolated microbes was analyzed with 1% soluble cellulose by maintaining pH 6.7 with phosphate buffer in 1mL of crude enzyme and incubated for 2 h at 50°C in a water bath. The supernatant was collected for centrifuge at 10000rpm for 6 min, and enzyme assay was calculated with the DNS method on spectrophotometric readings (Nguyen & Hoang 2020).

β-Glycosidases

For determination of the beta glycosidases enzyme activity, 0.5mL of the crude enzyme was mixed with 1% cellobiose/ lactose in 100 mL by adjusting pH at 6 in 50mM phosphate buffer for 2 h at 40°C in a shaking incubator. The mixture was centrifuged at 10,000 rpm, and the supernatant was collected for DNS assay on UV –A visible spectrophotometer at 450nm.

Lignin Peroxidases: To perform the assay, 1mL of culture broth from each isolate was placed in Eppendorf tubes, and the culture broths were subjected to centrifugation at 4°C and 7000 rpm in a cooling centrifuge. Given that lignin peroxidase is extracellular, the supernatant from each centrifuge tube containing the enzyme was collected for further analysis. For the enzyme test, a reaction mixture was prepared by combining 1 mL of 50 mM sodium potassium tartrate (pH 4) buffer with 0.1 mL of a 0.1 mM H_2O_2 inducer. This solution was supplemented with 32 µM methylene blue, and 10 µl of the enzyme supernatant was added to initiate the reaction. To serve as a control, a separate test tube was prepared by adding 10 µl of distilled water to the aforementioned reaction mixture in lieu of the enzyme supernatant. The assessment of enzyme activity was based on the observed % decolorization of the methylene blue dye. This experimental design allows for the evaluation of LiP's demethylation capabilities under specified conditions, providing valuable insights into its enzymatic activity. (Rath et al. 2022). The final test solution was incubated at room temperature for 1 hour before the absorbance was measured using a UV-Vis spectrophotometer.

Identification of the Isolated Microbes

Microscopic identification was done of the isolated microbes. The isolate that exhibited the largest hydrolytic zone indicating the highest enzyme activity compared to the other isolates, was chosen for 16s RNA ribotyping sequencing. The 16s RNA sequencing was conducted using universal primers. The forward primer used was 926-(3' AAA CTC AAA GGA ATT GAC GG 5'), while the reverse primers used were -518-(5' ATT ACC GCG GCT GCT GG 3') and 1100-(5' TTG CGC TCG TTG 3') (Carvalho et al. 2021, Rashid et al. 2022). Phylogenetic analysis of the isolated microbes. With the obtained Fasta sequence files of the isolated microbes, the FASTA sequences were aligned in the Nucleotide blast of the NCBI site and found most similar microbes and again aligned with multiple sequence sites. The construction of a phylogenetic tree was performed using the MegaX software. This involved comparing the detected isolate with a set of neighbor-joining organisms and determining the degree of similarity between them (Carvalho et al. 2021)

Biodegradation of Wastes for Utilization in Bioethanol Production

The aim of the select different cellulosic wastes to be degraded with cellulolytic microbe and utilization of the reducing sugars with yeast. There were different cellulosic wastes taken including newspaper, colored paper, cartons, and paper cups. These four wastes were added to the MSM mentioned above medium, and cellulosic wastes were utilized to make reducing sugars as the sole of carbon. Therefore, various cellulosic substrates were successfully utilized in minimum essential medium, and the presence of reducing sugars was observed with the DNS method. The process of biodegradation involved – chopping down different cellulosic or lignocellulosic wastes into fine pieces

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and added into the minimum essential medium with w/v in 100 mL of distilled water each. The medium was autoclaved at 121 and 15 psi pressure for 20 min. After cooling down of the medium, in the LAF hood, crude enzyme 0.5 mL was inoculated and kept for incubation at 34°C for 24 h-114 h. The determination of the reducing sugars released by crude enzyme was calculated with three different parameters: first was the traditional method DNS, second was the refractometer, and third was the glucometer. A glucometer is a device that can be used as a biosensor in human blood and biological reactions. The amount of sugar mg.dL^{-1} in the glucometer can be converted into mg.mL^{-1}. Once the monitoring of reducing sugars was done, the inoculation of yeast cells was performed. The local baker's yeast was purchased from the bakery and activated with a sugar solution for 24 h at 28°C. The inoculum of activated yeast cells of 100 µL was then procured into the reaction mixture. The reaction mixture was analyzed with different parameters from 0 h to 114 h, like sugars, pH, alcohol, and cell growth (Ega et al. 2020, Deka et al. 2013, Dadwal & Satyanarayana 2020).

Estimation of Alcohol with Potassium Dichromate Method

The presence of ethanol content was estimated with the potassium dichromate method and a standard curve of ethanol was prepared. Estimation of acid production with pH paper- the production of acids in the reaction was observed from time to time with litmus paper and a pH meter.

Effect of Different Parameters on Biodegradation

There were four different parameters taken to optimize the best possible cell growth, enzyme activity, and biodegradation in the reaction mixture. These parameters were as follows: Obviously, the pH of the mixture and different sets were taken in triplicates to enhance maximum cell growth and enzyme activity. Different temperature sets from lowest to highest range 15 degrees C to 60 degrees

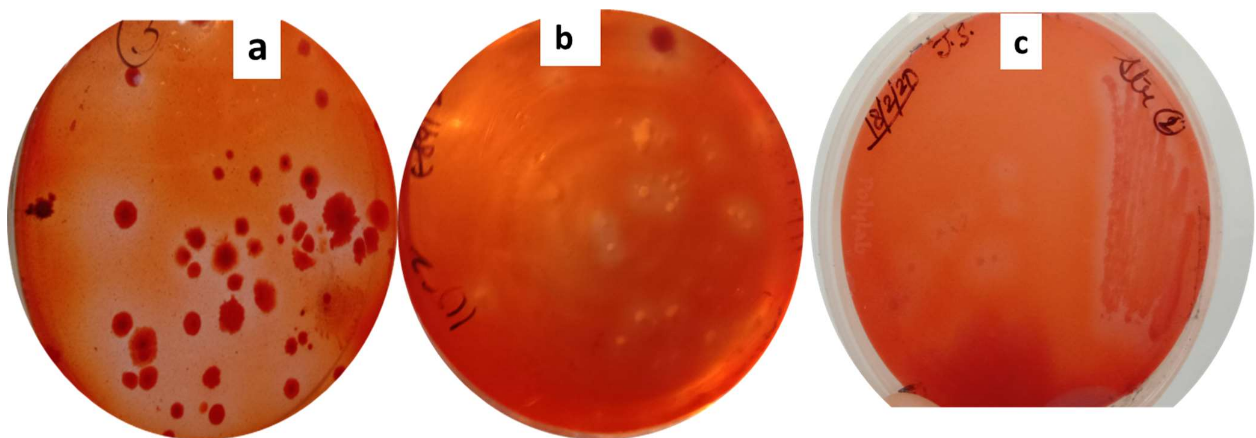

Fig. 1: An illustration of screening of cellulases producing microbes a), b), and c) describes the zone of clearance on CMC agar plates.

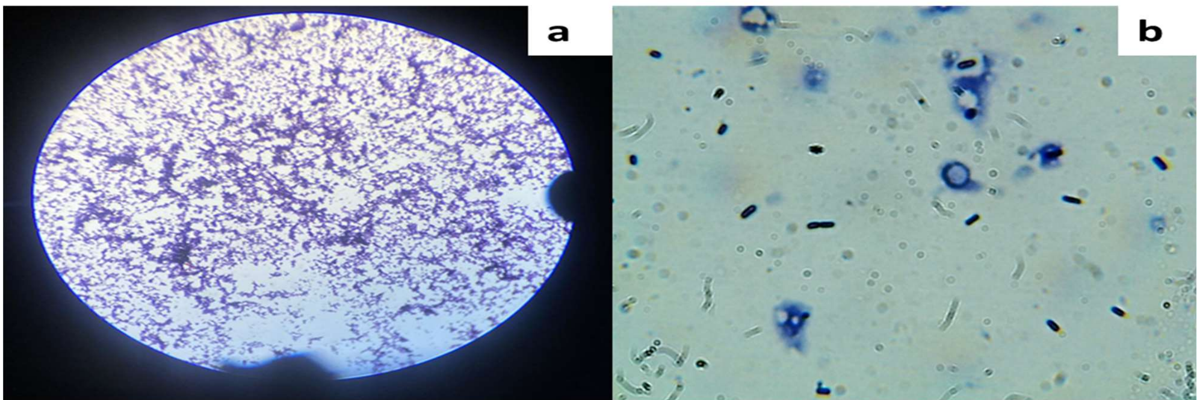

Fig. 2: Microscopic studies of isolated bacteria under a) compound microscope and b) Metzer Vision Plus-5000 microscope.

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Celsius. Similarly, there were different pH ranges taken from pH 4 -12 (Yaya et al. 2021, Restuhadi & Silaturahmi 2019, Zeng et al. 2016, Liggieri et al. 2009). Effect of inoculum size from 0.1mL to 2mL and different concentrations of the cellulosic wastes or substrate were also determined to achieve the maximum degradation (Rojas et al. 2009). At last, for every optimization parameter, the DNS assay was done.

RESULTS AND DISCUSSION

During the isolation process, the soil samples were collected from the sites where cellulosic wastes were discarded. The isolation procedure was done with sterile conditions. The screening of various microorganisms was done with a nutrient agar (Himedia) medium, and there were 10 microbes selected for the isolation process. Isolates were purified with the streaking method and further screening. Screening of cellulase enzymes was done with CMC media plates, and the hollow zone appearance was an indication of the utilization of cellulose in the medium by the microbes. Screening of cellulases-producing bacteria among 10 microbes with similar zones of clearance was further analyzed with cellulases assay. After qualitative analysis of cellulases, microscopy studies were performed with gram staining (Bose & Sarwan 2023), and the colonies of microbes were observed under two different microscopes. Fig. 1 describes the screening of cellulases-producing microbes with a zone of inhibition on CMC agar plates. One picture was captured in a light microscope, and another was observed under Metzer Vision plus-5000 under a 100x lens. With microscopic studies, it was revealed that isolated microbes were gram-positive, elongated, and more similar to bacilli as well as IMVIC tests of the isolated microbes revealed gram-positive and bacillus family of bacteria. Fig. 2 describes the microscopic studies of isolated bacteria under a compound microscope and Metzer under 100x resolution. Fig. 1 describes 3 different strains with higher and lower cellulase activity: JCA, JCB, and JCC.

A bacterial strain was identified – with 96% similarity to *Bacillus paramycoides,* and the phylogenetic tree was built with the software MegaX. Neighbor-joining method opted to identify closely related species with *Bacillus paramycoides*. Fig. 3. showing phylogenetic analysis and relation with neighbor-joining species (Carvalho et al. 2021). The Data was submitted to NCBI and got an accession number in Genebank with ON150894 (Thakur et al. 2019, Sarwan & Bose 2021, 2022, Ganesan et al. 2021).

Fig. 2 describes the fact that after the isolation of cellulases-producing enzyme microbes, morphological studies were performed on the isolated strain of the microbes. With morphological studies, it was gram-positive, bacilli-structured, and bead-like bacteria under the microscope. Other studies revealed that it was catalases negative, Simmon citrate positive, IMVIC positive strain and sent for further genomic sequencing. During isolation among 10 isolates, 3 isolates were giving similar cellulase activities. They were named JCA, JCB, and JCC. The JCB giving maximum enzyme activity was 2.901 UI (Bose & Sarwan 2023b).

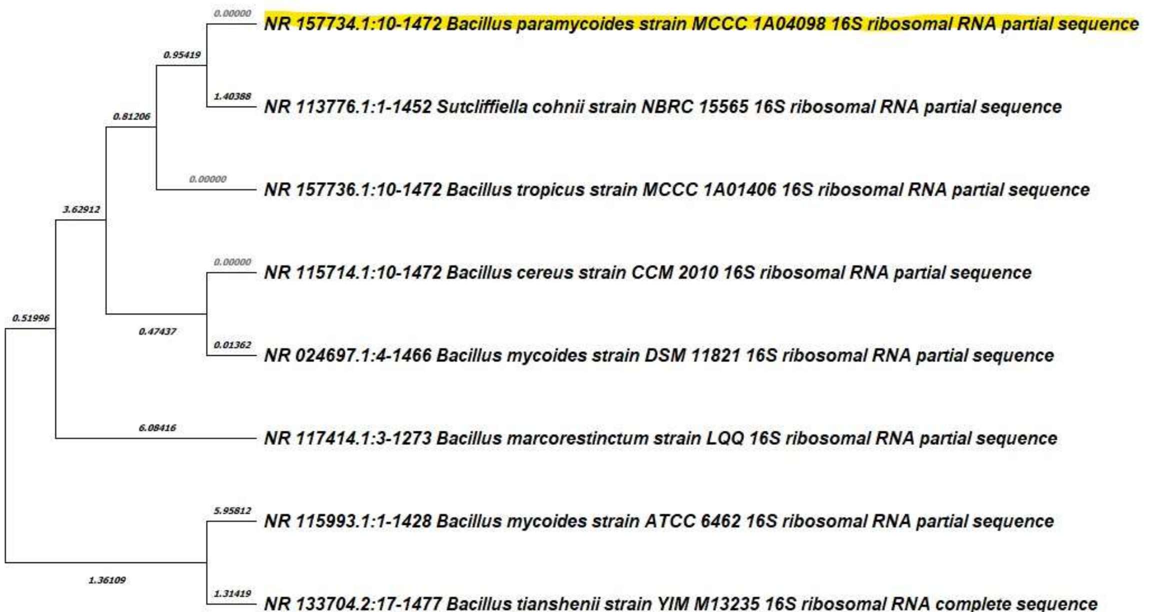

Fig. 3: Phylogenetic tree of isolated bacterial strain.

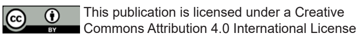
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Fig. 3 illustrates the similar neighbor species. During the construction of the phylogenetic tree from the NCBI nucleotide blast, there was the presence of numerous fumigates- unidentified species. Therefore, the authors recognized with most similar strains and made a phylogenetic tree with Mega X software.

Figs. 4 and 5 describe the effect of pH and temperature on different factors of cells like cell growth. The growth curve of bacteria was maximum achieved, giving 1.209 spectrophotometric readings after 48 h at 37°C and pH 7.8. Similarly, enzyme activity was 2.98 UI at the same temperature and pH, biodegradation, called ethanol production, was maximum achieved at pH 8 and temperature 34°C was 60-70% according to ethanol standard. Figs. 6 and 7 showing the maximum activity was found in 1mL of inoculum, and 1.5 g of substrate concentration was 2.189 UI and 3.156 UI.

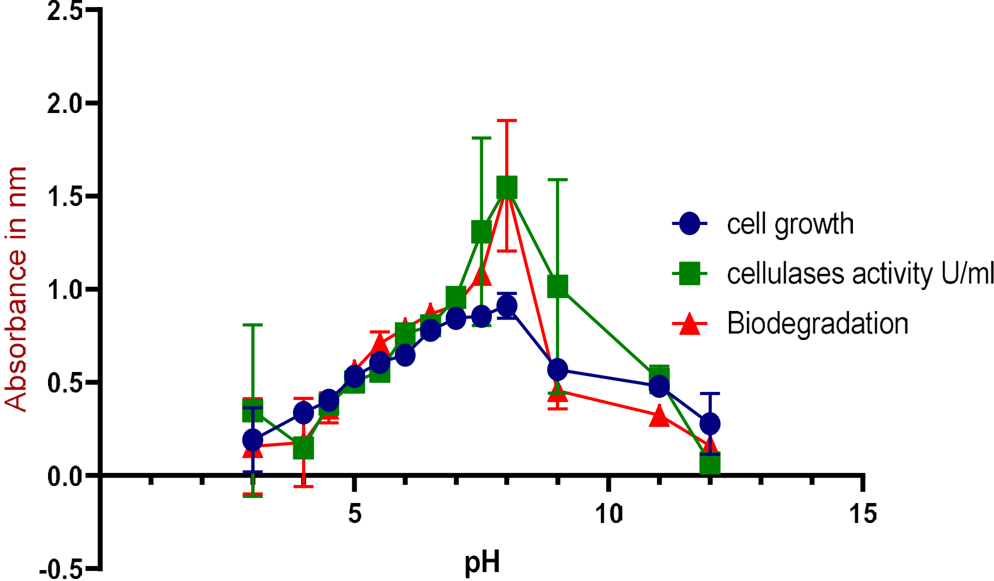

Fig. 4: Optimization of pH for maximum cell growth, cellulase activity, and biodegradation.

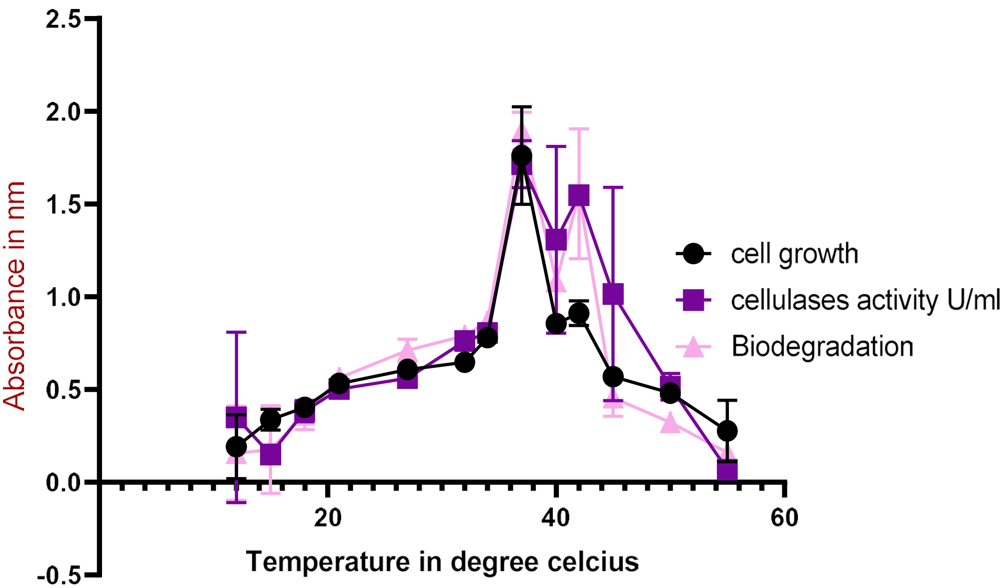

Fig. 5: Optimization of Temperature for achieving maximum cell growth, cellulase activity, and biodegradation.

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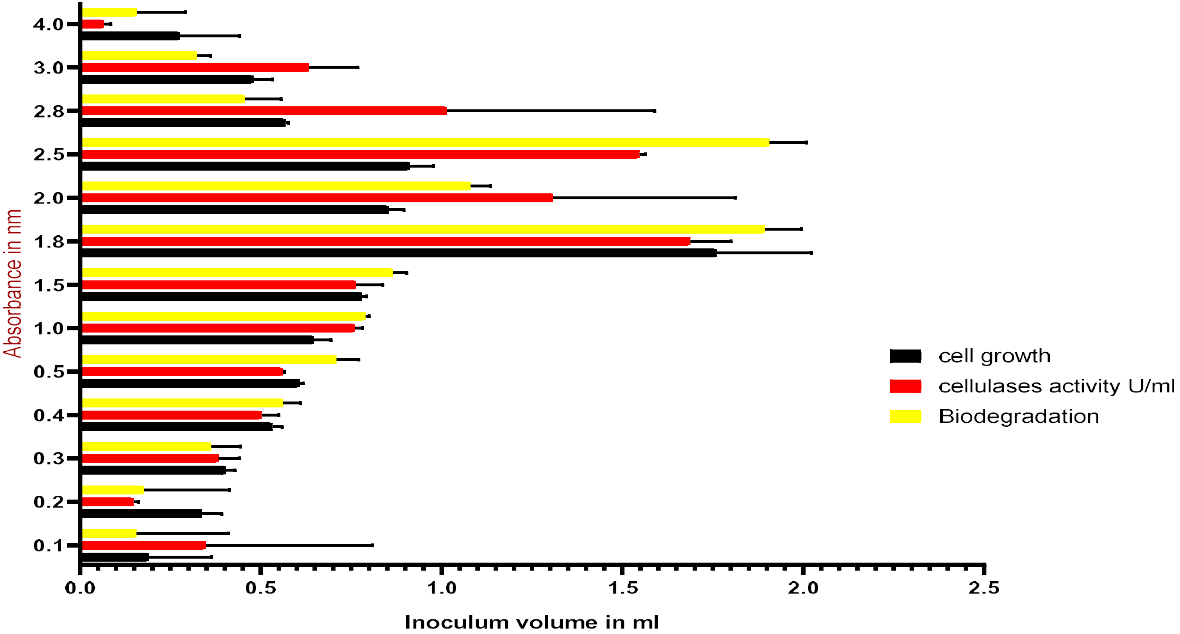

Fig. 6: The effect of inoculum size on cellulases, cell growth, and biodegradation.

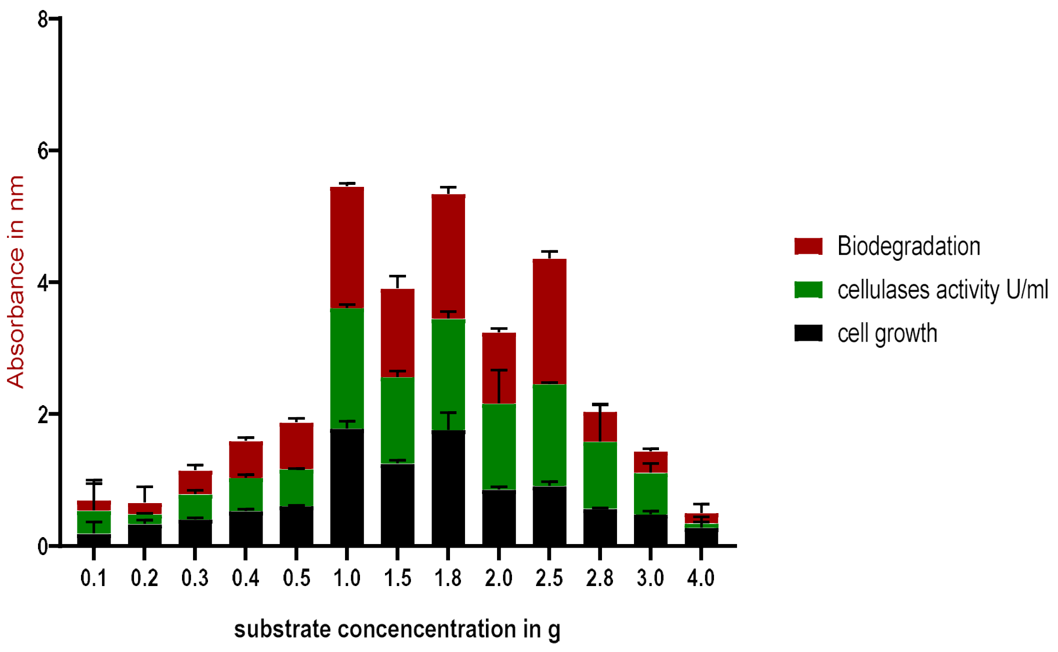

Fig. 7: Representing the effect of substrate concentration on enzyme activity.

Fig. 8 describes the list of various cellulosic wastes for biodegradation. Before being used as a substrate, they were finely chopped. Next, Fig. 9 describes the evaluation of sugar during biodegradation. During cellulase activity, sugars were released and calculated. Still, when reducing sugars were completely utilized, the amount of free reducing sugars decreased, but again, inoculation of the yeasts cells, the sugar release was observed as in Fig. 4 (Fu et al. 2022). Among all four cellulosic wastes, Newspapers are called NPS, Paper cups as PCS, Cartons CTS, and Purple paper PPS. Per the studies, paper cups were not as disposable as they claim, it takes at least 15 days to

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Fig. 8: Different cellulosic substrates for biodegradation.

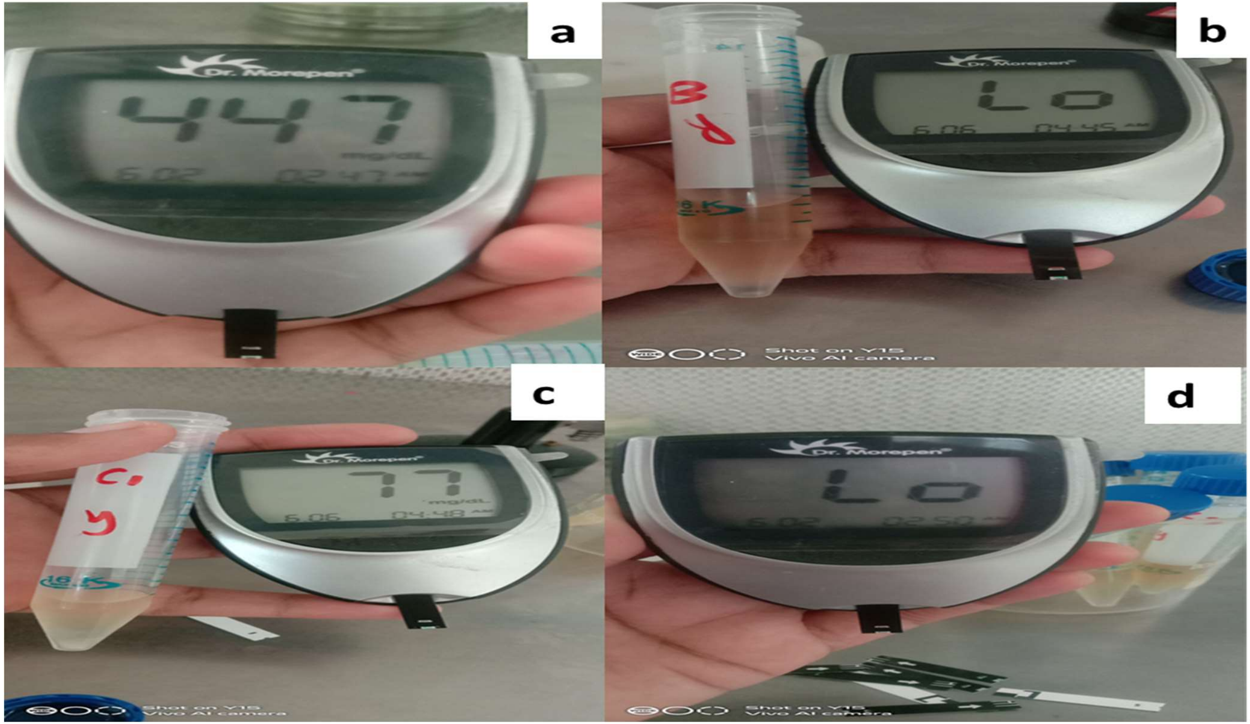

Fig. 9: Evaluating sugar molecules released during fermentation.

the degradation of Paper cups. The maximum alcohol produced by NPS, CTS, and PPS was 70-80% as the ethanol standard curve, but the lowest in PCS was 30% (Darwesh et al. 2020)

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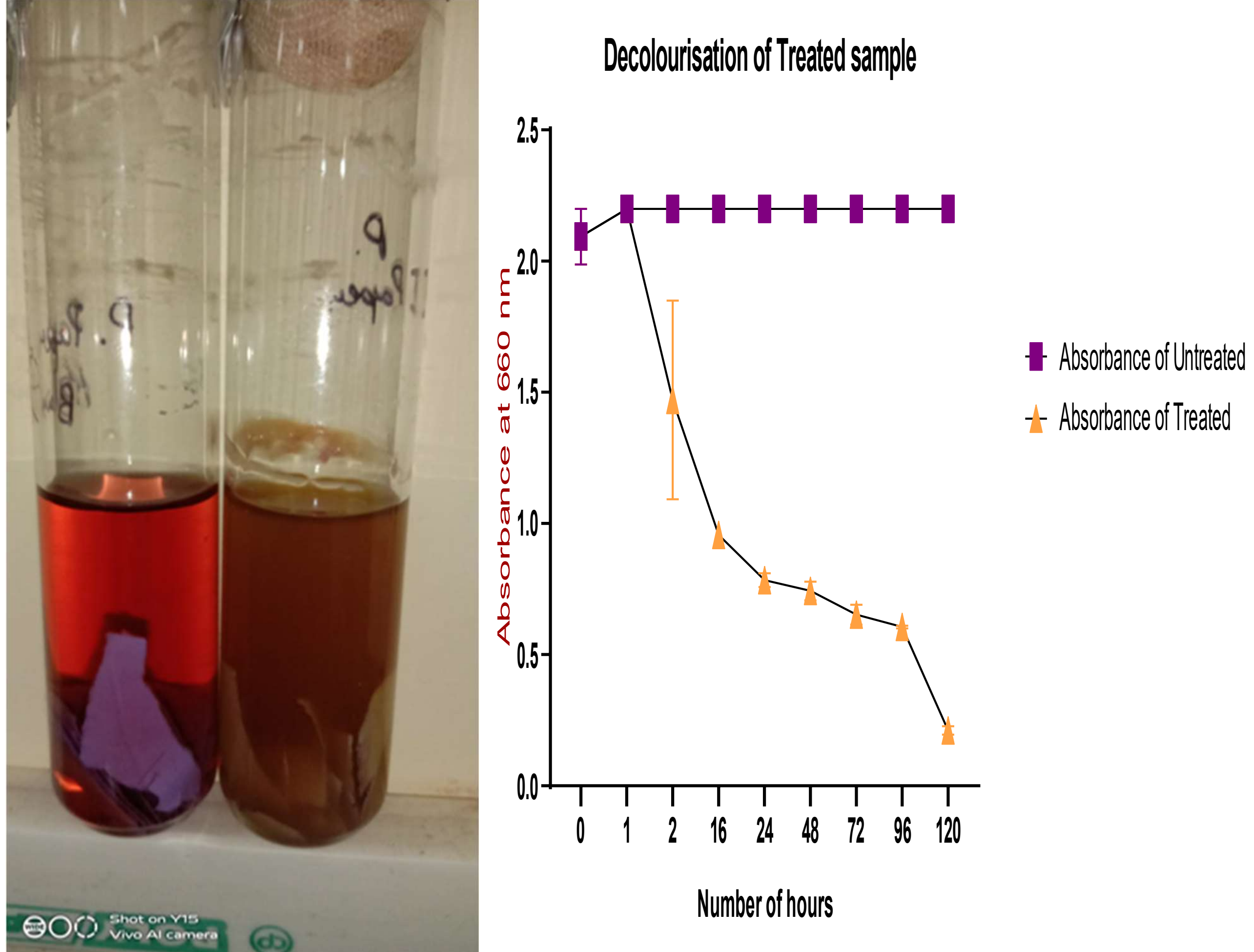

Fig. 10: The degradation and decolorization of colored craft paper with h.

Fig. 10 shows the unexpected decolorization of colored paper as PPS and Fig. 10 shows the decolorization of the PPS with control. The paper dye was unknown for the study; therefore, the spectrum of UV-visible spectrophotometer at 660 nm of both control and treated samples. The treated sample contained an isolated strain for biodegradation (Boonchuay et al. 2021) (Gea et al. 2023), but it was able to reduce colored dye from the sample. The formula for decolorization is absorbance control-absorbance sample/ absorbance control ×%62 =100 after 7 days (Rath et al. 2022) for complete decolorization. In the last section of the study, the ethanol bonds were confirmed with FTIR spectroscopy in all four samples. Table 1 describes the different bonds present in the samples, and Fig. 11 compares four samples with ethanol standard peaks in FTIR.

FTIR Interpretation

X– AXIS (Infrared Spectrum): The horizontal axis, also known as the x-axis, illustrates the infrared spectrum by plotting the intensity of the spectrum. The peaks observed, referred to as absorbance bands, align with the distinct vibrations of sample atoms when exposed to the infrared region of the electromagnetic spectrum. The wave number on the infrared spectrum is typically plotted within the range of 400 to 4000 cm^-1. Table 1 expresses different bonds present in the sample for the confirmation of the biodegradation from cellulose to ethanol. Therefore, the peaks detected on 3200, 2900, and 900 identify the presence of alcohols in the biodegradation. All the samples were giving 0.8 optical density in the biodegradation, confirming the 70-80% alcohol produced during the fermentation. The methylene blue control gave 0.812 absorbance, and the sample gave 0.234, describing 50%-60% decolorization. Y-AXIS (Absorbance/Transmittance Percentage): The vertical axis, or y-axis, represents the level of infrared light either transmitted through or absorbed by the sample

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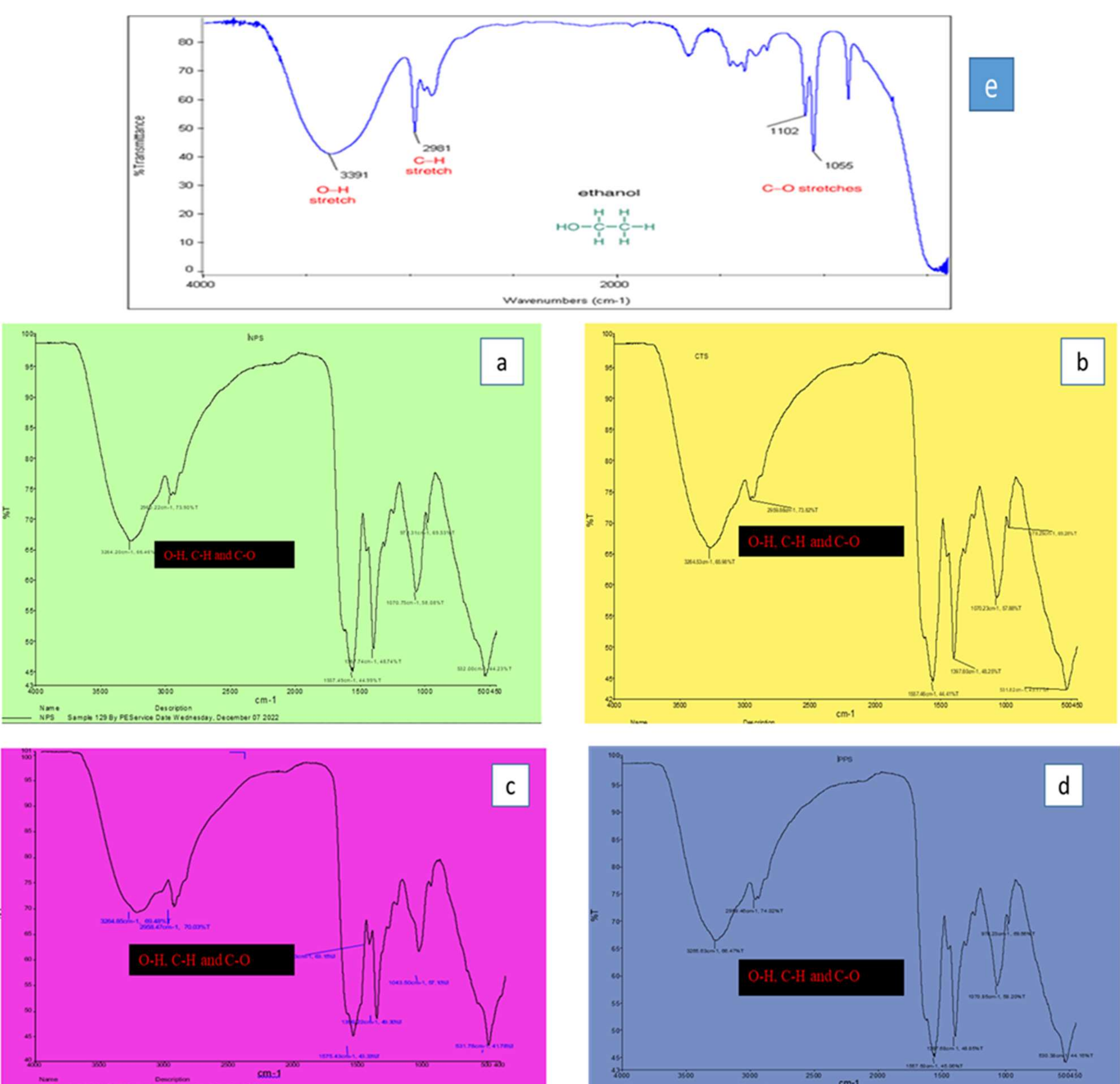

Fig. 11: FTIR bond confirmation of alcohol in the biodegradation process.

material under analysis. This axis provides a measure of the absorbance or transmittance percentage, offering insights into how the sample interacts with infrared radiation (Anjos et al. 2015, Campos, 2015, Gantumur et al. 2023).

CONCLUSIONS

According to the research, microbial enzymes are the cheapest source for biodegradation and if they can provide optimum pH and temperature, they can provide the maximum degradation. In this study authors have tried to explain different evaluation strategies for biodegradation. During the process of biodegradation, the acid produced continues till the end of the process. Cell growth is also affected in the medium, increasing or decreasing the pH and temperature of the medium. Inoculum size and substrate concentration can also impact cell growth, ethanol production, and cellulase activity. This study also concluding ethanol production from the various cellulosic wastes and the isolated microorganism similar to *Bacillus paramycoides*

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Table 1: Expressing the FTIR bond's presence in biodegradation.

Sample name	Wavenumber [cm^{-1}]	Functional group expected
NPS and PPS	3264.20	Hydroxyl group H bonded O-H stretch
	2960.22	Methyl C-H asym./sym. stretch
	1557.49	Primary amine N-H bend, secondary amine >N-H bend,
	1397.74	Phenol or tertiary alcohol O-H bend, organic sulfate, ammonium ion
	1070.75	Cyclic ether, C-O stretch, primary amine C-N stretch, skeletal C-C vibrations (Adiani et al. 2022)
		Vinyl C-H (out of plane) bend (Sidi-Yacoub et al. 2019)
	978.31	(Hamden et al. 2022, Landari et al. 2018, Hirphaye 2022, Ana et al. 2021)
PCS	3264.85	Normal or polymeric O-H Stretch Hydroxyl group H bonded
	2958.47	Methyl C-H Asymmetric Symmetric Stretch
	1399.22	Phenol or Tertiary Alcohol, O-H Bend, organic sulfate, ammonium ion (Sindhu et al. 2012, Akhabue et al. 2019, Imamura et al. 2008, Aging & Munajad, 2018)
CTS	3264.80	Normal or polymeric O-H Stretch Hydroxyl group H bonded
	2959.66	Methyl C-H Asymmetrical and Symmetrical Stretch
	1557.46	Carboxylate (Carboxylic acid salts)
	1070.23	Aromatic Ethers, Cyclic ether, C-O stretch, primary amine C-N stretch, skeletal C-C vibrations
	1397.60	Organic Sulfates Phenol organic sulfate, ammonium ion (Rios-Corripio et al. 2011, Sindhu et al. 2010, Aging & Munajad, 2018, Bayu et al. 2019)

can completely decolorize the colored origami craft paper with a shorter time of 24 h, as well as laccases activity was observed. Therefore, more studies can be done in the same direction to explore more utilization of the bacillus strain. Hence, the novel cellulolytic bacteria are successfully biodegrading the cellulosic wastes and help provide the sugars for bioethanol production. The process of deinking during the recycling process of the paper wastes requires lots of water and chemicals for the deinking and recycling process, However, the process of degradation with novel cellulolytic bacteria is quite cheap and eco-friendly approach to saving the environment. With the greater shift from relying on natural resources to bioethanol and biodiesel, the novel cellulolytic bacteria can work efficiently, reduce the risk of environmental pollution, and serve as an important industrial enzyme.

REFERENCES

Adiani, V., Gupta, S. and Variyar, P.S. 2022. FTIR-based rapid microbial quality estimation of fresh-cut jackfruit Artocarpus heterophyllus bulbs. J. Food Meas. Charact., 16(3): 1944-1951. https://doi.org/10.1007/s11694-022-01312-6

Aging, T. and Munajad, A. 2018. Fourier transform infrared FTIR spectroscopy analysis of transformer paper in mineral oil-paper composite insulation under accelerated. Energies, 11(2): 364. https://doi.org/10.3390/en11020364

Akhabue, C.E., Otoikhian, K.S., Adeniyi, A.G. and Ighalo, J.O. 2019. Effect of dilute acid pre-treatment on the functional complexes and surface morphology of wood sawdust for bioethanol production process modeling and biofuels production. Int. Mater. Congr., 12: 719. https://www.researchgate.net/publication/337946719

Ana, L., Santiago, R., Malvar, R.A. and Mart, D. 2021. FTIR screening to elucidate compositional differences in maize recombinant inbred lines with contrasting saccharification efficiency yields. Agronomy, 11(6): 1130.

Anjos, O., Campos, M.G., Ruiz, P.C. and Antunes, P. 2015. Application of FTIR-ATR spectroscopy to the quantification of sugar in honey. Food Chem., 169: 218-223. https://doi.org/10.1016/j.foodchem.2014.07.138

Asem, D., Leo, V. V., Passari, A. K., Tonsing, M. V., Joshi, J. B., Uthandi, S., Hashem, A., Abd-Allah, E. F. and Singh, B. P. 2017. Evaluation of gastrointestinal bacterial population for the production of holocellulose enzymes for biomass deconstruction. PLoS ONE, 12(10): 1-17. https://doi.org/10.1371/journal.pone.0186355

Bayu, A., Nandiyanto, D., Oktiani, R. and Ragadhita, R. 2019.. How to read and interpret FTIR spectroscope of organic material. Indon. J. Sci. Technol., 1: 97-118.

Boonchuay, P., Techapun, C., Leksawasdi, N., Seesuriyachan, P., Hanmoungjai, P., Watanabe, M., Srisupa, S. and Chaiyaso, T. 2021. Bioethanol production from cellulose-rich corncob residue by the thermotolerant saccharomyces cerevisiae TC-5. J. Fungi, 7(7): 547 https://doi.org/10.3390/jof7070547

Bose, J.C. and Sarwan, J. 2023. Multi-enzymatic degradation potential against wastes by the novel isolate of Bacillus. Biomass Convers. Biorefinery, 7: 4620. https://doi.org/10.1007/s13399-023-04620-z

Campos, M.G. 2015. Application of FTIR-ATR spectroscopy to the quantification of sugar in honey Application of FTIR-ATR spectroscopy to the quantification of sugar in honey. J. Food Chem., 7: 138.

Carvalho, J.K., Panatta, A.A.S., Silveira, M.A.D., Tav, C., Johann, S., Rodrigues, M.L.F. and Martins, C.V.B. 2021. Yeasts isolated from a lotic continental environment in Brazil show the potential to produce amylase, cellulase, and protease. Biotechnol. Rep., 30: e00630. https://doi.org/10.1016/j.btre.2021.e00630

Dadwal, A., Sharma, S. and Satyanarayana, T. 2020. Progress in ameliorating beneficial characteristics of microbial cellulases by genetic engineering approaches for cellulose saccharification. Front. Microbiol., 11: 1-18. https://doi.org/10.3389/fmicb.2020.01387

Darwesh, O.M., El-Maraghy, S.H., Abdel-Rahman, H.M. and Zaghloul, R.A. 2020. Improvement of paper waste conversion to bioethanol using novel cellulose-degrading fungal isolate. Fuel, 262: 651. https://doi.org/10.1016/j.fuel.2019.116518

Deka, D., Das, S.P., Sahoo, N., Das, D., Jawed, M., Goyal, D. and Goyal, A. 2013. Enhanced cellulase production from bacillus subtilis by optimizing physical parameters for bioethanol production. Biotechnology, 2: 1-11. https://doi.org/10.5402/2013/965310

Dini, I.R., Restuhadi, F. and Silaturahmi, K. 2019. The effect of

This publication is licensed under a Creative Commons Attribution 4.0 International License

purification on Snail (Achatina fulica) cellulase enzyme characteristic. IOP Conf. Ser. Earth and Environ. Sci., 250(1): 356-370. https://doi.org/10.1088/1755-1315/250/1/012051

Ega, S.L., Drendel, G., Petrovski, S., Egidi, E., Franks, A.E. and Muddada, S. 2020. Comparative analysis of structural variations due to genome shuffling of Bacillus Subtilis vs15 for improved cellulase production. Int. J. Mol. Sci., 21(4): 1299. https://doi.org/10.3390/ijms21041299

Fu, Y., Gao, H., Yu, H., Yang, Q., Peng, H., Liu, P., Li, Y., Hu, Z., Zhang, R., Li, J., Qi, Z., Wang, L., Peng, L. and Wang, Y. 2022. Specific lignin and cellulose depolymerization of sugarcane bagasse for maximum bioethanol production under optimal chemical fertilizer pretreatment with hemicellulose retention and liquid recycling. Renew. Energy, 200: 1371-1381. https://doi.org/10.1016/j.renene.2022.10.049

Ganesan, M., Remitha, M.V, Muniraj, I. and Uthandi, S. 2021. Thermophilic cellulase for biomass deconstruction Thermophilic cellulase for biomass deconstruction. Int. Environ. J., 56: 684-692.

Gantumur, M.A., Sukhbaatar, N., Shi, R., Hu, J., Bilawal, A., Qayum, A., Tian, B., Jiang, Z. and Hou, J. 2023. Structural, functional, and physicochemical characterization of fermented whey protein concentrates recovered from various fermented-distilled whey. Food Hydrocoll., 135: 108130. https://doi.org/10.1016/j.foodhyd.2022.108130

Gea, S., Bulan, R., Zaidar, E., Piliang, A. F., Oktari, N., Rahayu, S., Hutapea, Y. A. and Sari, R. M. (2023). Ultrasound pretreatment and enzymatic deinking by using bacterial cellulases from rice husk. J. Wood Chem. Technol., 43(3): 117-128. https://doi.org/10.1080/02773813.2023.2190594

Giuliano, A. 2023. The Transition of Scientific Research from Biomass-to-Energy/Biofuels to Biomass-to-Biochemicals in a Biorefinery Systems Framework. Energies, 16(5), 4-7. https://doi.org/10.3390/en16052261

Hamden, Z., El-ghoul, Y., Alminderej, F.M. and Saleh, S.M. 2022. High-quality bioethanol and vinegar production from Saudi Arabia date : Characterization and evaluation of their value and antioxidant efficiency. Environ. Technol., 1: 6-9.

Hirphaye, B.Y. 2022. Bio-ethanol production potential of Water Hyacinth (Eichhornia crassipes) as alternative energy feedstocks. Genes, 14: 1-17.

Imamura, K., Ohyama, K., Yokoyama, T., Maruyama, Y., Imanaka, H. and Interscience, W. 2008. Temperature scanning FTIR analysis of interactions between sugar and polymer additive in amorphous sugar–polymer mixtures. J. Polym. Sugar, 97(1): 519-528. https://doi.org/10.1002/jps

Islam, F. 2019. Isolation and characterization of cellulase-producing bacteria from sugar industry waste. Am. J. BioSci., 7(1): 16. https://doi.org/10.11648/j.ajbio.20190701.13

Islam, M., Sarkar, P. K., Mohiuddin, A. K. M. and Suzauddula, M. 2019. Optimization of fermentation condition for cellulase enzyme production from Bacillus sp. Malay. J. Halal Res., 2(2): 19-24. https://doi.org/10.2478/mjhr-2019-0009

Landari, H., Roudjane, M., Messaddeq, Y. and Miled, A. 2018. Pseudo-continuous flow FTIR system for glucose, fructose, and sucrose identification in the mid-IR range. MI, 14: 1-18. https://doi.org/10.3390/mi9100517

Liggieri, C., Obregón, W., Trejo, S. and Priolo, N. 2009. Biochemical analysis of a papain-like protease isolated from the latex of Asclepias curassavica L. Acta Biochim. Biophys. Sin., 41(2): 154-162. https://doi.org/10.1093/abbs/gmn018

Mothe, T. and Sultanpuram, V.R. 2016. Production, purification, and characterization of a thermotolerant alkaline serine protease from a novel species *Bacillus caseinilyticus*. 3 Biotech., 6(1): 1-10. https://doi.org/10.1007/s13205-016-0377-y

Nguyen, B.L. and Hoang, A.T.P. 2020. Screening of cellulolytic actinomycetes for decomposition of agricultural waste. Chem. Eng. Trans., 78: 283-288. https://doi.org/10.3303/CET2078048

Rashid, A., Mirza, S.A., Keating, C., Ali, S. and Campos, L.C. 2022. Indigenous Bacillus paramycoides spp. and Alcaligenes faecalis: a sustainable solution for bioremediation of hospital wastewater. Environ. Technol., 43(12): 1903-1916. https://doi.org/10.1080/09593330.2020.1858180

Rath, S., Paul, M., Behera, H. K. and Thatoi, H. 2022. Response surface methodology mediated optimization of Lignin peroxidase from *Bacillus mycoides* isolated from SimLipal Biosphere Reserve, Odisha, India. Journal of Genetic Engineering and Biotechnology, 20(1). https://doi.org/10.1186/s43141-021-00284-2

Rios-Corripio, M. A., Rios-Leal, E., Rojas-López, M. and Delgado-Macuil, R. 2011. FTIR characterization of Mexican honey and its adulteration with sugar syrups by using chemometric methods. J. Phy. Conf. Ser., 274(1): 12098. https://doi.org/10.1088/1742-6596/274/1/012098

Rojas, J.A., Cruz, C., Mikán, J.F., Villalba, L.S., Cepero de García, M.C. and Restrepo, S. 2009. Isoenzyme characterization of proteases and amylases and partial purification of proteases from filamentous fungi causing biodeterioration of industrial paper. Int. Biodeter. Biodegrad., 63(2): 169-175. https://doi.org/10.1016/j.ibiod.2008.07.009

Sahoo, A., Das, P.K., Patra, S. and Veeranki, V.D. 2023. Engineered yeasts for the production of biofuel and platform chemicals. Adv. Biotechnol. Biofuels Sustain., 5: 411

Saleh, A.K., Salama, A., Badawy, A.S., Diab, M.A. and El-Gendi, H. 2023. Paper sludge saccharification for batch and fed-batch production of bacterial cellulose decorated with magnetite for dye decolorization by experimental design. Cellulose, 45: 546.

Sartaj, K., Patel, A., Matsakas, L. and Prasad, R. 2022. Unraveling metagenomics approach for microbial biofuel production. Genes, 13(11): 1942.

Sarwan, J. and Bose, J.C. 2021. Importance of microbial cellulases and their industrial applications. Ann. Roman. Soc. Cell Biol., 25(1): 3568-3575.

Sarwan, J. and Bose, J.C. 2022. Role of isolates of bacillus species for biodegradation of multiple contaminants. Ann. Roman. Soc. Cell Biol., 1(2): 292-298.

Sarwan, J., Pandey, P. and Bose, J.C. 2021. Proteases immobilization and pharmacological aspects of matrix metalloproteinases. Ann. Roman. Soc. Cell Biol., 25(1): 3604-3615.

Shanmugapriya, K., Saravana, P.S. and Manoharan, M. 2012. Isolation, screening, and partial purification of cellulase from cellulase-producing bacteria. Int. J. Adv. Biotechnol. Res., 3(1): 509-514.

Sharma, H., Rana, N., Sarwan, J., Bose, J.C., Devi, M. and Chugh, R. 2023. Nano-material for wastewater treatment. Mater. Today: Proceed., 15: 258. https://doi.org/10.1016/j.matpr.2023.02.258

Sidi-Yacoub, B., Oudghiri, F., Belkadi, M. and Rodríguez-Barroso, R. 2019. Characterization of lignocellulosic components in exhausted sugar beet pulp waste by TG/FTIR analysis. J. Thermal Anal. Calorimet., 138(2): 1801-1809. https://doi.org/10.1007/s10973-019-08179-8

Sindhu, R., Binod, P. and Ánsari, R. 2012. Organosolvent pretreatment and enzymatic hydrolysis of rice straw for the production of bioethanol. Cellulose, 27: 473-483.

Sindhu, R., Binod, P. and Satyanagalakshmi, K. 2010. Formic acid is a potential pretreatment agent for the conversion of sugarcane bagasse to bioethanol. Cellulose, 26: 121-139.

Singhania, S., Ansari, R., Neekhra, N. and Saini, A. 2018. Isolation, identification, and screening of alkaline protease from thermophilic fungal species of Raipur. Int. J. Life-Sci. Sci. Res., 4(2): 1627-1633. https://doi.org/10.21276/ijlssr.2018.4.2.1

Sun, W., Li, X., Zhao, J. and Qin, Y. 2022. Pretreatment strategies to enhance enzymatic hydrolysis and cellulosic ethanol production for biorefinery of corn stover. Int. J. Mol. Sci., 23(21): https://doi.org/10.3390/ijms232113163

Thakur, N., Goyal, M., Sharma, S. and Kumar, D. 2019. Industrial Applications and Approaches Used in Strain Improvement Proteases. Springer, Cham

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Xian, X., Fang, L., Zhou, Y., Li, B., Zheng, X., Liu, Y. and Lin, X. 2022. Integrated bioprocess for cellulosic ethanol production from wheat straw: new ternary deep-eutectic-solvent pretreatment, enzymatic saccharification, and fermentation. Fermentation, 8(8): 371.

Yaya, A. Gimba, Abubakar Idris, Abdullahi Hassan and Opeyemi N. Hassan 2021. Isolation and optimization of the fermentation condition of cellulolytic microbial isolates from cassava wastewater. GSC Biological and Pharmaceutical Sciences, 14(1): 011-017. https://doi.org/10.30574/gscbps.2021.14.1.0421

Zeng, R., Yin, X.Y., Ruan, T., Hu, Q., Hou, Y.L., Zuo, Z.Y., Huang, H. and Yang, Z.H. 2016. A novel cellulase produced by a newly isolated *Trichoderma virens*. Bioengineering, 3(2): 1-9. https://doi.org/10.3390/bioengineering3020013

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