OPEN ACCESS South Asian Journal of Life Sciences

Research Article

Rice Straw Biodegradation Employing the Cellulase Enzyme and a Local Bacterial Strain, *Bacillus subtilis*, Production and Purification

Soad A. Atiya[1], Iman H. Gatea[1]*, Shaymaa Rajab Farhan[1], Suad Gazi Alkufi[2], Ameena G. Abed[1]

[1]Scientific Research Commission, Baghdad, Iraq; [2]Al-Nahrain University, Baghdad, Iraq.

Abstract | Microbial cellulase enzyme plays a significant role in various industries, including biofuel production, paper pulp, textiles, pharmaceuticals, and agriculture This research aimed to study rice straw biodegradation and cellulase enzyme production and purification by culturing *Bacillus subtilis* on medium containing rice straw as a carbon source. Bacterial isolate *B. subtilis* was tested for cellulytic activity by using CMC medium, and the result showed that it has cellulytic activity. Bacterial isolate *B.subtilus* was grown on the liquid medium containing rice straw for cellulase enzyme production. The results of enzyme purification by using centrifugation, then concentrated by PEG 6000, and gel filtration chromatography by using Sephadex G-100 and concentrated by PEG 6000. In the final step of cellulase purification, the specific activity, purification fold, and yield were 45.714 U/mg, 4.636, and 89.72%, respectively. Optimum conditions for the activity of the enzyme were studied, and the results gave the optimum temperature of 40 °C, incubation time of 30 minutes, at a pH of 6.0, with an enzyme concentration of 75% and 70 mg of the weight of the filter paper.

Keywords | Cellulase enzyme, Purification, Rice straw, *Bacillus subtilis*

Editor | Muhammad Nauman Zahid, Quality Operations Laboratory, University of Veterinary and Animal Sciences, Lahore, Pakistan.
Received | April 04, 2026; **Accepted** | June 16, 2026; **Published** | August 13, 2026
***Correspondence** | Iman H. Gatea, Scientific Research Commission, Baghdad, Iraq; **Email:** eman77aa@yahoo.com
Citation | Atiya SA, Gatea IH, Farhan SR, Alkufi SG, Abed AG (2026). Rice straw biodegradation employing the cellulase enzyme and a local bacterial strain, *Bacillus subtilis*, production and purification. S. Asian J. Life Sci. 14: 46-53.
DOI | https://dx.doi.org/10.17582/journal.sajls/2026/14.46.53
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

Agricultural wastes are composed principally of cellulosic or lignocellulosic matter. These materials are considered to be the inexpensive basis for the production of different utilizable products throughout the world (Sulyman *et al.*, 2020). Cellulose is a polymer made up of linear D-glucose molecules connected by 1,4-glycosidic linkages and is a crucial structural component of plant cell walls. Its partially crystalline structure provides extra strength and makes it less susceptible to enzymatic breakdown than other polysaccharides in the cell wall (Demissie *et al.*, 2024).

The development of methods for the effective processing and use of cellulosic wastes as low-cost carbon sources has been crucial for the business world. In recent decades, cellulose's hydrolysis has attracted economic interest due to the pressing need for green energy. Microbial hydrolysis is one of the most widely used techniques for converting cellulose into reducing sugars, which can subsequently be converted into ethanol and other chemicals (Elsababty *et al.*, 2022).

All cellulolytic enzymes, systems, and structures are referred to as cellulase, including those made by extracellular or cell-bound microorganisms as well as those with various modes of action (Korsa *et al.*, 2023). Cellulase is an enzyme complex consisting of three enzymes, namely endocellulase, exocellulase, and β-glucosidase (Elida *et al.*, 2023). These three enzymes are involved in the hydrolysis of cellulose through synergistic action, resulting in efficient and effective hydrolysis (Ezea, 2025). A particular class of glucanohydrolase known as endoglucanase (EC 3.2.1.4) binds to the noncrystalline portion of cellulose and randomly cleaves glycoside linkages faster hydrolysis of

amorphous portions due to weaker hydrogen interactions. It produces single polysaccharides or oligosaccharides of different lengths by randomly breaking irregular cellulose chain sites (Berisio *et al.*, 2022). Enzymes called EC 3.2.1.91 are exoglucanases. Crystalline cellulose is produced by the binding and breaking of primary fibrils by cellobiohydrolases I and II and 1, 4-β-D-glucan. It cleaves the ends of cellulose fibers to form cello-oligosaccharides or disaccharides like cellobiose or glucose (Elsababty *et al.*, 2022). β-glucosidase (EC 3.2.1.21) breaks down the disaccharide molecule cellobiose into simpler sugars and releases glucose monomers. It converts the nonreducing terminal glycosyl residues of cello-oligosaccharides, which are present in cellobiose and other cello-oligomers, into single sugars known as glucose monomers (Raj *et al.*, 2022).

True cellulolytic microorganisms are those that have hydrolysis natural cellulose; they are referred to as cellulolytic microorganisms (Olaitan *et al.*, 2025). Additionally, cellulolytic enzymes are produced by microorganisms like bacteria, actinobacteria, and fungi (Korsa *et al.*, 2023). Bacteria are becoming increasingly favored for the purification and production of various enzymes due to their faster growth rate, numerous enzyme complexes, and ability to withstand a wide range of external difficulties. Furthermore, they have been demonstrated to use a wide range of substrates, including solid organic waste leftovers from agriculture, forestry, and mills, to provide waste management benefits as well as economic enzyme production. Bacteria are an intriguing microbial group for faster cellulase production due to their faster growth rate than fungi, ease of handling, and ability to react to a wide range of genetic changes (Demissie *et al.*, 2024).

The main industries where cellulases are being utilized more and more are textile, food, beverage, healthcare, paper, and pulp, according to recent reports on the enzyme market. The removal of excess dye from fabrics, garment softening, bio-stoning of denim, and wet processing of textiles are among its many uses. Furthermore, their primary use in the detergent sector comes from their capacity to remove stains. To enhance the fabric's softness, color brightness, and ability to remove particle soil, these detergents alter the fibers within the fabric. The textile sector is subject to several regulations as a result of growing environmental concerns (Nisar *et al.*, 2023).

Production of an enzyme includes many steps, such as selection of a suitable organism, screening for the production of the enzyme, and fermentation. The end products of the fermentation are liberated into the fermentation broth (Olaitan *et al.*, 2025). The objective of the research is to study rice straw biodegradation and cellulase enzyme production and purification by using a local bacterial isolate, *B. subtilis*, grown on rice straw medium, as well as determining the optimal conditions for the cellulolytic enzyme activity.

MATERIALS AND METHODS

Rice straw preparation

Rice straw is cleaned, milled, and kept in a sterile container. Chemical treatment of rice straw was done by adding 100 ml of 1% sodium hydroxide to 10 gm of prepared rice straw, putting the mixture in a water bath at 100 ºC for 1 hour, then cooling, taking the precepetate washed many times with distilled water, filtered by Wattman No. 4 (Abd El-Zaher *et al.*, 2010).

Cellulytic activity of bacterial isolate

The nutritional agar medium was used to isolate pure bacterial colonies, which were then grown on CMC medium without Congo red for 48 hours at 37 ºC to test for cellulolytic activity. To enable the red dye to penetrate the cellulose, the medium was submerged in an aqueous solution of Congo red (1 mg/ml) for 15 minutes. Sodium chloride NaCl (1M) was added to the medium, and the surplus Congo red solution was removed (Huang et al., 2012).

Enzyme production medium

The cellulolytic bacterial isolate was cultivated in the salt medium containing chemically treated rice straw to produce the cellulolytic enzyme, which was then prepared in accordance with Table 1. The medium's pH was brought to 7.2. An autoclave with 1.5 bar of pressure and 121 ˚C in temperature was used to sterilize the medium.

Preparation of crude enzyme

After an (18–24) hour incubation period at 37 ºC, the cultures were centrifuged for 20 minutes at 4000 rpm to extract crude enzyme from the supernatant (Kotchoni *et al.*, 2003). Purification, estimation of protein content, and enzyme activity were all performed using the crude enzyme solution.

Enzyme activity assay

Using the DNS (3,5-dinitrosalicylic acid) method, cellulase activity was uantified by measuring the amount of reducing

Table 1: Enzyme production medium.

Substrate	Rice straw	Yeast extract	$MgCl_2.6H_2O$	$(NH_4)_2SO_4$	$NaHPO_4.12H_2O$	KH_2PO_4
g/l	10	2	0.09	0.5	4.5	1.5

sugars that were dissolved in 50 mM citrate buffer (pH 4.8) enzyme solution and letting the mixture sit at 37 °C for 60 minutes, the reaction was halted by adding DNS solution. Following a 10-minute boil, the treated samples were chilled in water to achieve stability of their color before having their optical density measured at 550 nm. A glucose calibration curve was used to calculate the cellulase activity. The quantity of enzyme that released one µmol of glucose per minute was one unit of enzyme activity (Dalagnol *et al.*, 2017).

Glucose estimation

Dinitrosalicylic acid was used to estimate glucose, and an Optima UV/VIS spectrophotometer was used to measure optical density at 550 nm (Ariffin *et al.*, 2006).

Protein estimation

Using bovine serum albumin (BSA) as a standard, the Lowry's method was used to quantify the protein content (Adeleke *et al.*, 2017).

Purification of enzyme

The culture fluid from the production medium was taken and centrifuged for 20 minutes of centrifugation at 4000 rpm to separate the cells after being incubated at 37 °C for 18–24 hours. The culture supernatant was collected as a crude enzyme extract. The culture supernatant was concentrated by using PEG 6000, then concentrated extract was subjected to gel chromatography Sephadex G-100 column (2.5×40 cm) adjusted to pH 4.8 using a 50 mM sodium citrate buffer, The cellulase was eluted from the column at a flow rate of 0.7 ml/min., fractions (5 ml each) were collected, and the protein content was measured with a spectrophotometer at 280 nm. Fractions with high protein content were pooled together, and then the activity of cellulase was measured.

Study of the optimal conditions for enzymatic activity

The crude enzyme produced after cultivating the bacterial isolate efficient in producing the cellulolytic enzyme was used to study the optimal conditions for enzymatic activity. The optimal conditions were determined by measuring the enzymatic activity of the crude enzyme compared to the control (without enzyme solution). Additionally, the concentration of glucose produced under all conditions studied was estimated, and the enzymatic activity was calculated (Pratiksha and Gireesh, 2012).

The effect of temperature

The effect of temperature on enzymatic activity was studied by incubating the enzyme with filter paper in the presence of citrate buffer at different temperatures (30, 40, 50) °C for one hour. The glucose concentration was measured, and the cellulase activity was calculated.

The effect of the time period

The effect of the time period on enzymatic activity was studied by incubating the enzyme extract with filter paper in the presence of citrate buffer for time periods of (30, 6090, 120) min, while keeping the other incubation conditions constant.

The pH effect

The effect of pH on enzyme activity in cellulose hydrolysis was studied under different values of 3.0, 4.0, 4.8, 5.8, and 6.0. All samples, including the control, were incubated at 40°C for one hour, then the glucose concentration was measured, and enzyme activity was estimated to determine the optimal pH.

The effect of enzyme concentration

The effect of enzyme concentration on the activity of the cellulolytic enzyme was studied, and the concentrations were prepared using a citrate buffer solution with concentrations of (100, 75, 50, 25, 12.5%).

The effect of substrate concentration

The effect of substrate concentration on the activity of the cellulolytic enzyme was studied by using Wattman No. 1 filter paper with weights (70, 60, 50, 40, 30, 20, 10 mg) and incubating it with the enzymatic extract under constant incubation conditions.

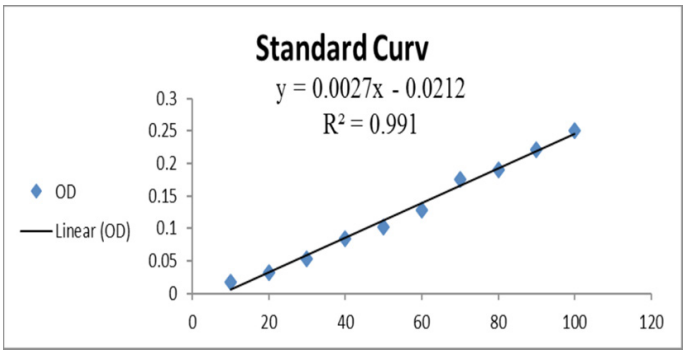

Figure 1: Estimate of the glucose concentration standard curve.

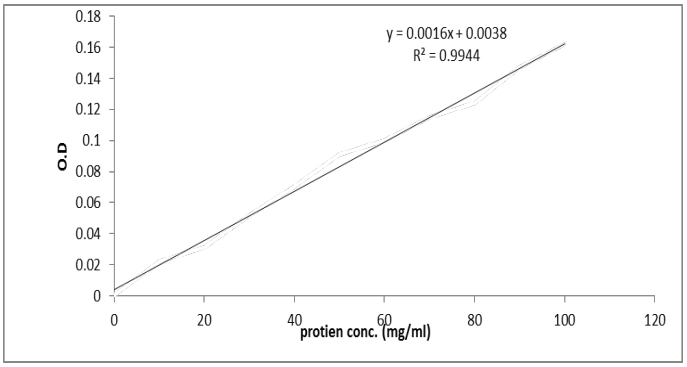

Figure 2: Standard curve of protein concentration estimation.

RESULTS AND DISCUSSION

Cellulytic activity of bacterial isolate

The bacterial isolate was tested for cellulolytic activity by using CMC medium. The bacterial isolate, *B. subtilis*, has cellulytic activity by the formation of a clear zone surrounding bacterial growth, as shown in Figure 3. Bacterial ability to lyse cellulose because of cellulytic enzyme production can degrade the cellulose polymer's (ß-1-4) bond (Sethi *et al*., 2013). Cellulase-producing bacteria were identified as *Pseudomonas fluorescens*, *E. coli, B. subtilus* and *Serratia marscens*. Higher-growth-rate bacteria have more potential for application in cellulase production than do fungi. But it's not common practice to use microorganisms to produce cellulase (Madwadza *et al*., 2000). *Bacillus* species are known to produce a variety of extracellular polysaccharide-hydrolyzing enzymes (Saini *et al*., 2017).

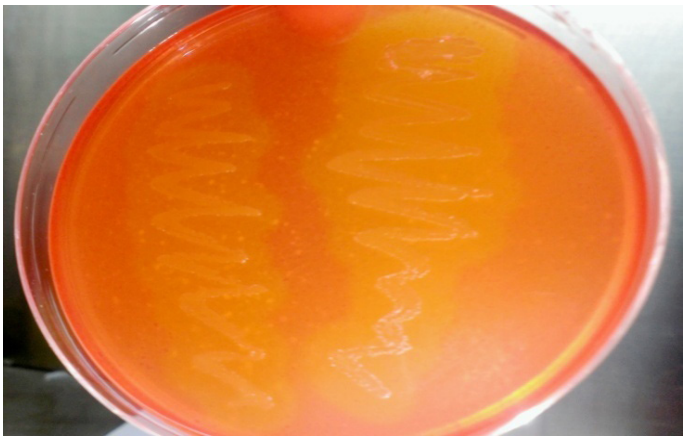

Figure 3: The effectiveness of local isolated bacteria in cellulose lysis with the appearance of a transparent region surrounding bacterial growth.

Cellulase enzyme production

As seen in Figure 4, the bacterial isolate *B. subtilus*, which possesses cellulolytic activity, was cultivated on a liquid medium containing rice straw as a carbon source for the production of cellulase.

Because of their high growth rate relative to fungi, ease of handling, and resistance to different genetic manipulations, bacteria are an interesting microbial group for the faster production of cellulase (Vadala *et al*., 2021). While many microorganisms can degrade cellulose, only a small number can produce large amounts of cell-free bioactive compounds that can hydrolyze crystalline cellulose completely in vitro. Using a fermentation technique, cellulases were first isolated and identified from bacteria (Gaur and Tiwari, 2015). According to (Prapulla and Karanth, 2014), the optimal carbon sources for *B. amy-loliquefaciens* DL-3 and *B. halodurans* CAS 1 to produce cellulase were rice husk and rice bran, respectively. Because they are inducible, the best carbon sources for cellulase production were cellulosic waste products such as rice bran, sugarcane bagasse, and rice hulls.

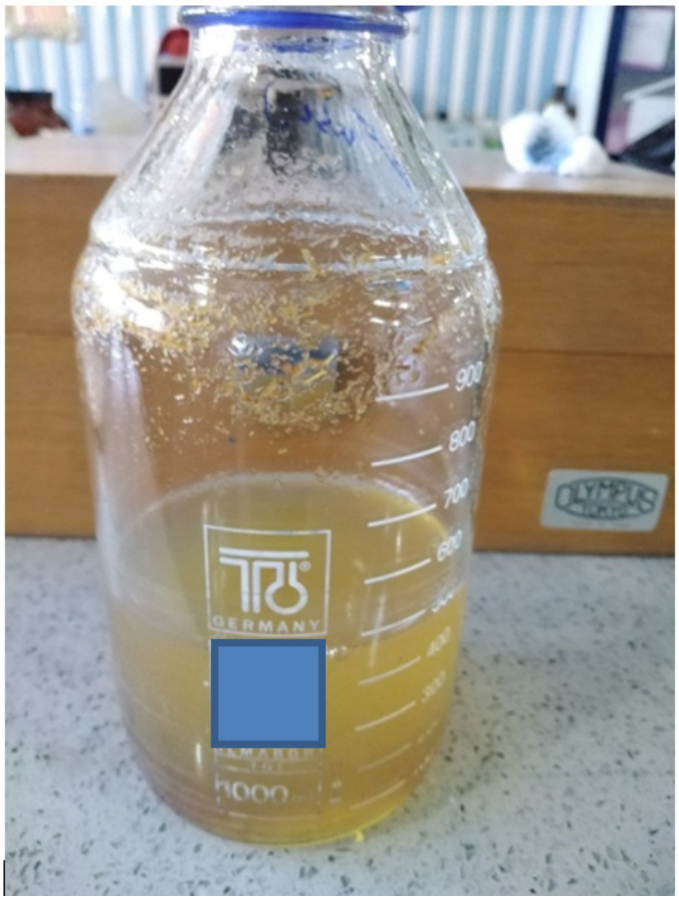

Figure 4: Cellulase production rice straw medium by bacterial isolate *Bacillus subtilus*.

Purification of cellulase enzyme

The cellulase enzyme was produced and extracted from the local isolate *Bacillus subtilis* using the liquid production medium. A series of physical and chemical procedures was carried out to produce a pure enzymatic extract that would remove as much protein, impurities, dyes, and other undesirable materials as possible. These purification steps shown in Table 2 were included: Centrifugation after incubation at 37 ºC for (18-24) hours, the culture fluid from the production media containing 10% rice straw as carbon source was collected and centrifuged at 4000 rpm for 20 min to separate the cells. 20 ml of the culture supernatant was collected as crude enzyme extract for the purpose of using it in purifying the enzyme, which was specific activity of 9.86 (u/mg), a purification fold of 1.0, and a yield of 100%. Then the concentration process was carried out with PEG-6000 in this step: specific activity 15.83 u/mg, purification fold 1.605, and yield 83%. Then gel filtration chromatography using a Sephadex G-100 column. Molecular-sieve chromatography and size-exclusion chromatography are other names for gel filtration. The basis for separation in this procedure is

Table 2: Cellulase purification from crud extract produced by *B. subtilis*.

Purification steps	Volume (ml)	Protein concentration (mg/ml)	Total Protein (mg)	Activity (u/ml)	Total activity (u)	Specific activity (u/mg)	Purification fold	Yield %
Crude extract	20	0.3	6.0	2.96	59.2	9.86	1.0	100
Concentration By PEG- 6000	9.5	0.48	3.12	5.20	49.4	15.83	1.605	83
Gel filtration by sephadex G-100.	15.3	0.105	1.606	3.4	52.02	32.391	3.28	87.87
Concentration by sephadex G-100.	8.3	0.14	1.162	6.4	53.12	45.714	4.636	89.72

the sample's molecules' varying capacities to enter the gel-filtration medium's pores as a result of their different molecular sizes (Prapulla and Karanth, 2014). Fractions were collected, and the protein content was measured with a spectrophotometer at 280 nm as shown in Figure 5. Fractions with high protein content were pooled together, and then activity of cellulase was measured, specific activity 32.391 (u/mg), purification fold 3.28, and yield 87.87%. The specific activity, purification fold, and yield in the last stage of the purification were 45.714U/mg, 4.636, and 89.72%, respectively, as shown in Table 2.

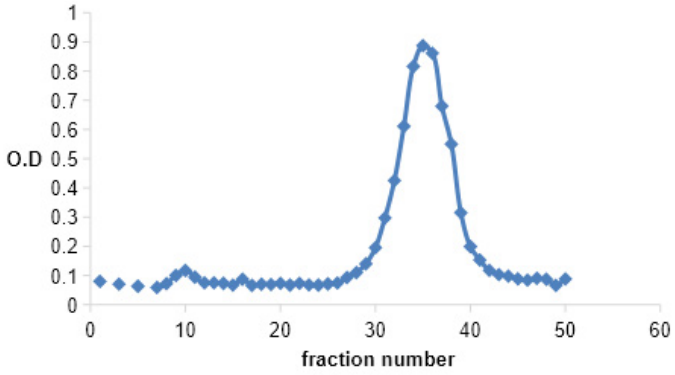

Figure 5: Gel chromatography for cellulase enzyme produced by *Bacillus subtilis* isolate.

Purified cellulase produced by *B. subtilis* (Madwadza *et al.*, 2000) and partially purified cellulase enzyme produced by *B. licheniformis* strain Z9 were identified by precipitation with $(NH_4)_2SO_4$ and Sephadex G-100 gel chromatography with 356.5 U/mg specific activity, 2.1-fold purification, and 3.07% yield (Elsababty *et al.*, 2022). Purified cellulase from cellulase-producing bacteria present in sugar industry waste by precipitation with ammonium sulfate, DEAE-cellulose, and CM-cellulose chromatography, respectively. In the final step of purification, specific activity, recovery, and purification fold were 2655 U/mg, 35.7%, and 9.7, respectively (Singh and Kumar, 1998). Chung *et al.* (2009) used the ultra-filtration method and achieved a purification fold of 7 and an enzyme yield of 96% during purification of cellulase enzyme from *B. brevis*. Also purified the cellulase enzyme from *Salinivibrio* sp. through a series of procedures that included concentration by ultra-filtration, precipitation with ammonium sulphate, then ion-exchange chromatography and gel filtration (Rawat and Tewari, 2012). The purification fold obtained was 29.5, and the enzymatic yield was 18.9%. Chen *et al.* (2004) also used precipitation with ammonium sulfate with a saturation rate of 80% as a first step in the purification of the cellulase enzyme; they obtained a purification fold of 2.3 and an enzymatic yield of 45%. Regarding (Mansour *et al.*, 2025), they used a series of procedures to purify the cellulase enzyme that was extracted from *Sinorhizobium frederii* bacteria. The first step involved precipitating the ammonium sulfate at a saturation rate of 40–60%, and the second step involved ion exchange using the DEAE-Sepharose ion exchanger. He was successful in achieving a purification fold of 1.9. While (Goyal *et al.*, 2014) purified the enzyme by sequential steps, resulting in a purification fold of 20.39 and a yield of 11.49%.

Study of the optimal conditions for cellulase activity

The effect of temperature

The effect of temperature on enzymatic activity was studied at different temperatures (30, 40, 50 °C) for 1 hour, and the glucose concentration was measured. The activity of the cellulase enzyme was calculated based on the glucose concentration. It was observed that as the temperature increased, the enzyme activity also increased, reaching its highest activity at 40 °C, as shown in Figure 6 (Abbas *et al.*, 2016). Found that cellulase activity due to *P. chrysogenum* was optimally observed at 40 °C. The temperature range reflects the mesophilic nature of the microorganisms producing the enzyme, while Iqbal *et al.* (2011) found that the highest activity was at a temperature of 55 °C.

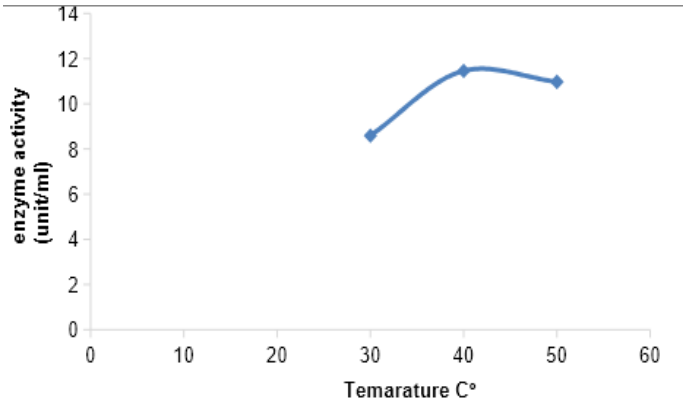

Figure 6: Temperature effec on enzyme activity.

The time period effect

The effect of the time period on the enzyme activity was studied by incubating the enzyme extract with filter paper in the presence of citrate buffer at a temperature of 40 °C for time periods of (30, 60, 90,120 min). By estimating the enzyme activity, it was found that the enzyme gave activity for all time periods under study, while it gave the highest activity at 30 minutes, and its value decreased with the increase in the time period, as shown in Figure 7.

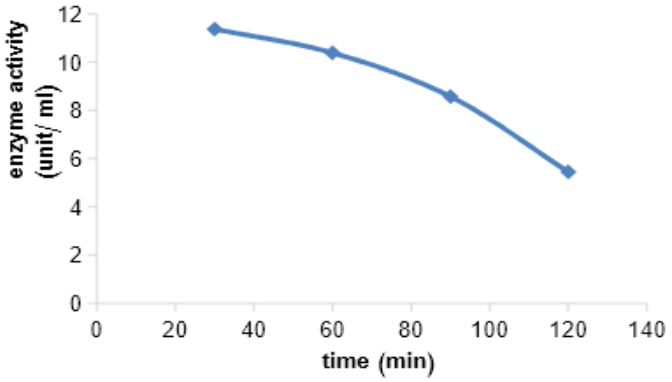

Figure 7: Time period effec on enzyme activity.

The effect of pH

The effect of pH on enzyme activity in cellulose hydrolysis was studied under different pH values of 3.0, 4.0, 4.8, 5.8, and 6.0. All samples, along with the control, were incubated at 40 °C for 30 minutes, then the glucose concentration was measured, and enzyme activity was estimated to determine the optimal pH. It was found that the highest activity was at pH 6.0, as shown in Figure 8. These results were consistent with those reached by Naz (2015), while the researchers (Iqbal *et al*., 2011) found that the optimal pH for the effectiveness of the cellulose-degrading enzyme is 6.0.

The instability of these enzymes at lower or very high pH was because they are proteins that are denatured at extreme pH levels. This is in line with the works of (Abbas *et al*., 2016).

The effect of enzyme concentration

The effect of enzyme concentration on the activity of the cellulase enzyme was studied at concentrations of (100, 75, 50, 25, 12.5%). The incubation conditions for all concentrations were kept constant at a temperature of 40°C for 30 minutes and a pH of 6.0 for 30 minutes. All concentrations showed similar enzymatic activity, with the highest activity observed at a concentration of 75%, as shown in Figure 9.

The effect of filter paper weight

It was investigated how the weight of filter paper weight on cellulolytic enzyme activity was investigated. Weights of 10, 20, 30, 40, 50, 60, and 70 mg of Whatman No. 1 filter paper were tested. The enzymatic extract was incubated with each weight at a 75% concentration for 30 minutes at 40 °C and a pH of 6.0. As shown in Figure 10, enzymatic activity was observed at all weights, with the maximum activity occurring at 70 mg. A weight of 70 mg was shown to support optimum enzyme activity. Substrate concentrations caused significant variation in enzyme activity. This can be explained by the greater availability of cellulose at 70 mg. A decrease in enzyme activity beyond the optimum concentration of substrate may be due to the unavailability of active binding sites or inhibitors (Abbas *et al*., 2016).

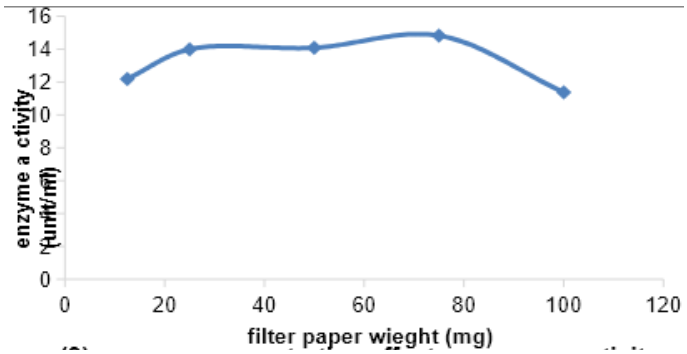

Figure 9: Enzyme concentration effect on enzyme activity.

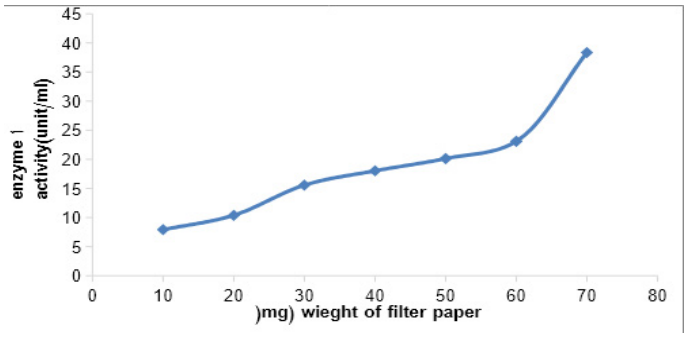

Figure 10: The effect of filter paper weight.

CONCLUSIONS

This study focuses on the biodegradation of rice straw and cellulase enzyme production by a local bacterial isolate, *B. subtilis*. The bacterium was used in cellulase production by bacterial metabolism through lignocellulosic agricultural waste as a substrate, representing an alternative approach for the sustainable bioconversion of biomass. After the enzymes were produced, partial purification of cellulase was conducted through a series of steps, including centrifugation followed by concentration using polyethylene glycol (PEG 6000) and consequent gel filtration on Sephadex G-100 to increase the purity and activity of the enzyme. In addition, we also found the most suitable conditions of cellulase activity, which were best at 40 °C for 30 minutes with a pH value and substrate concentration of 6.0 and 75% (mL), respectively, and a filter paper mass of 70 mg. This study demonstrates the conversion of lignocellulosic

waste into valuable enzymes using a novel Bacillus subtilis strain and lays the foundation for eco-friendly strategies regarding agricultural residue disposal and industrial enzyme production.

AUTHOR'S CONTRIBUTION

All authors contributed equally to the manuscript.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abbas AM, Milala MA, Isa MA, Allamin IA, Ismail HY (2016). Optimization of cellulase activity by *Aspergillus niger* and *Penicillium chrysogenum* using some agricultural wastes. UMYU J. Microbiol. Res., 1(1): 189–196. https://doi.org/10.47430/ujmr.1611.025

Abd El-Zaher FH, Fadel M (2010). Production of bioethanol via enzymatic saccharification of rice straw by cellulase produced by *Trichoderma reesei* under solid-state fermentation. N. Y. Sci. J., (**Vol and Pages?**).

Adeleke BS, Ojo SO, Oluwafemi YD, Olaniyi OO (2017). Chemical mutagenesis of *Bacillus subtilis* for improved mannanase biosynthesis. J. Adv. Microbiol., 3(2): 1–6. https://doi.org/10.9734/JAMB/2017/30911

Ariffin H, Abdullah N, Kalsom M, Shirai Y, Hassan M (2006). Production and characterization of cellulase by *Bacillus pumilus* EB3. Int. J. Eng. Technol., 3(1): 47–53.

Berisio R, Barra G, Romano M, Squeglia F, Ruggiero A (2022). Structural and biochemical characterization of endo-β-1,4-glucanase from *Dictyoglomus thermophilum*, a hyperthermostable and halotolerant cellulase. Catalysts, 12(3): 302. https://doi.org/10.3390/catal12030302

Chen PJ, Tao W, Yao-Tsung C, Liang-Ping L (2004). Purification and characterization of carboxymethyl cellulase from *Sinorhizobium fredii*. Botan. Bull. Acad. Sin., 45: 111–118.

Chung-Yi W, Yi-Ru H, Chang-Chai N, Helen C, Hsin-Tang L, Wen-Sheng T, Yuan-Tay S (2009). Purification and characterization of a novel halostable cellulase from *Salinivibrio* sp. strain NTU-05. J. Enzy. Microb. Technol., 44: 373–379. https://doi.org/10.1016/j.enzmictec.2009.02.006

Dalagnol LMG, Silveira VCC, Silva HB (2017). Improvement of pectinase, xylanase, and cellulase activities by ultrasound: Effects on enzymes and substrates, kinetics, and thermodynamic parameters. Process Biochem., 61: 80–87. https://doi.org/10.1016/j.procbio.2017.06.029

Demissie MS, Legesse NH, Tesema AA (2024). Isolation and characterization of cellulase-producing bacteria from forest, cow dung, Dashen brewery, and agro-industrial waste. PLoS One, 10: 1–12. https://doi.org/10.1371/journal.pone.0301607

Elida FS, Ramadhani DF, Winarsa RHT, Wiyono S, Siswoyo, Muzakhar K (2023). Production and partial purification of cellulase from *Aspergillus* sp. VT12 by solid-state fermentation using coffee pulp. ABSR, 27: 467–474. https://doi.org/10.2991/978-94-6463-062-6_47

Elsababty ZE, Abdel Aziz SH, Ibrahim AM, Guirgis AA, Dawwam GE (2022). Purification, biochemical characterization, and molecular cloning of cellulase from *Bacillus licheniformis* strain Z9 isolated from soil. J. Genet. Eng. Biotechnol., 20(34). https://doi.org/10.1186/s43141-022-00317-4

Ezea IB (2025). Microbial cellulase production – Microorganisms, technologies and factors: A review. Int. J. Biochem. Res. Rev., 34(3): 172–186. https://doi.org/10.9734/ijbcrr/2025/v34i3996

Falade AO, Mabinya LV, Okoh AI, Nwodo UU (2019). Studies on peroxidase production and detection of *Sporotrichum thermophile*-like catalase-peroxidase gene in a *Bacillus* species isolated from Hogsback forest reserve, South Africa. Heliyon, 5: e03012. https://doi.org/10.1016/j.heliyon.2019.e03012

Gaur R, Tiwari S (2015). Isolation, production, purification and characterization of an organic-solvent-thermostable alkalophilic cellulase from *Bacillus vallismortis* RG-07. BMC Biotechnol., 15: 19. https://doi.org/10.1186/s12896-015-0129-9

Goyal V, Mittal A, Bhuwal AK, Singh G, Yadav A, Aggarwal NK (2014). Parametric optimization of cultural conditions for carboxymethyl cellulose production using pretreated rice straw by *Bacillus* sp. 3135 under stationary and shaking conditions. Biotechnol. Res. Int., 2014: Article ID 651839. https://doi.org/10.1155/2014/651839

Hunag S, Sheng P, Zhang H (2012). Isolation and identification of cellulolytic bacteria from the gut of *Holotrichia parallela* larvae (Coleoptera: Scarabaeidae). Int. J. Mol. Sci., 13(3): 2563–257. https://doi.org/10.3390/ijms13032563

Iqbal H, Ahmed I, Zia MA, Irfan M (2011). Purification and characterization of the kinetic parameters of cellulase produced from wheat straw by *Trichoderma viride* under SSF and its detergent compatibility. Afr. J. Biotechnol., 10(50): 10109–10119.

Islam F, Roy N (2018). Screening, purification, and characterization of cellulase from cellulose-producing bacteria in molasses. BMC Res. Notes, 11: 445. https://doi.org/10.1186/s13104-018-3558-4

Korsa G, Konwarh R, Masi C, Ayele A, Haile S (2023). Microbial cellulase production and its potential application for textile industries. Ann. Microbiol., 73(13). https://doi.org/10.1186/s13213-023-01715-w

Kotchoni OS, Shonukan OO, Gachomo WE (2003). *Bacillus pumilus* BpCRI 6, a promising candidate for cellulase production under conditions of catabolite repression. Afr. J. Biotechnol., 2(6): 140–146. https://doi.org/10.5897/AJB2003.000-1028

Madwadza C, Kaul RH, Zvauya R, Mattiassori B (2000). Purification and characterization of cellulases produced by *Bacillus strains*. J. Biotechnol., 83(3): 177–187. https://doi.org/10.1016/S0168-1656(00)00305-9

Mansour SA, Al-Younis ZK, Swadi WA (2025). Purification and characterization of cellulase from Lactobacillus for enhanced essential oil extraction. Basrah J. Agric. Sci., 38(Special Issue): 289–300. https://doi.org/10.37077/25200860.2025.38.sp.26

Naz SH (2015). Purification and characterization of cellulase

production from *Aspergillus niger* (Van Tieghem, 1867). Int. J. Sci. Res., 4(7): 28–32.

Nisar K, Abdullah R, Kaleem A, Iqtedar M, Aftab M, Saleem F (2022). Purification, characterization, and thermodynamic analysis of cellulases produced from *Thermomyces dupontii* and its industrial applications. Saudi J. Biol. Sci., 29: 103483. https://doi.org/10.1016/j.sjbs.2022.103483

Olaitan CO, Kareem SO, Shittu OB, Akinloye OA, Marvelous EO, Musa AO (2025). Cellulase production from *Bacillus* spp. and purification with activated charcoal. World J. Adv. Res. Rev., 25(2): 87–93. https://doi.org/10.30574/wjarr.2025.25.2.0054

Prapulla SG, Karanth NG (2014). Cellulases. In: C.A. Batt M.L. Tortorello (Eds.), encyclopedia of food microbiology (2nd ed., pp. 369–377). Academic Press.

Pratiksha P, Gireesh B (2012). Isolation and identification of methanogenic bacteria from cow dung. Int. J. Adv. Biotechnol. Res., 4(7): 28–32.

Raj T, Chandrasekhar K, Kumar AN, Banu JR, Yoon JJ, Bhatia SK, Kim SH (2022). Recent advances in commercial biorefineries for lignocellulosic ethanol production: Current status, challenges and future perspectives. Bioresour. Technol., 344: 126292. https://doi.org/10.1016/j.biortech.2021.126292

Rawat R, Tewari L (2012). Purification and characterization of an acid other mophilic cellulase enzyme produced by *Bacillus subtilis* strain LFS3. Extremophiles, 16: 637–644. https://doi.org/10.1007/s00792-012-0463-y

Saini A, Aggarwal NK, Yadav A (2017). Isolation and screening of cellulose-hydrolyzing bacteria from different ecological niches. Bioeng. Biosci., 5(1): 7–13. https://doi.org/10.13189/bb.2017.050102

Sethi S, Datta A, Gupta BL, Gupta S (2013). Optimization of cellulase production from bacteria isolated from soil. ISRN Biotechnology, Article ID 985685: 7 pages. https://doi.org/10.5402/2013/985685

Singh VK, Kumar A (1998). Production and purification of an extracellular cellulase from *Bacillus brevis* vs-1. J. Biochem. Mol. Biol. Int., 45(3): 443–452. https://doi.org/10.1080/15216549800202832

Sulyman AO, Igunnu A, Malomo SO (2020). Isolation, purification, and characterization of cellulase produced by *Aspergillus niger* cultured on *Arachis hypogaea* shells. Heliyon, 6(e05668): 1–10. https://doi.org/10.1016/j.heliyon.2020.e05668

Vadala B, Deshpande S, Deshpan A (2021). Soluble expression of recombinant active cellulase in *E. coli* using *B. subtilis* (natto strain) cellulase gene. J. Genet. Eng. Biotechnol., 19. https://doi.org/10.1186/s43141-020-00103-0