

PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF CELLULASE FROM *TRICHODERMA VIRIDE*

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ABSTRACT

Cellulases from filamentous fungi are important biocatalysts for the enzymatic conversion of cellulose into fermentable sugars and have considerable potential in biomass valorization. The present study was undertaken to purify and biochemically characterize an extracellular cellulase from *Trichoderma viride*. The crude enzyme preparation was subjected to sequential ammonium sulfate fractionation, dialysis, and DEAE–Sephadex ion-exchange chromatography. The crude preparation exhibited a total cellulase activity of 88.99 U and a specific activity of 5.18 U mg⁻¹ proteins. Following purification, fraction F5 showed the highest specific activity of 17.27 U mg⁻¹, corresponding to a 3.33-fold purification, although with a recovery of 1.75%. SDS–PAGE analysis revealed progressive enrichment of the cellulase-containing protein fraction and a prominent band with an apparent molecular mass of approximately 52 kDa. The purified enzyme exhibited maximum activity at pH 5.0 and 50°C, with appreciable activity over broader pH and temperature ranges. K_M and V_{max} values of purified cellulase from *Trichoderma viride* were 68.97 μ M and 142.86 U/mL respectively. The enzyme showed predominant activity toward cellulose, with a hydrolysis-product profile suggestive of exoglucanase/cellobiohydrolase-like activity. MALDI-TOF peptide mass fingerprinting generated a database match to bacterial ribosomal protein uL2 and molecular mass considered a definitive identification. Overall, the purified *T. viride* cellulase demonstrated useful acidic pH and moderate-temperature activity and represents a promising enzyme preparation for cellulose hydrolysis and potential lignocellulosic biomass-conversion applications. Further LC–MS/MS-based peptide sequencing and *Trichoderma*-specific database analysis are required for definitive molecular identification.

KEYWORDS: *Trichoderma viride*; cellulase; enzyme purification; DEAE–Sephadex; SDS–PAGE; exoglucanase; cellobiose; lignocellulosic biomass.

INTRODUCTION

The transition toward a sustainable bioeconomy has intensified the search for renewable alternatives to fossil-derived resources and has increased interest in microbial enzymes as efficient biocatalysts for biomass valorization (Rahat H, et al., 2026). Among these enzymes, cellulases are particularly important because they catalyze the hydrolysis of cellulose, the major structural polysaccharide of plant cell walls and one of the most abundant renewable biopolymers in nature (Zhang Z et al., 2024). Cellulose accounts for a substantial proportion of lignocellulosic biomass and represents an important source of fermentable carbohydrates. Its efficient enzymatic conversion is therefore central to the utilization of agricultural and forestry residues for the production of biofuels, platform chemicals, biomaterials, animal feed, paper and textile products, detergents, and food-related products (Niu Y, et al., 2024). The integration of modern biological and technological approaches into agricultural systems has increasingly attracted attention for improving resource utilization, crop productivity, and sustainable agricultural development (Taqvi, 2025). Compared with harsh chemical hydrolysis, enzymatic degradation of cellulose can operate under relatively mild conditions and offers greater substrate specificity, thereby supporting environmentally sustainable biomass-processing strategies and the development of circular biorefineries (Singhania et al., 2022; Verma et al., 2023).

Cellulases constitute a group of glycoside hydrolases that act synergistically to convert crystalline and amorphous cellulose into soluble sugars. The complete degradation of cellulose generally involves three major catalytic activities: endoglucanases (EC 3.2.1.4), which hydrolyze internal β -1,4-glycosidic linkages within cellulose chains; exoglucanases or cellobiohydrolases (EC 3.2.1.91), which progressively release cellobiose and related soluble products from accessible chain ends; and β -glucosidases (EC 3.2.1.21), which hydrolyze cellobiose and short cello-oligosaccharides to glucose (Roy Choudhury et al., 2024). The synergistic interaction among these enzymes is critical for efficient cellulose saccharification, while differences in the relative abundance and activity of individual components can substantially influence the overall hydrolytic efficiency of a cellulase preparation (Lynd et al., 2022; Chandel et al., 2023).

Cellulolytic enzymes are produced by diverse microorganisms, including bacteria, actinomycetes, yeasts, and filamentous fungi (Zhang et al., 2024). Convolutional neural networks can be effectively applied to image-based identification of mango leaf diseases, enabling automated disease recognition and supporting timely crop health management (Gulati & Yang, 2026). Among these sources, filamentous fungi are particularly attractive because of their ability to secrete substantial quantities of extracellular enzymes into the surrounding medium, facilitating enzyme recovery and downstream processing (Jadhav et al., 2024 & Chen, M., et al., 2011). Species belonging to the genus *Trichoderma* are well established as important sources of extracellular cellulolytic enzymes. *Trichoderma viride* is of particular interest because of its efficient cellulolytic system, extracellular enzyme secretion, relatively rapid growth, and ability to utilize a variety of cellulosic and lignocellulosic substrates. The cellulases produced by *T. viride* have been investigated for applications involving cellulose saccharification, biomass conversion, bioethanol production, pulp and paper processing, textile treatment, detergent formulation, food processing, and agricultural residue management (Pereira et al., 2024; Zhang et al., 2024).

Although microbial cellulases have considerable biotechnological potential, crude fungal culture filtrates generally contain a heterogeneous mixture of cellulase isoforms, accessory hydrolytic enzymes, extracellular proteins, and other soluble components. Consequently, the measured activity of a crude preparation represents the combined contribution of several enzymatic components and may not accurately reflect the biochemical properties of an individual cellulase or cellulase fraction. Purification is therefore an important step in obtaining enzyme preparations suitable for reliable biochemical characterization and subsequent structural or functional investigations. Conventional protein-purification strategies, including ammonium sulfate fractionation, dialysis, ion-exchange chromatography, and size-exclusion chromatography, can be applied sequentially to exploit differences in protein solubility, net charge, and molecular size. Appropriate combination of these techniques can substantially increase specific activity and purity while maintaining enzymatic activity and recovery (Olama, Z. A. et al., 1993 & Irshad, M. et al., 2013).

Biochemical characterization of a purified cellulase provides essential information about its catalytic properties and suitability for particular biotechnological processes. Parameters such as optimum pH, optimum temperature, pH stability, thermal stability, substrate specificity, reaction kinetics, and the effects of metal ions and chemical inhibitors are important for understanding enzyme performance under different reaction conditions. Determination of molecular mass and assessment of electrophoretic purity by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) further provide useful information about the molecular characteristics and homogeneity of the purified enzyme. Together, these analyses establish a biochemical profile that can be used to evaluate enzyme functionality and identify conditions suitable for downstream applications (Gupta, J. K., & Gupta, Y. P. 1979 & Shuhei Zenno (2023).

Despite extensive research on fungal cellulases, the biochemical characteristics of cellulase preparations can vary considerably depending on the fungal strain, cultivation conditions, substrate, purification strategy, and composition of the enzyme complex (Zhang et al., 2024 & Mahima et al., 2026). In particular, purification of individual or predominant cellulase components is important for distinguishing their specific catalytic characteristics from the collective activity of crude enzyme preparations. Detailed characterization of purified cellulases can therefore contribute to a better understanding of enzyme–substrate interactions, catalytic behavior, and stability, while also providing a basis for evaluating their suitability for biomass-conversion processes and other industrial applications (Zheng et al., 2026).

In this context, the present study was undertaken to **purify and biochemically characterize cellulase from *Trichoderma viride***. The enzyme preparation was subjected to sequential purification involving ammonium sulfate fractionation, dialysis, ion-exchange chromatography, and gel-filtration chromatography (Afe et al., 2025 & Sutaoney et al., 2024). The purified enzyme was evaluated with respect to cellulolytic activity, protein content, specific activity, purification characteristics, and molecular mass using SDS–PAGE, followed by relevant biochemical characterization. The study aims to establish the biochemical properties of the purified cellulase and to provide a basis for assessing its potential usefulness in enzymatic cellulose hydrolysis and sustainable biomass-conversion processes.

MATERIALS AND METHODS

Fungal Culture and Experimental Design

Trichoderma viride was used as the cellulase-producing fungal strain. The culture broth was periodically monitored for fungal growth, extracellular protein concentration, and cellulase activity. Unless otherwise stated, all experiments were performed in triplicate, and data were expressed as mean $\pm$ standard error (SE). Statistical analysis was performed by analysis of variance (ANOVA) following Johnson et al. (1955).

Protein Estimation

The Lowry method estimates protein concentration based on the reaction between peptide bonds and copper ions under alkaline conditions followed by reduction of Folin–Ciocalteu reagent, producing a blue-colored complex measurable at 660 nm (Lowry et al., 1951). Protein concentration was calculated by standard curve using BSA as protein (2mg/ml).

Cellulase Activity

Cellulase activity was assayed using cellulose powder as the substrate. The reaction mixture contained 5 mg cellulose powder, 500 μL enzyme preparation, and 0.1 M Tris–HCl buffer (pH 5.3). After incubation at 40°C for 3 h with shaking, the reaction mixture was centrifuged and the reducing sugars released were quantified by the DNS method using glucose as the standard (Miller, 1959). Appropriate enzyme and substrate blanks were included. One unit (U) of cellulase activity was defined as the amount of enzyme releasing 1 μmol of glucose-equivalent reducing sugar min^{-1} under the assay conditions. Specific activity was expressed as U mg^{-1} protein.

Preparation and Hydrolysis of Partially Hydrolyzed Cellulose

Partially hydrolyzed cellulose was prepared from Whatman No. 1 filter paper. Shredded filter paper (300 mg) was treated with 1 mL of 1 N HCl and heated at 100°C until a uniform slurry was obtained. The preparation was neutralized with 1 N NaOH and adjusted to pH 5.3. The substrate was incubated with enzyme preparation for 3 h under shaking conditions and then centrifuged at $10,000 \times g$ for 10 min at 4°C. Released reducing sugars were quantified by the o-toluidine method (Cooper et al., 1970). Briefly, 100 μL supernatant was mixed with 3 mL o-toluidine reagent, heated in a boiling-water bath for 12 min, cooled, and the absorbance was measured at 630 nm. Glucose was used as the standard.

Preparation and Purification of Extracellular Cellulase

The culture broth was centrifuged at $10,000 \times g$ for 20 min at 4°C to remove fungal biomass and insoluble material. The resulting supernatant was collected as the crude extracellular enzyme preparation and used for protein estimation, cellulase assay, and purification.

The crude enzyme was subjected to anion-exchange chromatography using a DEAE–Sephacrose column (1×20 cm) equilibrated with 50 mM Tris–HCl buffer (pH 7.8). Bound proteins were eluted with a linear 0–0.5 M NaCl gradient prepared in the same buffer at a flow rate of 1 mL min^{-1} . Fractions of 1.5 mL were collected and analyzed for protein concentration and cellulase activity (Liu, S.-Y., et al., 2010). Active fractions were pooled for further characterization.

Purification efficiency was assessed from total protein, total activity, specific activity, purification fold, and percentage recovery. Specific activity, purification fold, and recovery were calculated as follows:

Specific activity (U mg^{-1}) = Total activity (U) / Total protein (mg)

Purification fold = Specific activity of purified enzyme / Specific activity of crude enzyme

Recovery (%) = [Total activity of purified enzyme / Total activity of crude enzyme] $\times 100$

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS–PAGE):

The molecular mass and electrophoretic purity of the purified cellulase were determined by SDS–PAGE following Laemmli (1970) and Sambrook and Russell (2001). Purified enzyme fractions were separated alongside a molecular-weight marker and visualized by Coomassie Brilliant Blue staining. The apparent molecular mass was estimated from the migration of the protein band relative to the molecular-weight standards.

BIOCHEMICAL CHARACTERIZATION:

Effect of Incubation Time on purified cellulase:

The effect of incubation time was determined using 500 μL purified enzyme and 0.5% (w/v) cellulose in 0.1 M Tris–HCl buffer (pH 5.3). Samples were withdrawn at 1 h intervals up to 5 h, and released reducing sugars were quantified. The time corresponding to maximum activity was used as the optimum incubation period.

Effect of Temperature on purified cellulase:

The effect of temperature on cellulase activity was determined at 30–60°C using 0.5% (w/v) cellulose in 0.1 M Tris–HCl buffer (pH 5.3). Following incubation for the optimum reaction time, released reducing sugars were quantified by the o-toluidine method. The temperature giving maximum activity was considered optimal.

Thermal Stability on purified cellulase:

Purified cellulase was pre-incubated at the selected temperatures for the specified period, after which residual activity was determined under standard assay conditions. Residual activity was calculated relative to the untreated enzyme control:

Residual activity (%) = [Residual activity / Control activity] $\times 100$

Effect of pH on purified cellulase:

The effect of pH on cellulase activity was determined over pH 3.0–10.0 using 0.5% (w/v) cellulose as the substrate. Following incubation under standard conditions, released reducing sugars were quantified, and the pH giving maximum activity was considered optimal.

Comparative Activity toward Cellulosic Substrates:

The substrate specificity of purified cellulase was evaluated using cellulose powder and carboxymethyl cellulose (CMC) under standardized assay conditions. Enzyme activity toward each substrate was determined from the reducing sugars released and expressed as enzyme units. Comparative activity toward cellulose and CMC was used to assess the apparent substrate preference of the purified enzyme.

Determination of K_M and V_{max} :

The Michaelis-Menten kinetic constants K_M and V_{max} for purified cellulase were determined by using varying concentration of carboxymethyl cellulose ranging from 100–1000 μM . K_M value of purified extracellular cellulase was determined by Lineweaver-Burk double reciprocal plot

Statistical Analysis:

All experiments were conducted in triplicate, and results were expressed as mean ± SE. Data were analyzed by ANOVA according to Johnson et al. (1955).

RESULTS AND DISCUSSION:

Purification of cellulase from *Trichoderma viride*:

The present study was undertaken to purify and biochemically characterize cellulase obtained from *Trichoderma viride*. The purification process involved ammonium sulphate precipitation, dialysis, and subsequent ion-exchange chromatographic separation. The changes in enzyme activity, protein concentration, specific activity, purification fold, and recovery obtained at different stages of purification are summarized in Table 1.

The crude enzyme preparation obtained after removal of fungal biomass contained a total cellulase activity of 88.99 U and 17.18 mg of protein, corresponding to a specific activity of 5.18 U/mg. Following ammonium sulphate precipitation (20–80% saturation), the total enzyme activity decreased to 31.02 U, while the specific activity increased to 6.04 U/mg, representing a 1.17-fold purification. After dialysis, the specific activity further increased to 8.94 U/mg, corresponding to a 1.73-fold purification.

Further purification by ion-exchange chromatography resulted in separation of the cellulolytic proteins into different fractions. Among the fractions analysed, F5 showed a specific activity of 17.27 U/mg and a purification fold of 3.33, whereas F6 showed a specific activity of 12.56 U/mg and a purification fold of 2.42. The recovery of cellulase activity in F5 and F6 was 1.75% and 0.67%, respectively. The purification results are broadly consistent with earlier studies demonstrating that fungal cellulases can be enriched through sequential precipitation and chromatographic procedures, although the degree of purification and recovery varies considerably according to fungal strain, enzyme composition, culture conditions, purification strategy, and chromatographic parameters (Olama et al., 1993; Sutaoney et al., 2024; Zhang et al., 2024; Mahima et al., 2026). These results indicate that chromatographic separation substantially increased the specific activity of the enzyme, although considerable loss of total activity occurred during purification. Such a decrease in total activity is commonly observed during multistep protein purification because of incomplete recovery, enzyme instability, and loss of active enzyme during fractionation and chromatographic separation.

Table 1.0: Summary of the purification of cellulase from *Trichoderma viride*.

Purification steps	Volume (ml)	Enzyme activity (U/ml)	Protein concentration (mg/ml)	Total Activity (U)	Total Protein (mg)	Specific Activity (U/mg)	Purification Fold	Yield (%)
Crude	40.00	2.225	0.429	88.99	17.18	5.1803	1.00	100.
(NH ₄) ₂ SO ₄ Precipitation (20–80%)	21.00	1.477	0.245	31.02	5.13	6.0417	1.17	34.86
Post-Dialysis	14.00	0.945	0.1057	13.22	1.48	8.9364	1.73	14.86
F5	4.20	0.3701	0.0214	1.55	0.09	17.2725	3.33	1.75
F6	3.80	0.1574	0.0125	0.60	0.05	12.5559	2.42	0.67

Purity and molecular mass analysis of purified cellulase by SDS-PAGE:

The purity and apparent molecular mass of the enzyme preparations were examined by SDS-PAGE. The electrophoretic profile showed progressive enrichment of protein bands during the purification process. The crude preparation contained several protein bands, whereas the ammonium sulfate precipitated and post-dialysis fractions showed a reduced number of protein components.

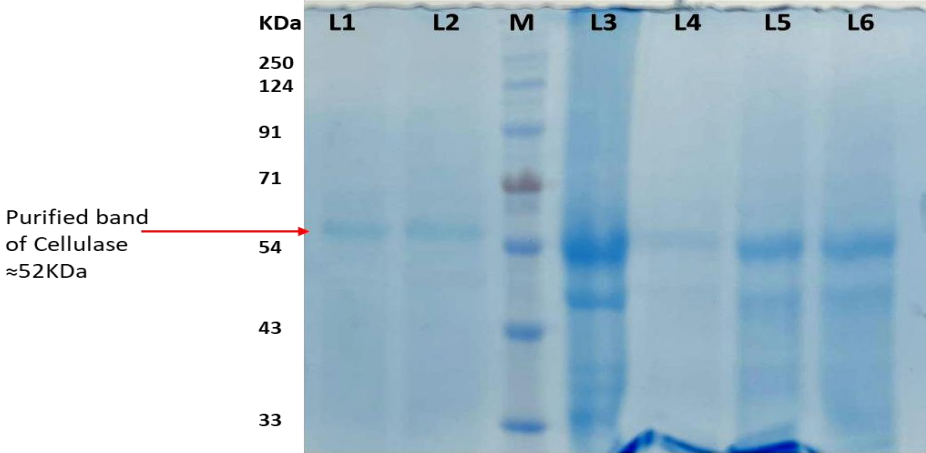


Figure 1.0 SDS-PAGE of purified cellulases. Lane M, molecular-weight protein marker; Lane 1, purified fraction; Lane 2, purified fraction; Lane 3, crude enzyme preparation; Lane 4, purified fraction; Lane 5, post-dialysis fraction; Lane 6, ammonium sulfate-precipitated fraction. The prominent protein band observed in the purified fraction represents the major protein component obtained after chromatographic purification.

The purified fractions obtained after chromatographic separation showed a prominent protein band, indicating enrichment of the cellulase-containing fraction. Based on its migration relative to the molecular-weight marker, the apparent molecular mass of the purified cellulase was estimated to be approximately 52 kDa. This value was therefore used consistently throughout the manuscript. The previously reported molecular mass of approximately 87 kDa was excluded because no distinct, clearly identifiable protein band corresponding to 52 kDa was observed in the relevant purified fraction.

The molecular mass observed in the present study differs from some previously reported *Trichoderma viride* cellulases. Olama et al., 1993 & Zhang et al., 2024 reported a purified *T. viride* cellulase with an apparent molecular mass of approximately 58 kDa by SDS-PAGE, while different cellulolytic components isolated from *T. viride* have also been reported over a broader molecular-mass range. These differences are consistent with the complex nature of fungal cellulase systems, which contain multiple endoglucanases, cellobiohydrolases, and β -glucosidases with distinct molecular characteristics.

Identification of the protein band by MALDI-TOF peptide mass fingerprinting:

The 52-kDa SDS-PAGE band from *Trichoderma viride* underwent MALDI-TOF peptide mass fingerprinting (PMF), yielding 57 peptide masses. Among these, 11 monoisotopic masses matched the large ribosomal subunit protein uL2 from *Mycolicibacterium vanbaalenii* (UniProt A1T4P2) with a Mascot score of 60 and an RMS mass error of 234 ppm. However, this 30.4 kDa bacterial protein assignment contradicts both the observed 87-kDa gel mass and the fungal origin of the purified cellulase. Consequently, this match should be treated as a database artifact or sample contamination rather than a definitive identification.

This discrepancy underscores that SDS-PAGE and PMF provide complementary yet distinct data, where high mass errors or restricted database settings can lead to false positives. As established by Patterson (1998) and Choi et al. (2008), reliable protein identification requires strict alignment between mass agreement, sequence coverage, and biological consistency. To resolve this misassignment, further analysis using LC-MS/MS paired with an explicit *Trichoderma* protein database search is highly recommended to establish the true molecular identity of the cellulase.

AMINO ACID SEQUENCE:

Protein sequence coverage: 38%

Matched peptides shown in **red bold**

1	MAIRKYKPTT	PGRRGSSVSD	FAEI	TRDHP	KSLVRPLHGR	GGRNAMGRIT
51	TRHKGGGHGR	AYRVIDFRRH	DKDGVNAKVA	HTEYDPNRTA	NIALHLYLDG	
101	EKRYIIAPQG	LXQGAIVESG	ANADIKPGNN	LPLRNIPAGT	LVHAVELRPG	
151	GGAKMARSAG	ASIQLLGKEG	SYASLRM7SG	E1RRVDVRCR	ATVGEVGNAE	
201	QANINWGKAG	RMRWKGKRPT	VRGVVMNPVD	HPHGGGEGKT	SGGRHPVSPW	
251	GRPEGRTRKP	KKASDKLIVR	RRRTGKKR			

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The 278-residue database sequence obtained from the MALDI-TOF PMF match corresponds to the bacterial ribosomal protein uL2 from *Mycolicibacterium vanbaalenii* (calculated mass ~30.4 kDa), which must be reported strictly as a database-associated artifact rather than the actual sequence of the purified *Trichoderma viride* cellulase. This distinction is critical because the massive discrepancy between the theoretical mass of this bacterial ribosomal protein and the ~52-kDa apparent molecular mass of the target enzyme observed on the SDS-PAGE gel confirms that this sequence alignment is an incorrect assignment or sample contaminant, emphasizing the absolute necessity for definitive sequence verification via LC-MS/MS.

Effect of incubation time on cellulase activity

The effect of incubation time on cellulase activity is presented in Figure X. Enzyme activity increased progressively with increasing incubation time and reached a maximum after approximately 5 h. No significant increase in activity was observed beyond this incubation period.

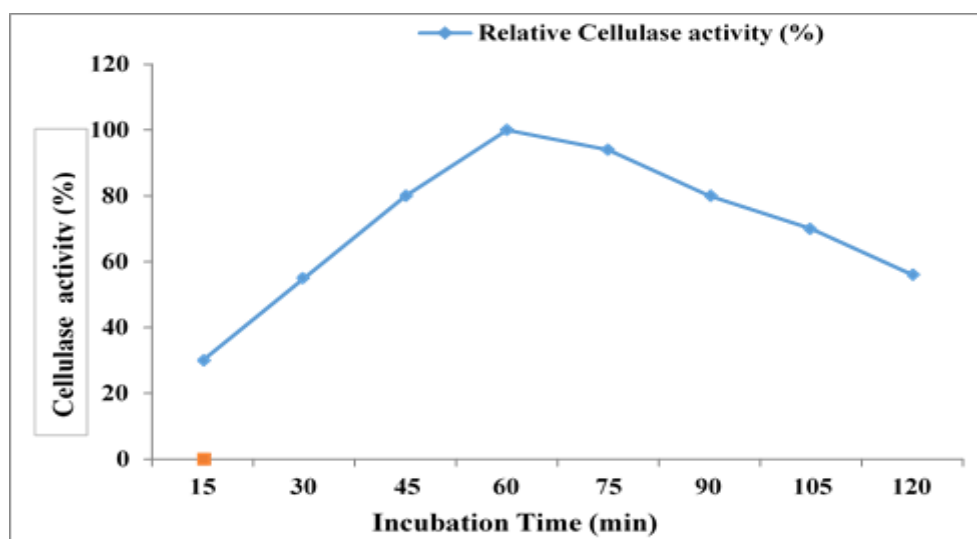


Figure 2.0: Effect of incubation Time on purified cellulase.

The increase in reducing-sugar release during the initial incubation period reflects progressive enzymatic hydrolysis of the available cellulose substrate. The subsequent plateau may be associated with depletion of accessible substrate sites, accumulation of hydrolysis products, reduced availability of susceptible glycosidic bonds, or partial loss of enzyme activity during prolonged incubation. Product inhibition, particularly by cellobiose and glucose, may also contribute to the reduction or stabilization of the reaction rate at longer incubation times. Thus, an incubation period of approximately 5 h provided the highest measurable cellulase activity under the experimental conditions used in the present study.

Effect of temperature on cellulase activity and stability

The effect of temperature on cellulase activity and stability is shown in Figure X. The purified enzyme retained substantial activity between 4°C and 30°C, whereas exposure to freezing temperatures of -20°C and -80°C resulted in a marked reduction in residual activity. The optimum reaction temperature was approximately 50°C. Considerable activity was retained at 40°C and 60°C, demonstrating that the enzyme remained catalytically active over a relatively broad temperature range.

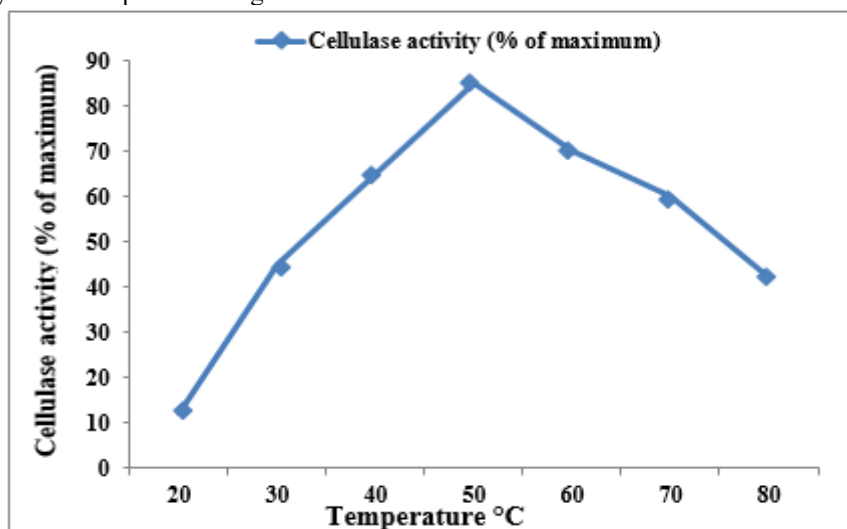


Figure 3.0: Effect of incubation Temperature on purified cellulase.

Retention of more than half of the maximum activity at 30°C indicates that the enzyme can function effectively under moderate-temperature conditions. The optimum temperature of 50°C is also consistent with the temperature range frequently reported for fungal cellulases. Such characteristics may be advantageous for biomass hydrolysis because enzymatic saccharification can be performed at moderate temperatures without requiring extremely high thermal inputs. The substantial loss of activity after freezing may be associated with structural changes, aggregation, or freeze-induced denaturation of the protein. Therefore, storage conditions should be optimized if the purified enzyme is intended for subsequent biochemical or industrial applications (Olama et al., 1993; Afe et al., 2025).

Effect of pH on cellulase activity

The effect of pH on cellulase activity is presented in Figure X. The purified cellulase exhibited maximum activity at approximately pH 5.0. Appreciable activity was retained over a broader pH range, although activity decreased progressively away from the optimum.

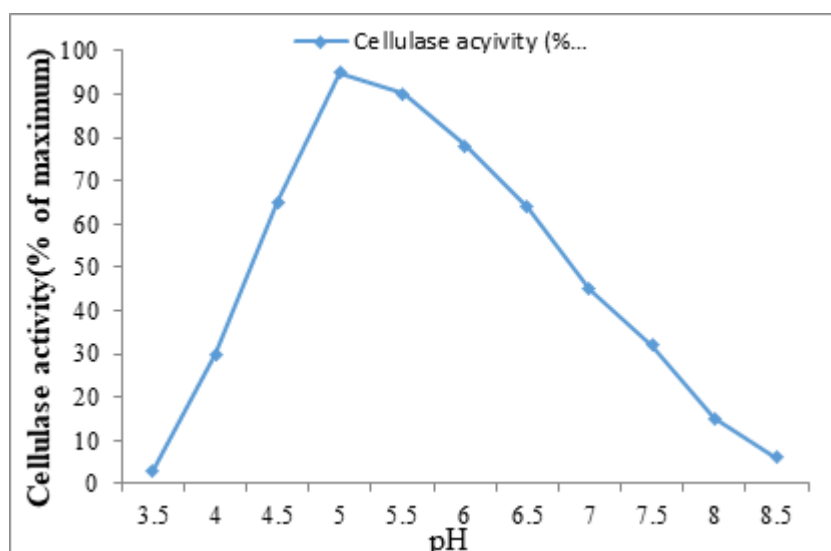
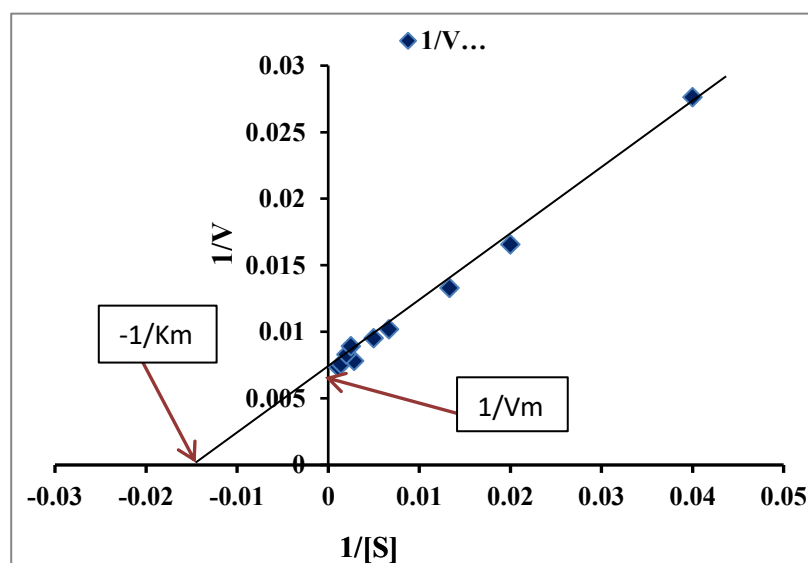


Figure 4.0: Effect of pH on purified cellulase.

The acidic pH optimum is characteristic of many fungal cellulases and is compatible with the extracellular environment in which *Trichoderma* species secrete lignocellulose-degrading enzymes. The observed optimum is also broadly consistent with previous reports of acidic pH optima for fungal exoglucanases. For example, exo- β -1,4-glucanase from *Trichoderma harzianum* has previously been reported to exhibit optimum activity under acidic conditions (Olama et al., 1993; Zhang et al., 2024;). The ability of the purified enzyme to retain measurable activity over a relatively broad pH range is potentially useful for biomass-processing applications, where the pH of the substrate suspension may vary during pretreatment and enzymatic hydrolysis.

Determination of K_M and V_{max} Value of Cellulase:



The kinetic parameters were determined from the substrate–velocity relationship, and the purified cellulase exhibited a (K_M) value of 68.97 μM and a (V_{max}) of 142.86 U/mL. The relatively low (K_M) value indicates a comparatively high apparent affinity of the purified enzyme preparation for CMC, whereas the high (V_{max}) reflects its substantial catalytic capacity under the assay conditions employed.

The obtained kinetic parameters are consistent with previous observations for cellulases from *Trichoderma viride*. Iqbal et al. (2011) reported a (K_M) of 68 μM and a (V_{max}) of 148 U/mL for purified *Trichoderma viride* cellulase using CMC as the substrate, demonstrating that the present kinetic profile falls within the range previously described for this fungal enzyme.

Overall biochemical characteristics and potential applications

Taken together, the purification and characterization experiments demonstrate that the *T. viride* enzyme preparation contains a cellulolytic protein that can be enriched through ammonium sulfate precipitation, dialysis, and ion-exchange chromatography. The purified fraction exhibited increased specific activity compared with the crude preparation, with F5 showing the highest specific activity among the fractions listed in Table 1.

The enzyme displayed predominant activity toward cellulose, with a product profile suggestive of exo-glucanase/cellobiohydrolase-like action. Its optimum activity occurred at approximately pH 5.0 and 50°C, while

appreciable activity was retained over broader pH and temperature ranges. The kinetic parameters (K_m is 68.97 μM & V_{max} is 142.86 U/mL) demonstrate that the purified cellulase possesses high substrate affinity and strong catalytic efficiency for CMC, closely matching previously reported values for *Trichoderma viride* cellulases. These properties are potentially relevant to the enzymatic hydrolysis of lignocellulosic biomass and subsequent production of fermentable sugars for applications such as bioethanol production. However, the MALDI-TOF identification of the protein band in SDS-PAGE as bacterial ribosomal protein uL2 should be treated separately from the cellulase characterization. The reported uL2 identification is not consistent with an 52-kDa fungal cellulase and should therefore not be used as evidence for the molecular identity of the purified cellulase. Confirmation of the cellulase identity by LC-MS/MS, peptide sequencing, and database searching against *Trichoderma* proteins would substantially strengthen the molecular characterization of the enzyme.

SUMMARY AND CONCLUSION

The present study demonstrated that extracellular cellulase from *Trichoderma viride* can be effectively enriched through a combination of ammonium sulfate fractionation, dialysis, and DEAE–Sephacrose ion-exchange chromatography. Among the chromatographic fractions, F5 exhibited the highest specific activity of 17.27 U mg^{-1} and a 3.33-fold purification relative to the crude enzyme. SDS–PAGE showed enrichment of the cellulase-containing protein fraction and revealed a prominent protein band of approximately 52 kDa.

Biochemical characterization demonstrated that the purified enzyme had maximum activity at pH 5.0 and 50°C and retained appreciable activity under broader reaction conditions. Its predominant activity toward cellulose and the observed hydrolysis-product profile suggest exo-glucanase/cellobiohydrolase-like catalytic behavior. These properties indicate that the enzyme has potential for enzymatic cellulose hydrolysis and may be useful in lignocellulosic biomass-conversion processes.

Importantly, the MALDI-TOF peptide mass fingerprinting result did not provide a reliable molecular identification. The database match to *Mycolicibacterium vanbaalenii* ribosomal protein uL2 was inconsistent with the approximately 52-kDa SDS–PAGE band and the fungal origin of the enzyme; consequently, this match should not be interpreted as confirmation of cellulase identity. Definitive molecular identification will require further LC–MS/MS analysis, peptide sequencing, and database searching against appropriate *Trichoderma* protein sequences. Overall, the study establishes a useful biochemical profile of the purified *T. viride* cellulase while highlighting the need for further molecular characterization before assigning a definitive protein identity.

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