Purification, characterization and thermodynamic analysis of cellulases produced from Thermomyces dupontii and its industrial applications

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Cellulases involved in the hydrolysis of cellulose and plays a vital role in different industries like textile, detergent paper and feed industry. Cellulases have been a prospective target for research by both the academic and industrial sectors because of the intricacy of the enzyme system and the enormous industrial potential. In the present work Thermomyces dupontii, which had previously been isolated and recorded as a promising cellulase producer were used. Both endoglucanases and betaglucosidases were purified to its homogeneity by ammonium sulfate followed by anion exchange and gel filtration chromatography. The recovery and purification fold for endoglucanases and betaglucosidases were 13.7, 10.7 % and 5.9, 2.7 respectively. The molecular weight of endoglucanases and betaglucosidases were estimated as 37 and 66 kDa on SDS-PAGE. Upon kinetic analysis the purified endoglucanases and betaglucosidases showed Km 0.63; 28.56 mg/ml and Vmax 82; 80 U/ml/min, respectively. Characterization revealed that enzyme was found to be acidophilic cellulase having optimal pH of 5.5 and 70 °C. Furthermore, cellulases were accelerated in the presence of Ca²⁺ and EDTA. The cellulases had activation energy (Ea) of −44.55; −50.02 kJ/mol for carboxy-methyl-cellulose hydrolysis and Enthalpy (AH) 42.20; 47.70 kJ/mol and entropy AS −5.1 and −5.7 kJ/mol for EG and BGL, respectively. In addition to this the enzyme had a secondary structure of protein as represented by FTIR spectrum. The current study suggested that purified cellulases can be used as a detergent additive to improve washing. Furthermore, it shows the biostoning ability when applied on jean fabric.

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1. Introduction

Cellulose is the most abundant organic compound and essential carbon source available on the earth. It is an unbranched polymer made up of β-glucose units that are joined together by β-1, 4-glycosidic bonds and are generally found in two forms i.e. crystalline and amorphous (Nehad et al., 2019). Microorganisms have the ability to produce several groups of enzymes that are collectively known as cellulases, which work in synergistic manner to hydrolyze the β-1,4-α-glycosidic linkages that is found in the cellulose molecules (Begum and Absar, 2009). Different studies show that, cellulose degrading enzymes are extensively produced by various microbes, including fungi, bacteria and protozoan. Among all the microbes, fungal genera such as Trichoderma, Penicillium and Aspergillus etc. have more potential to produce cellulases. Thermophilic fungi can grow at maximum temperature of 55 °C and minimum temperature of 20 °C. In recent years thermophilic fungi has gained more attention in many biotechnological applications due to their greater thermostability, tolerance to pH variation, and resistance to denaturing agents. (Moretti et al., 2012).

Recent reports of enzyme market state that, the key industries where the cellulases increasingly being used are textile, food, healthcare, beverages, paper and pulp. It is broadly used in wet processing of textile, bio-stoning of denim fabric, garment softening and excess dye removal from fabrics. In addition to this the ability to remove stains is the main reason for using them in the detergent industry. These detergents modify the fibers in the fabric to improve softness, color brightness, and particulate soil removal. Due to increasing environmental concerns, many restrictions have been made on the textile industry. However, treatment of cotton
fabric with cellulases having neutral pH is the eco-friendly method to obtain the desirable appearance of the fabrics (Juciene et al., 2014).

The physicochemical properties of the microbes such as the influence of substrate, temperature and pH etc. help in the up-scaling of enzymes. While growth kinetics and thermodynamic parameters further helps to evaluate the economic feasibility of microbial enzyme production at pilot scale. Investigations using thermodynamic and kinetic principles can reveal information about the thermo stability of enzymes at their operating temperature. However, enthalpy, entropy and activation energy among the unfolded and folded states of the enzyme are the factors used to define denaturation thermodynamics (Souza et al., 2015).

In current study, the growth kinetics and thermodynamics analysis of the cellulase produced from thermophillic fungal strain T. dupontii under submerger fermentation was studied. Moreover, according to our best of knowledge till date this was the first study upon purification characterization kinetics and thermodynamics analysis of cellulases produces from T. dupontii.

2. Materials and methods

2.1. Thermophillic fungal strain

The strain Thermomyces dupontii (Genbank accession no. MN685243) previously reported for cellulase production was obtained from the Biotechnology department, Lahore College for Women University. It was re-cultured and used for purification process (Nisar et al., 2020).

2.2. Enzyme assay

Endoglucanases activity was determined according to Gao et al. (2008) by taking 0.5 ml of supernatant and 0.5 ml of 1 % CMC prepared in citrate buffer (0.1 M; pH 5.0). A blank was run parallel by substituting enzyme with distilled water. Afterwards reaction mixture was incubated at 60°C for 30 min. Reducing sugar released after incubation was measured followed by Miller (1959) at 546 nm using spectrophotometer.

β-glucosidase activity was determined by Rajoka and Malik (1997). For this purpose 0.2 ml of enzyme extract was added in 0.2 ml of 10 mM p-nitrophenyl-β-D-glucopyranoside (pNPG) and 0.6 ml of acetate buffer (0.2 M; pH 5.5). The mixture was incubated at 45°C for 10 min. After incubation the reaction was stopped by adding 3.0 ml of 1 M sodium carbonate. A blank was also run parallel by substituting enzyme with distilled water. The absorbance was measured at 405 nm with the help of spectrophotometer.

2.3. Purification of cellulases

The fermented broth was centrifuged at 10000xg for 20 min at 4°C and clarified supernatant was used for the enzyme purification. Initially solid ammonium sulphate was gradually added in the crude extract and gently stirred at 4°C for 30 min in order to get different saturation (20–90) levels (Nasir et al., 2011). The precipitate was harvested by centrifugation at 14000xg for 15 min at 4°C and dissolves in 2 ml of 0.05 M Tris-HCl buffer (pH 7.5) and dialyzed for 24 h by using same buffer. All the procedure was carried out at 4°C. The dialyzed enzyme was subjected to Hi-trap anion exchange column pre-equilibrated and eluted with 0.05 M Tris-HCl, (pH 7) at a flow rate of 0.1 ml/min. The most active cellulase containing eluted fractions were collected and subjected to Sephadex G-100 column 0.05 M acetate buffer (pH 4.8) for EG and 0.05 M citrate buffer (pH 4.8) for BGL, respectively, were used to pre-equilibrate the samples before they were eluted with a linear gradient of (0–1 M) NaCl at a flow rate of 1.0 ml/min. Active fractions were pooled out and assayed for enzyme and protein (Shahriarinour et al. 2015).

2.4. SDS PAGE

Sodium dodecyl sulphate polyacrylamide gel electrophoresis with a 12% concentration was used to assess the molecular weight of purified cellulases (Zhang et al., 2017).

2.5. Characterization of cellulases

The effect of pH ranging from (4–9) and temperature between (40°C–100°C) on endoglucanase and β-glucosidase stability and activity were evaluated. Besides this, the influence of several metal ions (Mn²⁺, Cd²⁺, Zn²⁺, Hg²⁺ and Ca²⁺) and surfactants (EDTA, SDS, beta-mercaptoethanol and Tween 20) were evaluated at 10 mM concentrations.

2.6. Measurement of Km and Vmax

The kinetic parameters Vmax (maximal velocity) and Km (Michaelis–Menton constant) of EG and BGL activity were calculated by Lineweaver-Burk plot (LB) from the optimal assay conditions.

2.7. Thermodynamic analysis

According to Siddiqui et al. (1997) the activation energy (Ea) was estimated using the Arrhenius method and used to examine the thermodynamic parameters (ΔS) entropy and (ΔH) enthalpy change.

\[ \text{Ea} = -\text{slope} \times R \]

\[ \Delta H = \text{Ea} - R \times T \]

Whereas, Boltzmann constant \( R = 8.314 \text{ J/K-1 mol-1}, \) gas constant \( K_B = 1.38 \times 10^{-23} \text{ J/K}, \) Planck’s constant \( h = 6063 \times 10^{34} \text{ Js} \) and \( T \) is absolute temperature.

2.8. Shelf life of purified EG and BGL

Purified enzymes were kept for 30 days at 4°C and 30°C in order to check the shelf life.

2.9. Applications

2.9.1. Denim fabric treatment with purified endoglucanase

The role of purified endoglucanases on bio-stoning application was determined by cutting the denim fabric into two equal halves (4.5 X4.5 cm) and placing them in separate flasks. The Flask A contains a citrate buffer with 5.5 pH used as a control. However, in Flask B 25 ml of citrate buffer along with 25 ml of purified EG enzyme was added and kept for 24 h at 70°C. Enzyme activity was ceased by keeping the samples at 80°C for about 10 min. The samples were then carefully washed with tap water and left at room temperature for drying (Sahin et al., 2016).

2.9.2. Scanning electron microscopy

The surface morphology of denim fabric after cellulase treatment was determined by scanning electron microscopy. All microscopic images were obtained under the technological conditions:
Magnification 5000X, beam spot 5.0, electron beam accelerator voltage 20.00 kV and sample width 11 mm whereas fading effect of both the samples was examined by its physical appearance (Juciene et al., 2014).

2.9.3. Cellulases in application of detergent

In the current study the role of purified endoglucanase as an additive in detergents were also investigated. A clean 4x4 cm sized white cotton piece was stained with grass, marker, mud and ink and was allowed to dry. All the stained clothes were separately placed in different flasks with following combinations. In Flask A, a cotton cloth was dipped in 50 ml of distilled water and used as a control. In Flask B cotton cloth was soaked in 50 ml of detergent solution. Whereas, in Flask C stained cloth was placed in 50 ml of purified enzyme. 25 ml of the enzyme solution and 25 ml of solution. Whereas, in Flask C stained cloth was placed in 50 ml of detergent solution. For measuring the cleaning efficiency the stained clothes were separately washed with tap water and cleaning effect were noted (Nasir et al., 2011).

2.10. Fourier transformed infrared spectroscopy

The functional groups of purified EG and BGL were assessed through FTIR technique at 400 and 4000 cm⁻¹ frequency range and spectral resolution of 0.4 cm⁻¹. 

Data analysis: Using SPSS, version 32, experimental data were analyzed using statistical techniques for the determination of significance at P ≤ 0.05.

3. Results

3.1. Purification of endoglucanase and β-glucosidase

The purification results of cellulases from T. dupontii are summarized in Table 1. Total protein contents of cellulases were reduced by ammonium sulphate precipitation that exhibited 60 % and 80 % saturation level with 168, 184 mg/ml specific activity and enzyme activity of (13824; 17892 IU) for EG and BGL, respectively.

Elution profile of partially purified enzyme was obtained by anion exchange column (Fig SI). The protein of partially purified enzyme was further separated in two fractions by using Hi Trap QFF column filtration. The active fractions were pooled out and subjected to Sephadex G-100 for final purification step where single broad peak by Sephadex G-100 column were observed. Most of the enzyme activity is achieved in well separated single fractions which have been confirmed as endoglucanase and betaglucosidase under standard enzyme assay.

3.2. Determination of the molecular weight of the purified proteins

The molecular weight and homogeneity of the purified EG, BGL and partially purified enzyme extract was measured by SDS-PAGE gel-electrophoresis with reference to BLUltra prestained. Partially purified enzyme extract shows seven bands with various molecular (Fig 1c). However, a single prominent band of 37 and 66 kDa were obtained for purified EG and BGL, respectively (Fig. 1a &b).

3.3. The characterization of purified cellulases

3.3.1. Impact of different temperature on cellulases stability and its activity

EG and BGL activity and stability were determined at several temperatures (40–100 °C) and results are presented in Fig. 2. Enzyme activity was steadily increased after 40 °C and maximal activity was recorded at 70 °C. Reduction in enzyme activity was witnessed above or below the ideal temperature. Thermo stability was estimated by pre-incubating the EG and BGL at various time intervals between (1–7 h). Residual activity profile of purified EG and BGL revealed that after 5 h of incubation cellulases retained 100 % activity at 70 °C (Fig. 3). Afterwards the stability of both the enzymes dropped at 80 °C but they retained 70 % of their residual activity for 3 h. The activity dropped at 100 °C and only 7 % residual activity was recorded after 2 h of incubation.

3.3.2. Influence of pH on the activity and stability of enzyme

The activity and stability was assessed at various pH ranges (4–9) by incubating enzymes in appropriate buffers (Fig. 4). The pH profile revealed that the citrate buffer had the highest EG and BGL activity (pH 5.5). With further change in optimum level, the decreasing trend was observed.

The purified EG and BGL were remarkably stable in pH ranges from 4 to 6, as indicated by the residual activity profile. After 5 h of incubation the enzymes showed 74 % of residual activity at pH 4 while 100 % activity was maintained at pH 5.0. After 4 h of incubation, residual activity reduced to 82 %, 51 %, and 12 %, for pH 6, 7, and 8, respectively, while at pH 9 there was negligible activity (Fig. 5).

3.3.3. Influence of various metal ions on EG and BGL activity

The influence of various metal ions on EG and BGL activity was studied (Fig. 6). The results showed that Cd²⁺, Hg²⁺, Mn²⁺ and Zn²⁺ ions had an inhibitory effect on the activity of enzyme. Furthermore, the incorporation of Ca²⁺ (100 %) resulted in the highest residual activity, followed by Cd²⁺ (97 %), Hg²⁺ (95 %) Mn²⁺ (75 %) and Zn²⁺ (55 %).

3.3.4. Impact of various surfactants on enzyme activity

The effect of several surfactants on EG and BGL activity was evaluated. Among all the tested surfactants, EDTA retained 92 % of residual activity whereas less activity was noticed in the presence of SDS (87 %), Tween-80 (72 %) and BME (66 %) (Fig. 7).

3.4. Analysis of kinetics and thermodynamics

3.4.1. Km and Vmax determination

Fig. 8 showed the initial reaction rates of cellulases at various substrate concentrations (CMC and pNPG). For EG and BGL, the y-value intercept’s of (0.0115, 0.0125) was equivalent to 1/Vmax. The Km 0.63; 28.56 mg/ml and Vmax 82; 80 U/ml/min was evaluated by means of Line-weaver Burk plot.

3.4.2. Estimation of activation energy (Ea), the enthalpy of activation (AH) and the entropy of activation (AS)

The activation energy (Ea) –44.55; –50.02 kJ/mol and Enthalpy of reaction (AH) 42.20; 47.70 kJ/mol for EG and BGL, respectively was evaluated by using Arrhenius plot (Fig. 9). While entropy AS –5.1 and –5.7 kJ/mol for both the enzymes were calculated by plotting ln(Vmax/T) against the inverse of temperature (1/T) (Fig. 10).

3.5. Evaluation of shelf life

The shelf life was observed by storing the enzymes at 4 °C and 30 °C for 30 days. The results exhibit that EG and BGL were more stable at 4 °C for 20 days by showing 80 % of residual activity while on 30th day only 60 % of activity was recorded (Fig. 11).

3.6. FTIR analysis of purified EG and BGL

Fig. 12a &b showed the spectrum peaks between 611.00 and 665.00 cm⁻¹ region, by FTIR spectroscopy which indicated C═C
aromatic bonds. The spectrum peaks at 1400.00 cm\(^{-1}\) were specified with NH bending vibration and CH stretching vibration. The two strong sharp absorption peaks of 1635.64 cm\(^{-1}\) and 1639.49 cm\(^{-1}\) showed the EG and BGL, respectively. Such peaks represent the \(\alpha\)-helix structure that was formed by the symmetric stretching vibration of NH and C=O bonds. The bands between 1600 and 1640 cm\(^{-1}\) are due to the primary amides NH and represent as secondary structure of protein.

3.7. Treatment of denim fabric with endoglucanase

In the current experiment indigo dyed denim jeans were used as a test sample. Fig. 13 showed the untreated sample (Control) contained a large amount of pills and fuzz on its surface. However, after the treatment the fabric removed all the excess amount of fuzz and pills when observed under the electron microscope scanning (Fig. 14). Moreover, a large amount of indigo dye was released in the treated sample in contrast to the untreated sample.

3.7.1. Application of purified EG as an additive in detergent

Successful results were achieved when purified EG was used as an additive in detergent application. The results showed that stains of mud and grass on cotton cloth were slightly removed when soaked in detergent only, while the stain of marker ink was totally removed when rinsed with water. However, stains of marker ink and grass were slightly removed when treated with purified enzyme, whereas after washing mud stain on cotton cloth was completely removed.

After being treated with a combination of detergent and purified enzyme and washed the fabric successfully removed all stains (mud, marker and grass) (Fig. S3).

### Table 1

| Sr. No. | Purification steps for EG and BGL | Total enzyme activity (IU) | Specific activity (U/mg) | Total protein content (mg/ml) | Purification fold | % Yield |
|---------|---------------------------------|---------------------------|--------------------------|-----------------------------|------------------|--------|
| 1       | Crude                           | 29,945                    | 166                      | 180                         | 1                | 100    |
| 2       | Ammonium sulphate precipitation | 13,824                    | 168                      | 82                          | 1.01             | 100    |
| 3       | Dialysis                        | 10,800                    | 317                      | 34                          | 1.8              | 78.5   |
| 4       | Hi-Trap column                  | 6768                      | 752                      | 9                           | 4.7              | 23.5   |
| 5       | Sephadex G-100                  | 4123                      | 981                      | 4.2                         | 2.7              | 13.7   |

1600 and 1640 cm\(^{-1}\) are due to the primary amides NH and represent as secondary structure of protein.

4. Discussion

Sometimes the properties of enzymes are clearly revealed only with purified enzymes. In current research total protein of crude

![Fig. 1.](image1.png)

![Fig. 2.](image2.png)
Extract was reduced by ammonium sulfate precipitation at 80% level and successful purification was obtained by using anion-exchange chromatography (Hitrap column) and gel filtration chromatography (Irshad et al., 2013). Since the salting-out procedure depends on the protein’s hydrophobicity, high concentration of ammonium sulphate was employed. Thus, a high salt concentra-

**Fig. 3.** Purified Endoglucanase and β-glucosidase stability at various temperatures.

**Fig. 4.** The effect of various pH on Endoglucanase and β-glucosidase activity.

**Fig. 5.** Purified Endoglucanase and β-glucosidase stability at various pH range.
**Fig. 6.** Effect of metal ions on Endoglucanase and β-glucosidase.

**Fig. 7.** Effect of surfactants on Endoglucanase and β-glucosidase.

**Fig. 8.** Line-weaver burk plot (a) Endoglucanase (b) β-glucosidase.
tion disturbs the water structure and reduces the electrostatic repulsion between similar-charged groups which results in the aggregation of hydrophobic groups on the surface of the protein. Results of the present work are fairly similar to the researchers who implemented ammonium sulphate precipitation as an initial step for protein purification. For instance, current findings are in accordance with Bai et al. (2013) who mentioned 80% ammonium sulphate saturation for \( \beta \)-glucosidase activity from \( P. \) simplicissimum. After ammonium sulfate precipitation the purification of cellulases by anion exchange chromatography on Hi-Trap QFF column followed by gel filtration on Sephadex G-100 steps increased by 1.01 and 1.27 folds as compared to crude extract. In current investigation the \( T. \) dupontii produced the higher titers of EG and BGL under Smf indicating the potential use of the strain in contrast to previous studies (Ahmed et al., 2009). The homogeneity of cellulases was confirmed by a single prominent band on 12 % SDS-gel with 37 and 66 kDa molecular weight for EG and BGL, respectively. These findings resemble those of Goyari et al. (2015) in certain ways who mentioned 61 kDa for BGL. However, our findings are in contrast to Pol et al. (2012) who obtained 42 kDa for EG. The existence of carbohydrate content or biological aspects of different fungal strains may be the cause of the variance in the molecular weight of cellulases (Abdul-Hadi et al., 2016; Hamdan and Jasim, 2018).

In the present work the optimal temperature for purified EG and BGL was 70 °C. The data of this study agreed with Bhavsar et al. (2015) and Olajuyigbe et al. (2016) who mentioned 70 °C for endoglucanase and \( \beta \)-glucosidase, respectively. Fluctuation in optimal temperature resulted in decline enzyme activity. This could be due to the fact that enzymes denatured at high tempera-

**Fig. 9.** Estimation of \( E_a \) and \( \Delta H \) on the enzyme by Arrhenius plot (a) Endoglucanase (b) \( \beta \)-glucosidase.

**Fig. 10.** Determination of entropy change (\( \Delta S \)) (a) Endoglucanase (b) \( \beta \)-glucosidase.
Fig. 11. Shelf life of Endoglucanase and β-glucosidase.

Fig. 12. The spectrum of FTIR (a) Endoglucanase (b) β-glucosidase.

Fig. 13. Physical appearance of Denim fabric (a) Control (b) Fabric treated with enzyme.
tures and lowers the enzyme production. In addition to this thermal stability showed that EG and BGL were quite stable at 70 °C and retained 100 % of its residual activity for 5 h. Reduction in enzyme activity was noticed at 80 °C after 3 h of incubation and retained 70 % of its residual activity. This could be the reason that enzymes lose the catalytic activity with increase in incubation time. The current findings conflict with those of Brito et al. (2015), who found 50 % cellulases activity at 50–60 °C after 4 h of incubation.

In current study the effect of various pH values proved that purified EG and BGL activity are quite stable in acidic pH of 5.5. With subsequent increases in the optimal value, the tendency toward decline became apparent. The pH value had a significant impact on the enzyme activity as the binding of substrate and enzyme is influenced by the distribution of charge on substrate and enzyme molecules. Different observation was noticed by Dave et al. (2012) who reported 4 pH for cellulases activity. Findings from a wide range of pH values demonstrated that EG and BGL were 100 % stable for 5 h at pH 5. Current investigation disagrees with Santos et al. (2016) who claimed that cellulases had 100 % of its residual activity for 4 h at pH 5–6.

Metallic ions can activate or deactivate the enzyme through interaction with the amino acid amine or carboxylic group (de Cassia et al., 2017). In this study the effect of various metal ions on EG and BGL activity was also studied. Rise in EG and BGL activity was observed in the existence of Ca²⁺ ions whereas inhibition in enzyme activity was noticed by Cd²⁺ followed by Hg²⁺, Mn²⁺ and Zn²⁺. The attack of functional groups on the enzyme’s active sites may be the cause of the reduction in enzyme activity brought on by the effects of these metal ions (Tao et al., 2010). The obtained results are in line with Olajuyigbe et al. (2016) who mentioned Ca²⁺ ion as an activator for EG and BGL.

The impact of several surfactants on endoglucanase and β-glucosidase activity was investigated. Among all the tested surfactants EDTA stimulated the maximal enzyme activity. This might be as a result of the active sites of the enzyme becoming more accessible to the substrate when EDTA interacts with metal ions in the reaction mixture. The recent findings were supported by Naika and Tiku (2011) who claimed a significant effect of EDTA on cellulases production.

The catalytic efficiency and affinity of purified cellulases for their substrate are determined by kinetic parameters. The low values of Km 0.63; 28.56 % and Vmax 82; 80 U/ml/min for endoglucanases and β-glucosidase, respectively demonstrated the strong affinity of EG and BGL for their substrates. Current findings are in contrast to Maharana and Ray (2015) who stated 24.6 U/mg Vmax and 0.37 mg/ml Km for EG and 43.68 μmolmin-1 mg and 3.3 mM for BGL. Genetic variation among the fungus species may be the cause of the change in the values (Hamdan and Jasim, 2018).

The Arrhenius method was used to graphically derive the thermodynamic parameters (Siddiqui et al., 1997). Enthalpy (ΔH) and Energy of activation (Ea) were calculated to be 42.20; 47.70 kJ/mol and −44.55; −50.02 kJ/mol for EG and BGL respectively. A lower activation energy value indicated a strong interaction between the substrate and the enzyme. Enthalpy of activation with a positive value designated an endothermic reaction. Entropy of activation (ΔS) determined the dysfunction of a system proved to be −5.3 and −5.7 kJ/mol for EG and BGL respectively. Thermal denaturation of enzymes causes the disruption of non-covalent linkages, consisting of hydrophobic interactions, as well as an increase in the activation enthalpy. The disruption of the enzyme structure is linked with an increase in randomness, activation disorder or entropy. When the values of enthalpy and entropy were calculated, high value of the enthalpy were obtained with negative value of entropy. The Entropy of activation with a negative value has shown that the system is less random and entropically unfavorable.

FTIR analysis which is considered to be the most suitable and reliable tool for determining the functional groups of enzymes was also carried out. According to the current results the two distinct and strong absorption peaks at 1635.64 cm⁻¹ and 1639.49 indicated the occurrence of EG and BGL enzymes. Bai et al. (2013) also reported a similar sharp absorption peak at 1653.26 cm⁻¹ for cellulases. In the current study, a sample of denim jeans was used for bio-stoning application. The results show that denim that has been dipped in an enzyme and buffer solution releases indigo colour, as opposed to denim that has merely been dipped in a buffer solution. This may be because cellulases possess indigo dye domains and have the ability to transfer the insoluble dye from cloth to solution (Gusakov et al., 2000).

Furthermore, when the denim fabric is treated with cellulases, it breaks and hydrolyzes the small fibers that come out of it, causing the dye to be lost. Because of this, the beta – 1,4-linkages in cellulose chains may be disrupted, which could lead to the formation of simple water-soluble sugars, which would then remove the fibres that retain the indigo dye. The fabric then quickly loses the dye, giving it a faded appearance. Present results support the findings of Belgith et al. (2001), who used endoglucanase to release
indigo dye from denim fabric. Furthermore, the stain test revealed that purified endoglucanases significantly improved the cleaning effect when combined with detergents this may be the cause that cellulase alters the cellulose fiber and aid in removing the dirt from the fabric (Singh et al., 2007).

5. Conclusion

In current investigation thermophillic T. *dupontii* which has a high ability to produce cellulases were selected. The optimal environmental factors were determined for cellulase production. The purified EG and BGL were recovered, with the final yield of 13.7; 10.7 % with 8.9; 2.7-fold purification in anion-exchange chromatography followed by Sephadex G-100. The outcomes showed that *T. dupontii* cellulase produced under investigated conditions can be employed successfully in bio stoning and detergent applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2022.103483.

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