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(Research Article)

Applications of cellulase enzyme in textile industry purified from *Bacillus paramycoides S* 5

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Abstract

Cellulases are enzymes which breaks down β -1-4-glycosidic bonds present in a complex polysaccharide cellulose. In this study cellulase producing bacteria were isolated from soil samples and efficient cellulase producing bacteria was identified as *Bacillus paramycoides S 5 strain* by using 16 S rRNA sequencing method. By using the same strain, cellulase enzyme was purified with different methods such as ammonium sulfate precipitation, dialysis, chromatography. The purified enzyme was tested for its applications in textile industry. The applications of cellulase enzymes in txtile industry includes wet processing, biostoning, biopolishing, softening and removal of stain from the clothes.

In the present study of applications of purified cellulase enzyme in textile industry, after testing for wet processing and softening of fabrics giving the good biofinishing effects on selected fabric. In another application that is in removal of stain from cloth shows positive effect and found that cellulase has capability to remove stain colour from the cloth, later on cellulase applicability also tested on fabrics with respected to biopolishing which helps to remove the fabrics and microfibrils from the cloth giving a soft appearance to cloth. Finally cellulase was also tested for biostoning.

Keywords: Cellulase; Biopolishing; Biostoning; *Bacillus paramycoides*

1. Introduction

Cellulases are consisting of three different types of enzyme which includes -

- Exo-1-4, β-glucanase which are also called as exoglucanases. These enzymes attacks on short chains of cellulobiose produced by endoglucanase enzymes.
- Endoglucanase enzymes, are also called as endo β,1-4 glucanase attacking on the internal glycosidic bonds of cellulose to produce short chains of glucose molecules called as celluloboise.
- The third type of cellulase enzyme is called as β -glucosidase which attacks on short chains of cellulobiose and small celluloligosaccharides releasing individual glucose molecules.

In present research study cellulase producing bacteria were isolated from the soil and tested for efficient cellulase producers. After finding out efficient cellulase producer, this bacteria was identified by using 16 S r RNA sequencing as a *Bacillus paramycoides S 5 strain*, same bacterial strain was used to optimize the production medium by using response surface methodology and Plackett-Burman design experiment. In this response surface methodology and Plackett-Burman design experiment different chemical components are checked to optimize the production medium. In the same study crude cellulase enzyme was characterized for different parameters such as temperature optima, pH optima. Later on this crude cellulase enzymes was purified by using different purification methods such as dialysis, ammonium

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sulphate precipitation, chromatography, cellulase enzyme purified by above method is later on used to check applicability of this enzyme in textile industry.

In textile industry cellulases have many applications such as these enzymes can be used to remove dirt particles trapped in microfibrill network of clothes, in biostoning where this enzyme are used to biostoning of denim fabrics which gives a soft appearance to denim fabrics . In textile industry cellulases are also used to remove microfibrills from surface of cotton fabrics as well as mixed cotton fabrics. The removing of microfibrills from the clothes restores the original colour as well as smooth surface of clothes. Cellulases are also used for softening of clothes. In addition to this application of cellulases in textile industry; cellulases are also used to remove excess dye from the fabrics. In the present research, the purified cellulase enzyme was used to check its applicability in textile industry specifically in biostoning of denim fabrics, softening of fabrics, and removal of stain from the fabric and in biopolishing of fabrics. In all, applications of celluase enzyme in textile industry showed the positive results.

2. Material and methods

2.1. Biostoning of Denim Fabrics

For biostoning of denim fabrics by using cellulase enzyme the pH and temperature adjusted to 7.5 and 45^{0}C respectively. During this research to check application of purified cellulase various concentrations of cellulases such as 0.5% , 1 % and 1.5% were used. This part involves the three steps for enzymes washing which consist of

- De –sizing
- Bio-abrassion
- Back wash
    - De-sizing is the first step in biostoning or enzyme washing. In this process denim fabric was washed to remove any dirt and other impurities. The pH , temperature and concentrations of cellulase used during this process are pH- 7.5, Temp- 45^{0}C and concentrations of cellulase were 0.5% , 1% and 1.5% , for30 minutes .
    - In bioabrassion enzyme is added to get faded denim. To prevent the back staining, anti back staining that is nicosist -ABS powder was added and the duration of this was 1.5 hours. The pH, temperature and concentration of purified cellulase used was same as above.
    - The last step that is back wash involves removing of all of the enzyme from the denim fabric, along with colouring material. In this process also the pH, temperature and concentration of purified cellulase was same as in above steps. The antiback staining agent and the dye absorber used was nicosist -ABS powder and soda ash respectively.

2.2. Biopolishing of cotton fabrics by using purified cellulase enzyme.

During this process a fabric piece of size 12 X 12 cm was added to flask consisting of celluase in phosphate buffer and shaken at 50 rpm . The temperature, pH and different concentrations of celluase used were , 45^{0}C , 7.5 and 0.5% , 1%and1.5 % respectively. After these 10 minutes hot temperature at 85^{0}C was performed to end the biopolishing process. During this biopolishing process different tests are performed on cotton fabric. These tests are as follows

- Weight loss test
- Strength loss test
- Fabrics pilling test

2.2.1. Weight Loss Test

Weight Loss of bio polished cotton fabric was calculated by using following formula

Weight Loss % = (Fabric Weight before bio polishing) – (Fabric Weight after bio polishing) / Fabric Weight before bio polishing

2.2.2. Strength Loss Test

A bursting strength tester was used to measure strength. The following formula was used for this test.

Strength Loss % = (Fabric strength before bio polishing) - (Fabric strength after bio polishing) /Fabric strength before bio polishing

2.2.3. Fabric Pilling Test

By using pilling tester, the fabric resistance to pilling was assessed and rating scale was used to report observed resistance to pilling with a rate of pilling resistance from 1 that is extremely severe pilling to 5 that is no pilling.

2.3. Wet Processing and Softening Of Fabrics

The methodology for wet processing and softening of fabrics was involved cellulose hydrolysis test and wettability drop test.

2.3.1. Cellulose hydrolysis test

In this process assays for cellulase and reducing sugars were performed. The glucose content was measured by using spectrophotometer adjusted to 540 nm wavelength. In this method cotton fabric was fully submerged in 100 ml of enzyme preparation and allowed to set for 24 hrs at 45^0C.

2.3.2. Wettability Drop test

The test method was used AATCC test method, where cotton fabric was tested at room temperature. Wetting time of less than 1 second was considered to be a sign that the fabric absorbency was sufficient.

2.4. Stain removal from the fabric

In the methodology for the removal of stain from fabric, wash performance test was performed.

2.4.1. Wash performance test

The wash performance of cellulase was evaluated by gauging how it removed turmeric stain from cotton fabrics.

In this method 5 X 5 cm cotton piece was used and applied 300 microlitre turmeric stain, placed in hot air oven overnight to dry. Then each piece of stained cotton fabric was placed into flask containing

- 25 ml of tapwater (control)
- 25 ml of tap water and 5 ml of concentrated detergent (5%) and
  20 Ml of tap water and 5 ml of commercial detergent (1 %) without cellulase enzyme.

3. Results

3.1. Biostoning of Denim Fabrics

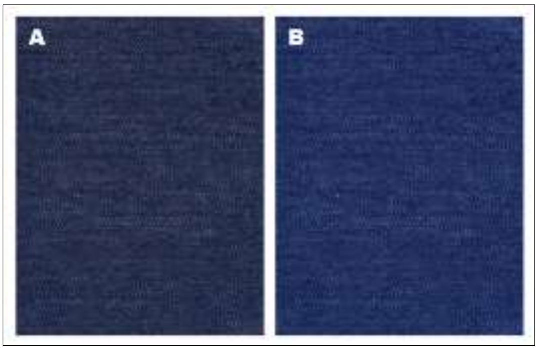

Figure 1 Biostoning of denim. (A) Raw (Indigo) dyed denim (B) Bio-stone washed denim using 0.5% of cellulose

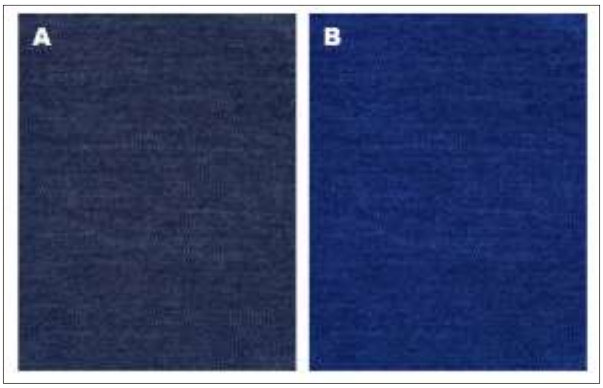

Figure 2 Biostoning of denim. (A) Raw (Indigo) dyed denim (B) Bio-stone washed denim using 1% of cellulase

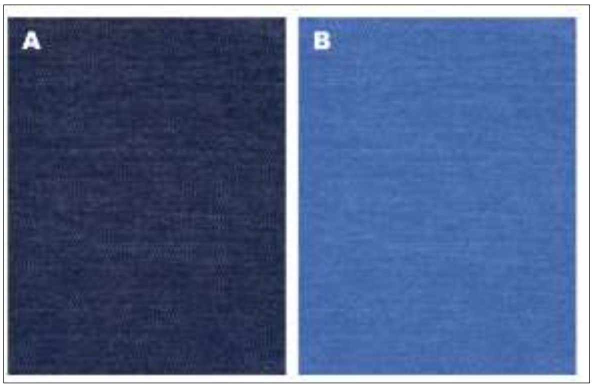

Figure 3 Biostoning of denim. (A) Raw (Indigo) dyed denim (B) Bio-stone washed denim using 1.5% of cellulase

On the denim fabric the coating of fuzzmakes the fabric hairier overall. Use of celluase enzyme on such fabric caused the colour fading effect as shown in above figures . As the cellulase treatment duration and concentration of cellulase increases, the colour fading effect also increased. This research also proved that instead of using pumice stone for denim washing , cellulase can be used to remove the exposed fibrils from the surface of fabric simultaneously removes the inside dye found in fabrics outer layer.

3.2. Biopolishing of Cotton Fabric

During checking the applicability of purified cellulase enzyme in biopolishing of cotton fabric 3 parameters are considered, these are temperature 45^{0}C, time 60 minutes and concentration of purified cellulose 0.5,1 and 1.5 % .The results obtained with respect to weight loss, strength loss and fabric pilling are as follows:

3.2.1. Weight Loss

Weight loss increased gradually as the percentage of cellulase from 0.5 % to 1.5 % increased. The results obtained for the weight loss are shown in following figure and table number 1.

Table 1 Cotton fabrics treated with cellulase exhibit weight loss

S. No.	Concentration (%)	Temperature	Time (min)	Weight Loss(%)
	0.5	45	60	3
	1	45	60	4
	1.5	45	60	5

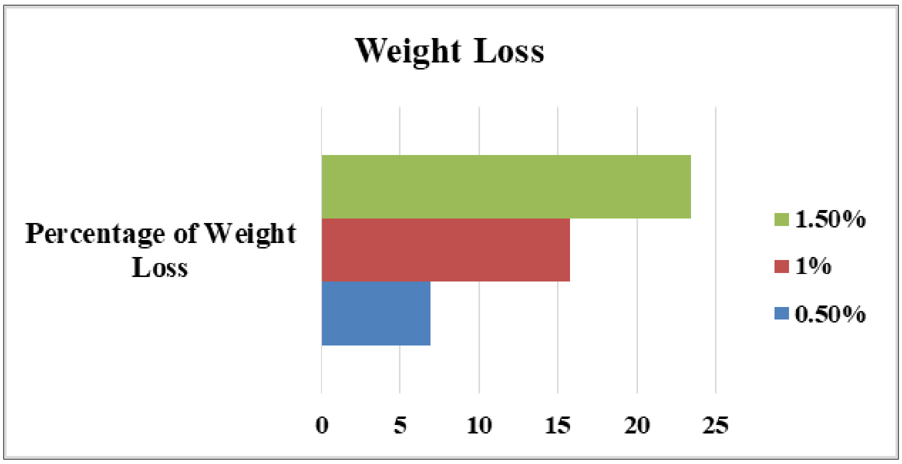

Figure 4 Weight loss of cotton fabric by various concentration of cellulase treatment

3.2.2. Strength Loss

After performing strength loss test, it is observed that strength loss increases as the concentration of cellulase increases.The results obtained are showed in following table number 2.

Table 2 Cotton fabrics treated with cellulase exhibit strength loss

S. No.	Concentration(%)	Temperature	Time (min)	Weight Loss(%)
	0.5	45	60	18.93
	1	45	60	24.40
	1.5	45	60	42.60

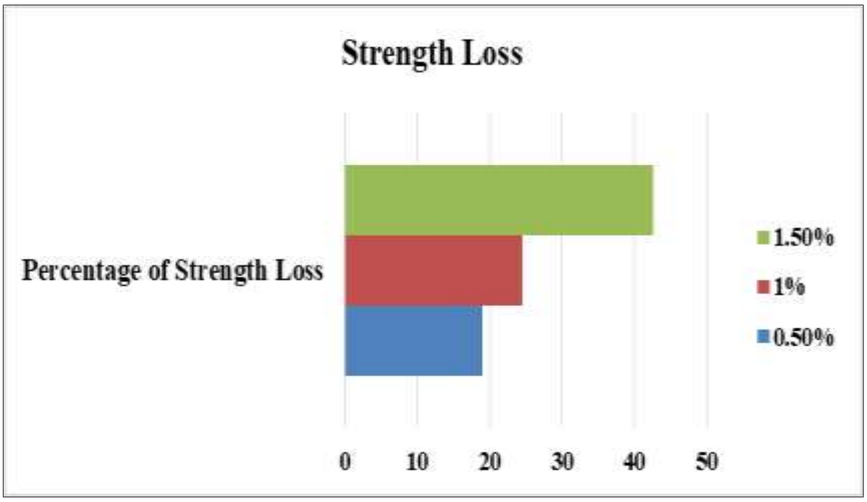

Figure 5 Strength loss of cotton fabric by various concentration of cellulase treatment

3.2.3. Pilling test

In the pilling test fabric ratings for pilling resistance were, 4. 3-4 and 5 under 125, 500 and 2000 cycles of operations respectively. The results obtained are shown in table number 3.

Table 3 Cotton fabrics treated with cellulase exhibit pilling

Sr. No.	Concentration (%)	Temperature	Time(min)	Pilling Cycles	Pilling Rating
	0.5	45	60	125	1
				500	2
				2000	3
	1	45	60	125	3
				500	3
				2000	4
	1.5	45	60	125	4
				500	4
				2000	5

3.3. Wet processing and softening of fabrics

3.3.1. Cellulase hydrolysis test

After testing fabric cloth with purified cellulase the yield of reducing sugars increases from the 14 th to 24 th hrs after testing with 1.5 % cellulase concentration at 45^{0}C for 24 hrs after which release of reducing was decreases. The results obtained shown in following graph.

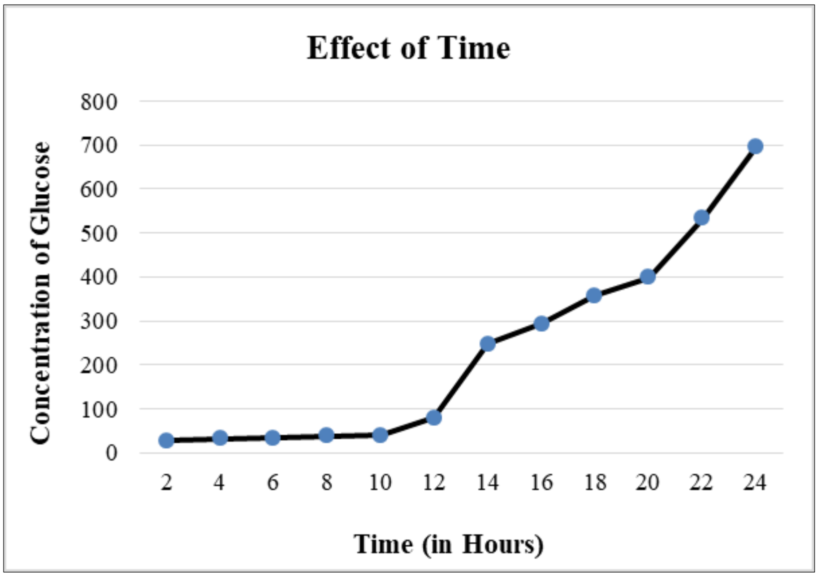

Figure 6 Effect of time on the yield of reducing sugars (glucose) when cellulase from *Bacillus paramycoides* biofinished cotton fabric

3.3.2. Wettability drop test

Less than one second was wetting time of textiles treated with cellulase for 24 hrs at 45^{0}C which is significantly less than corresponding values recorded. This results showed the increase in fabric absorbency after cellulase treatment.

Table 4 Wettability drop test of enzyme treated cotton fibres

Treatment time (in hours)	Wetting time in Seconds
14	20
16	13
18	11
20	7
22	5
24	< 1

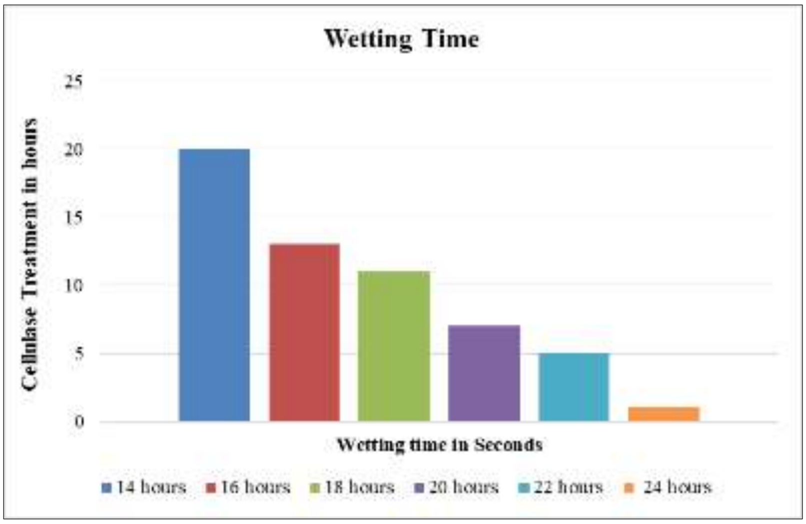

Figure 7 Wetting time of cellulase treated cotton fabrics

3.4. Stain removal from fabric

3.4.1. Wash performance test

Cellulases are effective in the removal of stains from fabric. These enzymes weaken the stain structure, making it easier to remove stain from fabric. In present study applicability of purified cellulase was checked to remove the turmeric stain from fabric. The results obtained for removal of turmeric stain from fabric are shown in following photos.

Figure 8 Wash performance test of turmeric stained cotton fabrics with cellulase. (A) Washed with 25 mL of tap water (control), (B) Washed with 20 mL of tap water and 5 mL of commercial detergent (5%), and (C) Washed with 20 mL of tap water and 5 mL of commercial detergent (5%), and cellulase enzyme(1% of 10 U/mL)

4. Discussion

In an effort to improve fabric quality, research and development efforts have recently concentrated on customizing the replacement of cellulase enzymes, whose configuration has been reformed through biotechnology, in order to better understand specific applications and stay up to date with the denim industry's latest fashion trends. The most recent use of microbial cellulase provided scientists with new tools to create better fabric products for textile applications.

Using cellulase and the enzyme concentration as the operating parameter, the biopolishing effect on cotton fabric was examined in this study. Strength loss and weight loss increased as concentration level rose. Higher concentrations of cellulase (1.5%) were found to increase the weight loss and strength loss of the fabric. Ultimately, based on the data collected, it can be concluded that cellulase performed better at a 1.5 % concentration for 60 minutes at 45 °C.

The amount of cellulase produced by *Bacillus paramycoides S5* and its potential for textile finishing processes are demonstrated in this study. When 1.5% (w/v) of cellulase were treated with cotton fabrics for 24 hours at 45°C, a good bio-finishing effect was observed. Treatment condition optimization is expected to improve bio-finishing action. In order to meet the increasing demand for cellulases in the textile industries, multidisciplinary research on the fundamental and practical aspects of optimization must continue. *Bacillus paramycoides S5* cellulase seems to be unique for biofinishing processes, which are more favoured for textile applications. By taking into account the wetting characteristics, pore volume distribution, and fiber strength following the treatment, additional gains can be anticipated.

5. Conclusion

The washing effect of cellulase on turmeric stained cotton fabric was analyzed. This study suggested that adding cellulase to detergent has a better washing effect in the removal of stain

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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