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# Treating denim fabrics with immobilized commercial cellulases

Nurdan Kaşikara Pazarlioğlu [a,*], Merih Sariişik [b], Azmi Telefoncu [a]

[a] *Biochemistry Department, Faculty of Science, Ege University, 35 100 Bornova-Izmir, Turkey*
[b] *Textile Engineering Department, Engineering Faculty, Dokuz Eylul University, 35 100 Bornova-Izmir, Turkey*

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**Abstract**

Immobilization of a commercial cellulase (C) onto chemically modified pumice (P) particles has been studied. $ZrOCl_2$ activated pumice was used as a carrier for the cellulase immobilization and some operational properties of this carrier were determined. Denim washing performance of immobilized cellulase (MP) was also investigated comparing with free enzymes (FE) and traditional denim washing procedure. Immobilized acid cellulases can efficiently abrade indigo dyed denim fabrics.


*Keywords:* Cellulase; Immobilization; Denim washing; Biostoning

## 1. Introduction

Cellulases have achieved their worldwide success in textile because of their ability to modify cellulosic fibres in a controlled and desired manner, so as to improve the quality of fabrics. Although, cellulases were introduced in textile only a decade ago, they have now become third largest group of enzymes used in these applications. Biostoning is one of the best known current textile applications of cellulases [1].

Cellulase treatment of cotton fabrics is an environmentally-friendly way of improving the property of the fabrics [2]. Although, traditionally, denim jeans manufacturers have washed their garments with pumice (P) stones to achieve a soft handle as well as a desirable worn look, nowadays, the aged look is obtained by non-homogeneous removal of the indigo dye trapped inside the fibres by the cooperative action of the enzymes and mechanical factors such as beating and friction [3,4]. Natural pumice stone is widely used in denim garment washing process [5] has disadvantages, the difficulty of removing residual pumice from processed clothing items and the damage to the equipment by the overload of tumbling stones and the pumice stones and particulate material can also clog machine drainage passages and the drains and sewer lines at the machine site [6]. Denim washing with cellulases is thus a standard technique, providing an environmentally friendly process to achieve a desirable appearance and soft handle for fabrics [6,7].

During the enzymatic treatment, the removed indigo dye can be redeposited on the white yarn of denim fabric [3,8–11]. This process is called backstaining, and it can diminish the look of denim garment. An ideal biostoning enzyme would posses high abrasive activity as well as low backstaining [12]. Since cellulases strongly adsorbed to their substrates [13], this phenomenon can be explained by dye binding to the bound enzyme protein [3].

Although, there were some studies on backstaining, focused on the mechanism of indigo redeposition on denim garments and to reduce the effect of backstaining, it is still going on to be a problem for the manufacturer.

Since cellulases were used in soluble form in liquor, backstaining will also occur because of the affinities: cellulase–cellulose and indigo–cellulase. To be able to bring a practical solution to this problem, we planned to use cellulase in immobilized form on pumice for denim washing process, instead of soluble form. At the same time, immobilization of cellulase can also reduce costs and raise the activity of the enzyme. As our knowledge, the usage of immobilized cellulase (MP) on pumice for the denim washing process was carried out for the first time in this study.

* Corresponding author. Tel.: +90-232-343-86-24; fax: +90-232-343-86-24.
*E-mail address:* nurdan@sci.ege.edu.tr (N.K. Pazarlioğlu).

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## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Carrier

Pumice, obtained from the region of Konya from Turkiye, was ground and fractionally sieved. Acid washed pumice particles were dried at room temperature and 110 °C in an oven for 1 h and then treated with 0.65 M $ZrOCl_2$ in 1.0 M HCl. The mixture was dried in an oven for 48 h at 55 °C [14] and then washed three times with distilled water. Acid washed pumice particles was also silanized in toluene. Pumice (10 g) was added to 40 ml of γ-APTS (3-aminopropyltriethoxysilane) solution (10%, v/v) in toluene and refluxed for 18 h. The support material was then washed with toluene and acetone, respectively and dried in vacuum oven [15]. Finally, heat treated pumice particles (in an oven at 350 °C) were used for the cellulase immobilization.

#### 2.1.2. Enzyme

Perizym α-amylase (AM) was kindly supplied by (Dr. Petry GmbH) for desizing, and acid cellulase (Roglyr A 1537) for stone washing was also supplied by Rotta.

#### 2.1.3. Fabric

Indigo dyed denim fabric from Bayraklı Denim (TR), was used in these experiments. The yarn count was Ne 6.2/5.5 (warp/filling), the fabric weight per area was 475 g/$m^2$ and yarn sett was 17/24 (warp/filling).

### 2.2. Enzyme assay

The enzyme activities of native and immobilized on modified pumice particles were determined according to Miller [16]. Reducing sugars were determined by dinitrosalicylic acid (DNS), carboxymethyl cellulose (CMC) and glucose as standard.The calculating formula is as follows: activity of cellulase (μmol/ml min) $= 1000w/Mvt$; where, $w$ is the amount of produced glucose, $M$ the molecular weight of glucose, $v$ the volume of the measured sample and $t$ the reaction time, respectively. The retained activity of immobilized cellulase, determined by the percentage of the activity of immobilized cellulase in the activity of free cellulase (FE), used for binding was calculated according to the following equation:

$$\text{retained activity (\%)} = \frac{\text{the activity of immobilized cellulose}}{\text{the activity of free cellulase used for binding}} \times 100$$

### 2.3. Immobilization method

Cellulase immobilization was carried out for 1 h in flasks which contained washed and/or activated pumice (12–16 mesh) on a shaker with a controlled speed (60 rpm), later the optimum immobilization period was determined as 30 min and the following immobilization studies was performed at this time interval. The amount of adsorbed enzyme was determined by monitoring the retained enzyme activity and protein.

### 2.4. Treating fabrics with free and immobilized cellulase

Enzymatic desizing and stone washing was performed with Linitest (Atlas, USA) at 45 rpm. According to the manufacturer, the assay conditions for Perizym AM were pH 6 and 60 °C. The dosage was 3 g/l, the liquor ratio 10:1 and time 60 min. Then 1 g/l detergent-nonionic (Perlavin NIC-Dr. Petry) were added to the liquor as auxiliary agent. After enzymatic desizing, enzymes were deactivated by immersing the samples for 10 min in 80 °C water, followed by rinsing in warm and cold water and drum drying.

In the acid cellulase treatments, the samples were treated with Roglyr A 1537 Acid Cellulase, as a commercial acid cellulase. The dosage was 2%, liquor ratio 5:1, temperature 50 °C, pH 4.5–5 (sodium acetate/acetic acid) and time 60 min.

After the enzymatic treatments, enzymes were deactivated by immersing the samples for 5 min in pH 9 liquor, followed by rinsing in warm and cold water and drum drying. Each experiment was also carried out for immobilized cellulase. The mechanical action was provided by adding five steel discs to the pots. Each experiment was repeated twice.

The degree of abrasion and the evaluation of backstaining were measured using a spectrophotometer (Minolta 3200 D). CIE $L$ values of denim fabrics were used to quantify the amount of abrasion after treatments whereas higher CIE $L$ values denote more abrasion. $K/S$ values (660 nm) of pocket material and absolute CIE $b$ values of inside of denim fabrics where as higher absolute CIE $b$ values and $K/S$ values denote more backstaining.

## 3. Results and discussion

### 3.1. Activation methods

Three activation methods of pumice for cellulase immobilization, drying in an oven at 350 °C (O), silanization with γ-aminopropyltriethoxysilane (S) and treating with $ZrOCl_2$ (Z) absorption were studied and the results are shown in Fig. 1.

Results indicate that the $ZrOCl_2$ activated pumice was more suitable than the other activation methods the cellulose immobilization. In this method, the hydrous metal oxide derivatives of pumice stone are produced by metal interaction with the silanol groups and the carrier which lead to a stable metal oxide layer on the pumice stone.

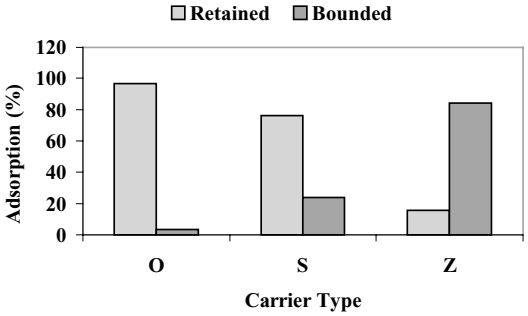


Fig. 1. Cellulase immobilization on different carriers.

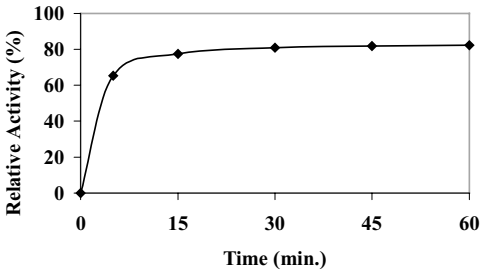


Fig. 2. Time course cellulase adsorption on $ZrOCl_2$ activated pumice.

### 3.2. Individual immobilization of cellulase

Different cellulase preparations (including acid and neutral, multicomponent and monocomponent samples) are available in the world market of textile enzymes [17]. In immobilization studies, commercial acid and neutral cellulases were tested. Since, the binding efficiency and the enzymatic activity of immobilized acid cellulase were higher than neutral cellulase, in the next studies, the usage of immobilized acid cellulase was preferred. Factors, such as enzyme concentration, binding time and enzyme leakage from the support material were studied to determine their effects on the activity of immobilized cellulase.

#### 3.2.1. Binding time

Fig. 2 shows the effect of binding time on the activity and the retained activity of immobilized cellulase. Results indicate that the activity of immobilized cellulase tends to increase with the increasing binding time, but it does not change very much after 15 min. The retained activity of immobilized cellulase has the same tendency. It was assumed that the amount of enzyme bound on to the surface of carrier reached saturation when the binding time is over 30 min.

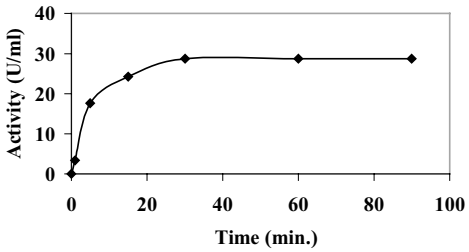


Fig. 4. Enzyme desorption from the support material.

#### 3.2.2. Enzyme concentration

Commercial enzyme was diluted at different ratios and then used for enzyme immobilization (Fig. 3). Results show that a high concentration of cellulase solution was favourable for the high activity and high retained activity, because it can provide more enzymes for binding [18]. Under given conditions, enzyme almost completely bound to the carrier above dilutions of 1/20. In industrial processes, the enzyme–garment ratio was generally 1:5 therefore the carrier–enzyme ratio and enzyme dilution ratios were determined as 1/5 (g/ml) and 1/10 (v/v), respectively.

#### 3.2.3. Enzyme desorption

In order to test for possible enzymes desorption during the process, enzyme leakage from the carrier was monitored at defined intervals in a batch enzyme reactor. It has been observed that, after 30 min, enzyme leakage reached equilibrium and the enzyme activity in solution was lower than the initial activity level of the immobilized enzyme (Fig. 4).

### 3.3. Treating fabrics with free and immobilized cellulase

#### 3.3.1. Abrasion efficiency

After desizing, denim fabrics were washed several times with commercial acid cellulase and the optimum washing period was found as 60 min. This period was also convenient with the prospectus of the enzyme used.

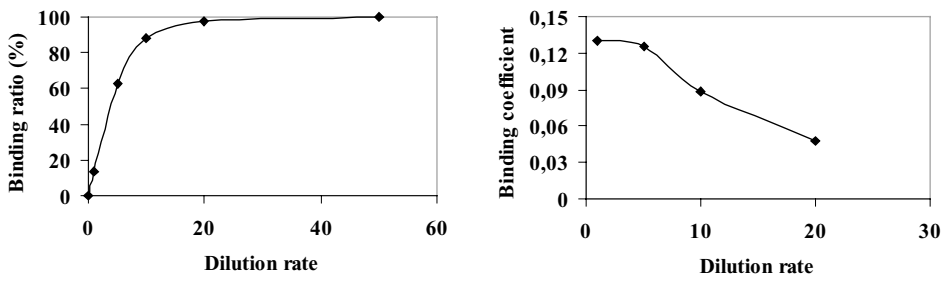


Fig. 3. The effects of enzyme dilution rate on enzyme binding levels.

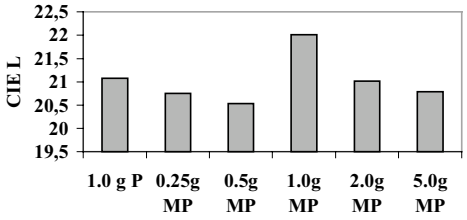


Fig. 5. CIE *L* values of denim fabrics according to immobilized cellulase dosage (P: pumice; MP: modified pumice (immobilized enzyme)).

Desized denim fabrics were treated with immobilized cellulase, in different amounts, at 50 °C, liquor ratio 5:1, time 60 min (Fig. 5). One gram of modified pumice (immobilized cellulase) had the most abrasive effect and the following studies were carried out by using this dosage.

To determine the effects of the pumice stones containing acid cellulase, the washing experiments were repeated five times under the same conditions. Fig. 6 shows CIE *L* values of denim fabrics treated with immobilized cellulase.

The results of commercial free acid cellulase and immobilized acid cellulase were compared, it seems that, in all treatments, immobilized acid cellulase gave rise to higher CIE *L* values than commercial acid cellulase and pumice and there were no significant differences between the first and fifth treatments.

### *3.3.2. Backstaining*

Acid cellulases are more aggresive on denim fabrics. Thus, more backstaining has to be expected. Furthermore, acid cellulases have a higher affinity to indigo than neutral cellulases (3,9). Backstaining can be determined by the measurement of *K*/*S* of the pocket materials, reflectance, or absolute CIE *b* values of the denim fabrics. Backstaining of the reverse side of denim fabrics is used as an indication of the degree of backstaining on the right side of the fabric.

In this study, to quantify the level of backstaining, the absolute CIE *b* values of reverse side denim fabrics (Fig. 7) and also *K*/*S* of pocket material were determined (Fig. 8) [19]. Results from both studies were almost in parallel.

Comparing indigo staining of the reverse side of fabrics in the presence of free cellulase, immobilized cellulases, and absence of cellulase, it seems that the levels of the backstaining appear to decrease after the first and second usage of MP (Fig. 8). This circumstance can be explained by an enzyme leakage from the pumice during the processing of the fabrics with the MP and these free enzymes (FE), by binding to cotton cellulose, caused the redeposition of indigo dye. In the next treatments, because strongly bounded enzymes remained on the pumice, staining was detected at lower levels than FE and P.

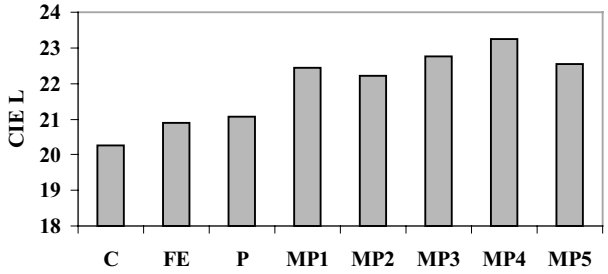


Fig. 6. CIE *L* values of control (C), pumice (P), free enzyme (FE) and immobilized cellulase (MP) treated denim fabrics.

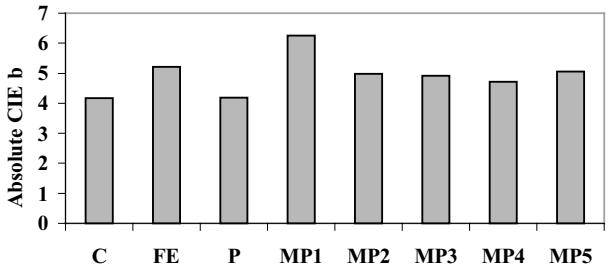


Fig. 7. Absolute *b* values of reverse side of denim fabrics with treated control (C), pumice (P), free enzyme (FE) and immobilized acid cellulase (MP).

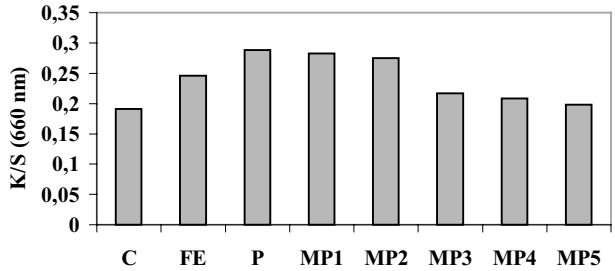


Fig. 8. *K*/*S* values of pocket material with treated control (C), pumice (P), free enzyme (FE) and immobilized acid cellulase (MP).

In conclusion, immobilized acid cellulases can efficiently abrade indigo dyed denim fabrics. Although, at this stage, it is difficult to say that the usage of cellulase in the immobilized form has a clear ability to prevent backstaining, immobilized cellulases have a great potential for the textile industry. In further studies, it is planned to investigate the performance of cellulase immobilized pumice in commercial denim washing processes.

## References

[1] Bhat MK. Cellulases and related enzymes in biotechnology. Biotechnol Adv 2000;18:355–83.
[2] Belghiht H, Ellouz-Chaabouni S, Gargouri A. Biostoning of denims by *Penicillium occitanis* (Pol6) cellulases. J Biotechnol 2001;89:257–62.
[3] Cavaco-Paulo A, Morgado J, Almeida L, Kilburn D. Indigo backstaining during cellulase washing. Textile Res J 1998;68(6):398–401.
[4] Csiszár E, Szakács G, Rusznák I. Combining traditional cotton scouring with cellulase enzymatic treatment. Textile Res J 1998;68(3):163–7.
[5] Yu JM, Szeto YS, Tao XM, Chong CL, Choy CL. Surface morphology of natural pumice stone and its abrading effect on denim fabrics. In: Proceedings of the 4th Assian Textile Conference. Hong Kong: August 2001.

[6] Heikinheimo L, Buchert J, Miettinen-Oinonen A. Treating denim fabrics with *Trichoderma reesei* cellulases. Textile Res J 2000; 70(11):969–73.
[7] Sinistyn AP, Gusakov AV, Grihutin SG, Sinistyna OA, Ankudimova NV. Application of microassays for investigation of cellulase abrasive activity and backstaining. J Biotechnol 2001;89:233–8.
[8] Andreaus J, Campos R, Gubitz G, Cavaco-Paulo A. Influence of cellulases on indigo backstaining. Textile Res J 2000;70(7):628–32.
[9] Campos R, Cavaco-Paulo A, Andreaus J, Gubitz G. Indigo cellulase interactions. Textile Res J 2000;70(6):532–6.
[10] Gusakov AV, Sinitsyn AP, Markov AV, Sinitsyna OA, Ankudimova NV, Berlin AG. Study of protein adsorption on indigo particles confirms the existence of enzyme–indigo interaction sites in cellulase molecules. J Biotechnol 2000;87:83–90.
[11] Gusakov AV, Sinitsyn AP, Markov AV, Skomarowsky AA, Sinitsyna OA, Berlin AG, et al. Indigo-binding domains in cellulase molecules. In: Biocatalysis-2000: fundamentals and applications, vol. 41, no. 6. Khimiya: Vestnik Moskovskogo Universiteta; 2000. p. 77–80.
[12] Gusakov AV, Sinitsyn AP, Grishutin S. Microassaya to control the results of cellulase treatment of denim fabrics. Text Chem Am Dyestuff Rep 2000;32(5):42–7.
[13] Gilkes NR, Jervis E, Henrissat B, Tekant B, Miller Jr RC, Warrant RAJ, et al. The adsorption of a bacterial cellulase and its two isolated domains to crystalline cellulose. J Biol Chem 1992;267(10):6743–9.
[14] Kennedy JF, Cabral JMS. Immobilization of enzymes on transition metal-activated supports. In: Methods in enzymology, vol. 135. Academic Press; 1987. p. 113–8.
[15] Uslan AH, Tarhan L, Ardic Z. The use of perlite for catalase immobilization. J Fac Sci Ege Univ Ser A 1991;14(2):65–71.
[16] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31:426–8.
[17] Gusakov AV, Sinitsyn AP, Berlin AG, Markov AV, Ankudimova NV. Surface hydrophobic amino acid residues in cellulase molecules as a structural factor responsible for their high denim-washing performance. Enzyme Microbiol Technol 2000d;27:664–71.
[18] Yuan X, Shen N, Sheng J, Wei X. Immobilization of cellulase using acrylamide grafted acrylonitrile copolymer membranes. J Membr Sci 1999;155:101–6.
[19] Yoon M-Y, Mcdonald H, Chu K, Garratt C. Protease, a new tool for denim washing. TCC & ADR 2000;32:25–9.