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Sustainable denim fading using immobilized cellulase on reversibly soluble-insoluble polymers

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This study introduces a sustainable approach to generic denim fabric washing by employing immobilized cellulase on two eco-friendly polymers (chitosan and Eudragit S-100). The immobilization process enhances the stability and reusability of cellulase, while the choice of chitosan and Eudragit S-100 allows for reversible solubility and insolubility, providing easy removal of the enzyme after the washing process. The study investigates the enzymatic activity and stability of cellulase when immobilized on chitosan and Eudragit S-100 and assesses their efficiency in denim fading. The experimental results reveal that the immobilized cellulase exhibits some decreased activity with increased *p*H and thermal stability. The immobilized cellulase grades equivalent denim decolouration with reduced back-staining for three consecutive cycles in comparison to native cellulase which is drained after a single use. The proposed method not only addresses the economic constraints of the reusability of enzymes but also contributes to the development of innovative and sustainable practices in textile industry.

Keywords: Cellulase, Chitosan, Denim fading, Eudragit S-100

1 Introduction

Denim is a durable cotton fabric that has grown very popular in the fashion industry because of its versatility and rugged appearance. Conventional denim washing has historically depended on chemical and mechanical methods to produce the appropriate faded, worn-out, or distressed appearance[1]. Traditional methods like stone washing, sandblasting, and hydrogen peroxide treatments often pose significant environmental challenges, particularly in water and energy consumption[2]. Enzymatic denim washing has emerged as a sustainable and eco-friendly alternative to traditional methods. Cellulase enzymes are used in this enzymatic treatment to catalyze the hydrolysis of cellulose, resulting in desirable effects, such as softening, fading, and distressing, contributing to the popular worn-in appearance[3,4]. This allows for greater control and precision in achieving specific denim finishes and when optimized, can significantly reduce back staining issues, where dye particles redeposit onto the backside of fabrics[5]. However, the practical implementation of cellulase treatment is currently limited by high enzyme production costs, low enzyme stability, and reusability issues. Furthermore, because native cellulase hydrolyses is uncontrollable, it significantly affects the physical characteristics of the fabric[6,7]. Immobilization involves the attachment of enzymes to a support material and a wide range of materials can be used for this. Immobilization allows the enzyme to be reused for multiple cycles to lower the cost of application and overcome such technical obstacles[8,9]. A lot of research work has been done on the immobilization of cellulase enzymes with a variety of support materials, such as nanoparticles[10], natural polymers[11], mesoporous materials[12,13], magnetic materials[14], and hybrid (inorganic-organic) supportmaterials[15]via covalent crosslinking and physical adsorption.

Chitosan, a natural polyaminosaccharide (copolymer of N-acetyl-D-glucosamine and D-glucosamine) is an ideal smart support for enzyme immobilization because of economy, its hydrophilicity, biocompatibility, biodegradability and antibacterial properties[16,17,18]. Similarly, Eudragit S-100, a synthetic soluble-insoluble copolymer of methacrylic acid and methyl methacrylate is extensively used to immobilize a variety of enzymes[6,19,20].

These polymers are suitable for bio-catalytic applications in the soluble phase at a favourable *p*H range, while can be recovered in the insoluble phase at a specific *p*H.This study focuses on the immobilization of commercial cellulase enzyme onto these reversible soluble-insoluble polymers, namely chitosan and Eudragit S-100, to enhance the enzymatic treatment of denim fabrics. The selection of these support materials was based on their distinct features that enable a

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controlled and reversible release of the immobilized cellulase. The immobilization enhances the stability and reusability of the immobilized cellulase ensuring its sustained efficacy throughout denim fabric treatments[3,9,15].After the immobilization, the immobilized cellulases were applied in denim fading to achieve the classic worn-out appearance, and the acquired results were carefully contrasted with the results from native cellulase applications. This study demonstrates the possibility of immobilized cellulase on chitosan and Eudragit S-100 as a workable and environmentally acceptable alternative, marking a significant advancement in the quest for efficient and sustainable enzymatic denim treatments.

2 Materials and Methods

2.1 Materials

Generic denim fabric (dark blue indigo dyed, 2/1 twill weave, 385 g/m^2, EPI × PPI 60×45, Warp7s and weft 10s count) and Cellusoft Conc. L (acid cellulase, Novozymes commercial product) having strength 1500 ECU/g were used with recommended conditions (*p*H 4.5 – 5.5 & temperature 40 – 50°C). Water-soluble chitosan (chitosan chloride, India Seafood Ltd. Coimbatore) and Eudragit® S-100 (Evonik India Pvt. Ltd, Mumbai, anionic copolymer of methacrylic acid and methyl methacrylate in approx. 1:2), glutaraldehyde (25%, Thomas Baker), DNSA (3,5-Dinitrosalicylic acid, SD Fine) and carbodiimide hydrochloride/carbodiimide [1-Ethyl-3-(3-diethylaminopropyl), Sigma-aldrich] were used. The other chemicals used were procured from SD Fine Chemicals Ltd., India, and were of analytical grade.

2.2 Experimental Methods

2.2.1 Immobilization of Cellulase Enzyme on Chitosan

The water-soluble chitosan was used to make the chitosan beads as per the method described earlier[21,22,23] and then the cellulase enzyme was immobilized on these chitosan beads using glutaraldehyde as a cross-linking agent. Chitosan beads were activated using 3% (v/v) aqueous glutaraldehyde solution for 3h with gentle magnetic stirring at room temperature (25°C). After that, unbound glutaraldehyde was removed from the beads by simply washing them in sodium acetate buffer. Now, the activated chitosan beads were immobilized with 5% (v/v) cellulase concentration at room temperature (25°C) with neutral *p*H for 12 h. The immobilized beads were filtered and cleaned to remove unbound enzymes and thereafter, the enzyme loading % and activity of the chitosan immobilized cellulase were evaluated[24,25].

2.2.2 Immobilization of Cellulase Enzyme on Eudragit S-100

Cellulase was immobilized on Eudragit S-100 using carbodiimide as a mediator[26,27]. Initially, a 2% (w/v) solution of Eudragit S-100 was prepared in phosphate buffer 0.3% (w/v) and carbodiimide coupling agent was added to this with continuous stirring for 10 min. Subsequently, 2% (v/v) of cellulase enzyme solutions were added at room temperature (25 °C) and kept under stirring for 6 h of immobilization time. To precipitate the cellulase-eudragit conjugate, acetic acid was used to lower the *p*H to 4.5 of the mixture. The precipitate was separated by centrifugation (11,000×g) at 25°C for 10 min and washed in 0.01M acetate buffer at *p*H 4.5 for 10 min. Further, the precipitate was dissolved in phosphate buffer at *p*H 7 and re-precipitated at *p*H 4.5 with acetic acid solution. Finally, the Eudragit-cellulase precipitate was re-dissolved in a 100mL buffer of *p*H 7.6 for use[24,25]. Similarly, the enzyme loading % and activity of the immobilized cellulase were also measured.

2.2.3 Enzymatic Treatment of Denim Fabric

Initially, generic denim fabric was desized for 60 min at 60–65°C using 5 g/L of the amylase enzyme. Acetic acid was used to keep the *p*H between 5 and 6, and 1:30 was the material-to-liquor ratio. The enzyme was rendered inactive following desizing by heating the water bath to 80°C for 10 min. Warm and then cold water were used to rinse the fabric. After drying in the oven, the removal of the size was evaluated using an Iodine drop test. The fabric was taken for additional treatment after passing the test.

The generic denim fabric was treated with free cellulase enzyme in a conventional water bath with a thermostatically regulated system that can hold stainless steel containers (Innolab Pvt. Ltd, India). Different dosages of cellulases (2%, 4%, 6%, and 8% owm) were applied to the denim textiles for 60 min at 50°C, a material-to-liquor ratio of 1:20, and a *p*H of 5.5 in a 0.2 M acetate buffer. The containers were rotated at a speed of 50 rpm with 10 stainless steel balls (5 mm diameter) to imitate the mechanical agitation. Next, the remaining cellulase was deactivated in deionized water at an 80°C temperature and the denim samples were then dried at 70°C. The aforementioned experiment was also conducted with

covalently immobilized cellulase that had the same activity units as the native cellulase at optimal treatment conditions. Three replications were carried out in each experiment.

2.3 Evaluation and Testing

2.3.1 Cellulase Activity

The activity of cellulase enzyme in native as well as immobilized form was evaluated by the amount of reducing sugar (glucose) formed by the enzymatic reaction on cellulose using the 3,5-dinitrosalicylic acid (DNS) method [12,14,15,28]. The amount of reducing sugar produced from the reaction was measured by a UV-visible spectrophotometer (Perkin Elmer, Lamda 25) at the wavelength (λ) of 540nm and cellulase enzyme activity was calculated in Endo Cellulase Unit (ECU)/mL using the following equation:

$$\text{Cellulase activity}\left(\frac{\text{ECU}}{\text{mL}}\right) = \frac{\text{Glucoseproduced}(\mu\text{g})}{\text{Enzymeamount}(\text{mL}) \times \text{Incubationtime}(\text{min})} \quad \ldots (1)$$

Further, the specific activity of the enzymes can be evaluated using the following equation, where the protein content was calculated using 2,4,6-trinitrobenzene sulfonic acid (TNBS) reagent[25,27].

$$\text{Specific activity (U/mg)} = \frac{\text{Enzymeactivity (ECU/mL)}}{\text{Totalprotein}(\text{mg/mL})} \quad \ldots (2)$$

2.3.2 Enzyme Loading %

The amount of enzyme immobilized was calculated from the protein content in the solution before and after immobilization, by detecting the changes in absorbance before and after enzyme immobilization using 2,4,6-trinitrobenzene sulfonic acid (TNBS)[25, 29],as seen below:

$$\text{Enzymeloading}(\%) = 1 - \frac{\text{absorbencevalueafterimmobilization}}{\text{absorbencevaluebeforeimmobilization}} \quad \ldots (3)$$

2.3.3 FTIR Spectroscopy

The FTIR-ATR spectra of native cellulase enzyme and immobilized cellulase with chitosan and Eudragit were recorded between 400 cm^{-1} and 4000 cm^{-1}on Brucker Eco Alpha FTIR spectrophotometer.

2.3.4 Measurement of Colour Characteristics

Colour characteristics of denim fabrics were measured by a Macbeth COLOUR-Eye spectro-photometer under the D65 illuminant using a 10° standard observer. The fabrics were folded twice to ensure opacity and were measured five times. The colour strength was denoted by the well-known *K*/*S* value. In addition, CIE colour coordinates, L* (lightness and darkness), a* (redness and greenness), and b* (yellowness and blueness) of the denim samples were also evaluated.

2.3.5 Back-staining Evaluation

The degree of back-staining on denim fabrics was determined by attaching a white cotton fabric to the reverse side, as enzyme action on denim frequently results in unwanted back-staining[5,6,30]. The measurement of the *K*/*S* and CIE L* values of the white cotton samples attached on the reverse side of the denim textiles was used for the determination of the degree of back-staining.

2.3.6 Physical Properties of Denim Fabric

The effect of the enzyme on the physical properties of cellulose was tested by evaluating the weight loss% as per ASTM D 3776 and the tensile strength of denim samples was measured in warp direction by ASTM D5034-95 using a digital tensile strength tester (Globe-Tex Industries).

2.3.7 Reusability

The reusability of immobilized cellulases was evaluated by using them for five consecutive cycles of denim washing. After each washing cycle, the enzyme was separated from the liquor by changing *p*H to its insoluble phase, and residual activity was estimated. The residual activity was measured by taking the initial activity for the first cycle as 100%[25]. Also, the fading of denim samples was evaluated in terms of *K*/*S* value after each cycle.

3 Results and Discussion

3.1 Characterization of Native and Immobilized Cellulase

The immobilized cellulases were characterized by their activity and application parameters(Table 1). It is evident from the results that after immobilization the activity of cellulase decreased. The reason for the decrease in cellulase activity after immobilization may be that the immobilization makes the enzyme larger and less soluble, which impairs its ability to interact with the substrate[9,19]. The only factor influencing these changes in the enzyme is the supporting material. Hence, the Eudragit immobilized cellulase has a greater activity than chitosan.

The immobilization may lead to a change in the thermal and *p*H stability of enzymes. Consequently, it

Table 1 — Activity and optimum conditions for native and immobilized cellulases

Cellulase enzyme	Specific activity, ECU/g	Enzyme loading, %	Optimum *p*H	Optimum temperature, °C
Native	1350	-	4.5 – 5.5	40 – 50
Chitosan immobilized	1020	72.4	4.5 – 5.5	60
Eudragit immobilized	1170	73.1	6 – 6.5	60

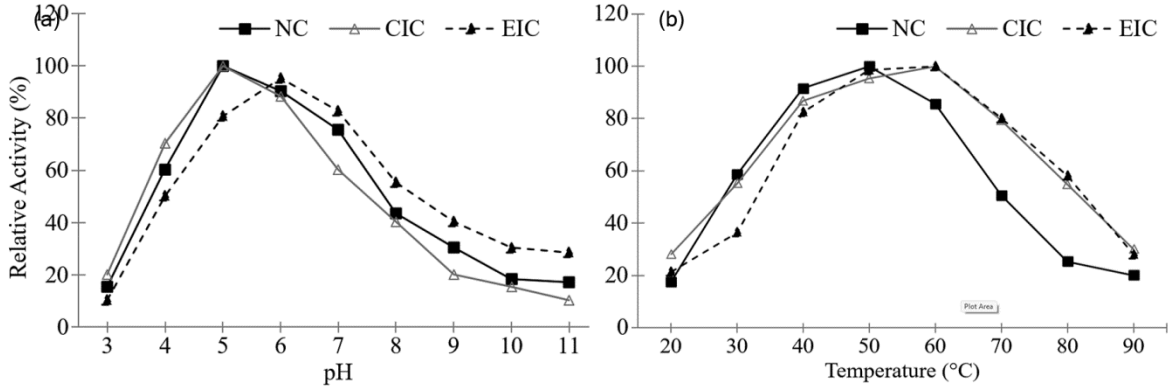

Fig. 1 — Effect of (a) *p*H and (b) temperature on relative activity of native and immobilized cellulose

is essential to evaluate the immobilized enzymes for their optimal *p*H and temperature of application concerning native cellulase. The effect of *p*H on the relative activity of native and immobilized cellulase has been studied over a broad range of *p*H values (3 – 11), [Fig.1(a)]using different acetate, phosphate, and carbonate buffer solutions at 50°C. In a similar vein, these cellulases are cultured at *p*H 5.5 and temperatures ranging from 20°C to 100°C to investigate the impact of temperature. The DNS method is used to measure the activity of immobilized cellulases at various *p*H and temperature values.

The ideal *p*H range for native cellulase is found 4.5–5.5 which shifts to 6 – 6.5 for Eudragit immobilized cellulase. Figure1(a) makes it evident that while the chitosan immobilized cellulose has slightly increased activity in the acidic *p*H range, the Eudragit immobilized cellulase exhibits higher activity in *p*H values above 6. According to Krajewska B[31], a *p*H of less than 6 promotes the solubility of chitosan and causes the ideal *p*H to move towards the acidic side. The fact that the Eudragit S-100 matrix is anionic and becomes soluble at *p*H 5 indicates this, shifting the optimal *p*H of conjugate activity toward the neutral side[26, 29, 32]. Similarly, cellulase immobilized on Eudragit S-100 shows a shift in optimum *p*H toward the neutral side[29, 32].

Similarly, the effect of temperature on the relative activity of free and immobilized cellulases is studied between 20°C and 80°C, under optimal *p*H conditions [Fig. 1(b)]. The native cellulase exhibits its maximum activity at 50°C and retains 80% of its activity in a temperature range from 40°C to 60°C. In comparison, both immobilized cellulases exhibit their highest activity at temperatures of 55°C and 60°C and retain over 80% of their maximum activity over a wide temperature range from 40°C to 70°C.

At temperatures below 40°C and above 60°C, the catalytic activity of the free biocatalyst significantly decreases, suggesting that the native enzyme is unstable in these conditions due to denaturation of the protein structure. In addition, the drop-in relative activity of immobilized cellulases above 70°C is less significant than the native one, and the immobilized cellulase exhibits over 50% of its relative activity even above 80°C. Thus, the enhanced thermal stability of the cellulase enzyme upon immobilization is amply supported by the results [19, 21, 24, 27].

3.2 FTIR Analysis of Immobilization

The immobilization of enzymes on chitosan beads takes place in two steps, where initially, glutaraldehyde is used to activate the chitosan beads. This activation process creates an imine bond between the amine group of the chitosan and the aldehyde groups of glutaraldehyde[21]. Following their reaction with the enzyme, the free aldehyde group on the activated beads forms an imine link. The distinctive peaks of the polysaccharide structure of chitosan are located at 1380 cm^{-1}, 1150 cm^{-1}, 1040 cm^{-1}, and 985 cm^{-1}[Fig.2(a)].

The covalent attachment of the enzyme over chitosan is confirmed by the shift of the peak at 1588

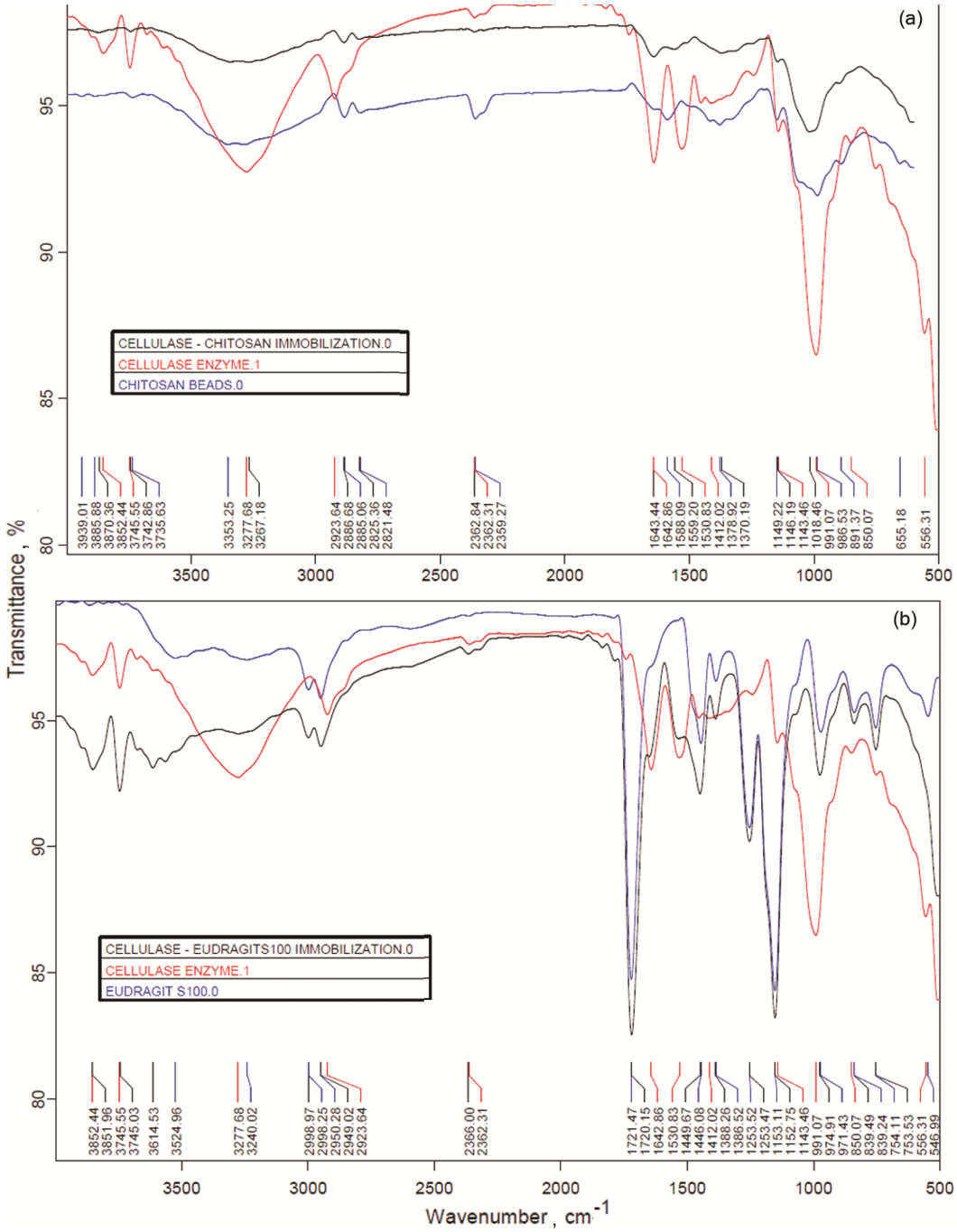

Fig. 2 — FTIR spectra of (a) chitosan beads, cellulase enzyme, and chitosan immobilized cellulase, and (b) Eudragit S-100, cellulase enzyme, and Eudragit immobilized cellulase

cm^{-1}[which corresponds to (-NH_2-) group in the chitosan spectra] to 1644 cm^{-1} in the cellulase immobilized chitosan [which corresponds to the (-C=N-) imine bond][23 – 25] (Table 2).

Similarly, there are two phases involved in immobilizing cellulase on Eudragit. Adding EDC activates the carboxy-groups of Eudragit first andthe result is an active acyl-isourea intermediate product. It is referred to as a zero-length cross-linking agent since it just activates carboxy groups and mediates the coupling with superficial main amino groups without the need for spacer molecules. Second, the enzyme is added and a peptide (-CO-NH-) link is established between the carboxy groups on the surface and the superficial amino side chains of the enzyme[33 – 35]. Enzyme bonding is confirmed by the appearance of a

Table 2 — FTIR analysis of chitosan and chitosan immobilized cellulase

Sr No	Peak, cm^{-1}	Corresponding group	Inference
1	3000-3500 1380 1150,1040, 985	Stretching vibrations of –OH and $–NH_2$ $–CH_2$ bending of chitin Anti-symmetric stretching of the C–O–C bridge Skeletal vibrations of C–O stretching	Characteristics of the polysaccharide structure of chitosan
2	1644 (region)	Attributed to imine bond –C=N– formation	Present in immobilized cellulase
3	1580-1585, 2360	Corresponds to $-NH_2$ group	Reduced upon bond formation in immobilized cellulase FTIR

Table 3 — FTIR analysis of Eudragit S-100 and Eudragit immobilized cellulase

S. No.	Peak, cm^{-1}	Corresponding group	Inference
1	3850 & 3745	-NH stretching vibrations	Presence of enzyme structure
2	2950-3000	-OH & $–CH_x$ stretching	Presence in Eudragit structure
3	1650-1655	amide –CO–NH–	Presence confirms enzyme bonding
4	1728	-C=O vibrations of esterified groups	Characteristics of Eudragit become prominent due to bonding
	1250 – 1275, 1150 – 1160	-COOR ester vibrations	
5	1385 – 1390, 1450	$–CH_x$ stretching vibrations	Characteristics of Eudragit polymer chain

Table 4 — Colour parameters and physical properties of cellulase-treated denim fabrics

Denim fabric	Cellulase % (owm)	Colour properties				Weight loss, %	Tensile strength, kgf
		K/*S* value (640 nm)	CIE L*	CIE a*	CIE b*		
Untreated	-	14.78	24.845	1.378	-13.604	-	66.19
Control	0	14.49	25.693	1.093	-15.015	0.4	66.21
Native cellulase	2	10.26	29.754	0.579	-16.732	3.8	50.42
	4	6.97	36.511	-1.560	-19.261	7.5	42.38
	6	4.52	38.982	-2.137	-20.325	9.7	35.63
	8	3.14	40.861	-3.946	-20.873	12.5	32.51
Chitosan immobilized cellulase	2	12.26	27.723	0.945	-16.981	3.1	58.26
	4	7.22	34.831	-1.638	-18.782	3.8	55.67
	6	5.13	36.773	-2.452	-19.003	4.6	53.96
	8	4.73	39.477	-3.082	-20.528	5.2	50.48
Eudragit immobilized cellulase	2	11.17	28.628	0.628	-16.874	2.8	57.89
	4	7.34	35.361	-1.486	-18.752	3.2	56.14
	6	4.53	38.472	-2.254	-19.019	4.7	52.14
	8	3.86	40.265	-3.569	-20.382	5.6	50.72

strong peak at about 1650 cm^{-1}, which corresponds to the amide (-CO-NH–) in the immobilized enzyme [Fig. 2(b)].The covalent bonding causes the distinctive bands of the C=O vibrations of esterified carboxyl groups at 1730 cm^{-1} as well as additional ester vibrations at 1150 – 1160 cm^{-1} and 1250 – 1275 cm^{-1} to become prominent[35, 36] (Table 3).

3.3 Application of Native and Immobilized Cellulase for DenimFading

3.3.1 Colour Fading

The effect of cellulase treatment on the denim fabric colour is evaluated in terms of colour strength (*K*/*S*) and CIE L* a* b* values. Table 4 shows the results of the application of native and immobilized cellulase treatment on the colour properties of the denim fabric. The untreated samples show a maximum *K*/*S* value of 14.78 and the *K*/*S* value decreases after the cellulase enzyme treatment as it is linearly correlated with the concentration of dyestuffs on the fabric, surfaces. This is because cellulases hydrolyze the surface fibres of denim fabrics, and the indigo dyestuffs on the fabrics are also removed along with the weaker surface fibres, resulting in denim fading[2 – 3,30].

The denim fabric treated with native and immobilized cellulases shows comparable fading results for all the cellulase concentrations, while the Eudragit immobilized cellulase is observed to be a little more effective in comparison to chitosan immobilized cellulase due to its higher activity (Fig.3). At a cellulase concentration of 6%, the denim

fabric treated with native and immobilized cellulases have K/S values of 4.52,5.13 (chitosan immobilized), and 4.53 (Eudragit immobilized) respectively.

CIE L* values of the denim fabric sample are also used to measure the effectiveness of the fading effect of enzymatic treatment. The higher L* values of the denim fabrics treated with native and immobilized cellulase are evident in the removal of colour as compared to the untreated denim fabric (Table 4). The denim fabric treated with native and immobilized cellulases have CIE L* values of 40.861, 39.477 (chitosan immobilized) and 40.265 (Eudragit immobilized) respectively, at a cellulase concentration of 8%. Table 4 also includes CIE a* and b* values of the denim fabrics after enzymatic treatment. The enzymatic treatments reduces both the CIE a* and b* values and denim fabrics treated with native and immobilized cellulases show a similar change in colour parameters. This result indicates that the denim fabrics are decoloured with comparable efficiency by the enzymatic treatments using native and immobilized cellulases.

The digital photographs of the generic denim fabrics treated with cellulase showa faded or worn-out look as compared to the untreated denim sample, [Fig. 4(a)]. Both native and immobilized cellulases give a good decolouration effect, where some faded streaks can be observed in digital photographs [Figs4(b)–(d)].

3.3.2 Back-staining Analysis

Back-staining is the most significant issue with denim fading where the indigo released during the fading process stains the reverse side of the garment[5,6,30]. Here, the back-staining is evaluated on the additional white cotton fabric attached to the denim for treatment. *K*/*S* and CIE L* values of this cotton fabric are evaluated and compared for the enzymatic treatments. The results of *K*/*S* values and CIE L* values are shown in Figs 5(a) and (b).

A higher *K/S* value and a lower CIE L* value indicate a higher degree of back-staining. It is clear from the results of *K*/*S* and CIE L* values that the degree of back-staining with the native cellulase is higher than the immobilized cellulose while the chitosan immobilized cellulase shows slightly reduced

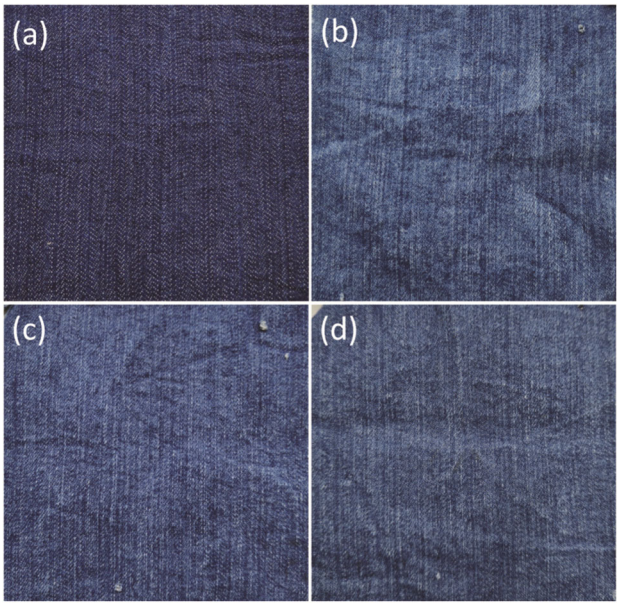

Fig. 4 — Digital photographs of generic denim fabric (a) untreated, (b) native cellulase, (c) chitosan immobilized cellulase and (d) Eudragit immobilized cellulase

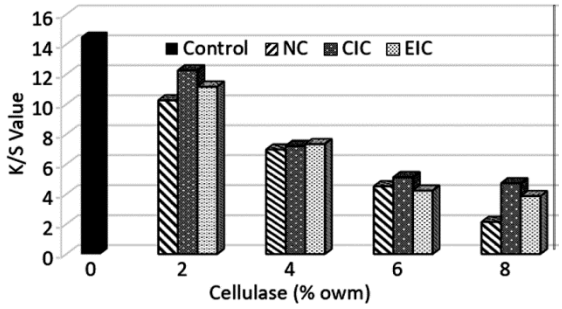

Fig. 3 — *K*/*S* values of denim fabrics treated with native and immobilized cellulase (NC – native cellulase, CIC – chitosan immobilized cellulase and EIC – Eudragit immobilized cellulase)

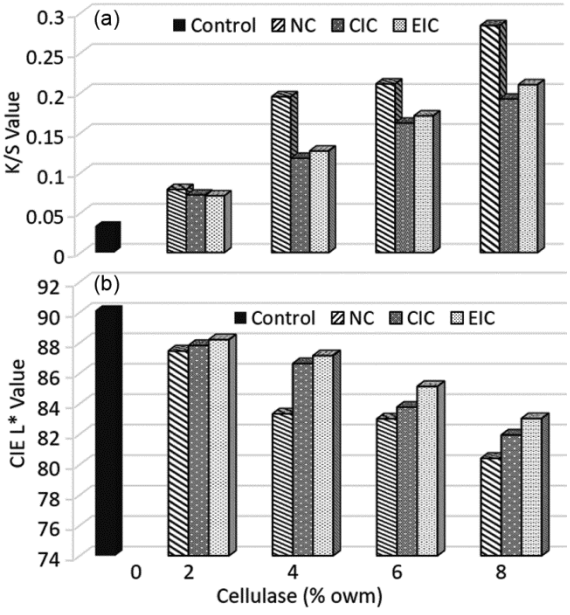

Fig. 5 — (a) *K*/*S* and(b) CIE L*values of white fabrics for back-staining(NC – native cellulase, CIC – chitosan immobilized cellulase & EIC – Eudragit immobilized cellulase)

back-staining in comparison to Eudragit immobilized cellulase. The back-staining depends upon the cellulase–cellulose and indigo–cellulase affinities, and this may be due to the reason that the support materials used for the immobilization also show some affinity for indigo which may result in lower back-staining [2,20,30]. Also, the degree of back-staining increases with increasing cellulase concentration, regardless of whether it is the native or immobilized cellulase.

3.3.3 Physical Damage

The results of weight loss % and tensile strength of the denim fabrics treated with the native and immobilized cellulases are also represented in Table 4. It is evident from the results that the weight loss percentage increases with increasing cellulase concentration. Denim fabrics treated with native cellulase exhibit a weight loss of 9.7% and 12.5% respectively for 6% and 8%concentrations. However, for the same concentrations and activity units, the immobilized cellulase treatment results in weight losses of just 4.6% and 5.6% respectively, suggesting less damage to the denim textiles (Table 4). Figure 6 illustrates the impact of the enzymatic treatment on the tensile strength of the denim fabrics. The results reveal that the tensile strength of denim fabrics decreases considerably after being subjected to cellulase treatment, especially at higher concentrations.

Denim fabric treated with a 6% concentration of native cellulase exhibits a tensile strength of 35.63 kgf, which is significantly lowered as compared to untreated denim (66.19 kgf), while the tensile strength of immobilized cellulase-treated denim samples is 52.96 kgf (chitosan immobilized) and 52.84 kgf (Eudragit immobilized) respectively, for same cellulase concentration.The denim fabric treated with the immobilized cellulase shows lower weight loss % and considerably higher retained tensile strength than those treated with the native cellulase.This is due to the fact that immobilization results in the increased molecular size of cellulase due to support attachment which restricts its hydrolytic action only to the surface of the cellulosic fibre[5,6,9], which enables the hydrolysis process of the denim to be efficiently controlled.

3.3.4 Reusability of Immobilized Cellulase

The reusability of enzymes is essential for their cost-effective industrial applications, and immobilization enables the recovery and reuse of enzymes[3,9]. The Eudragit immobilized cellulase is insoluble below *p*H 4 while the chitosan immobilized cellulase is insoluble in alkaline *p*H. Because of their *p*H-dependent soluble-insoluble nature, immobilized cellulases can therefore be readily separated and reused after the completion of the process. The immobilized cellulases are used for the 5 consecutive denim fading cycles and their retained activity is evaluated after each cycle[Fig. 7 (a)], where retained activity means activity of the immobilized cellulase after each cycle taking initial activity as 100%.

The reusability has been examined up to 5 cycles for the denim fading effect in terms of *K*/*S* values of the denim fabrics, as indicated in Fig. 7(b).The results unequivocally show that both the chitosan and

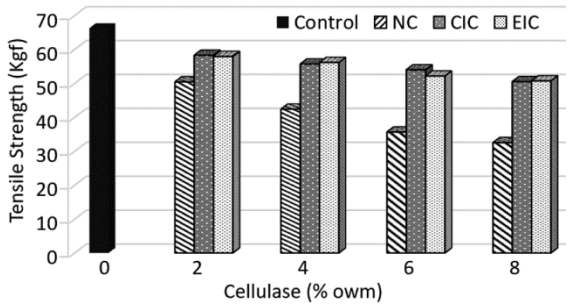

Fig.6 — Tensile strength of denim fabrics treated with native and immobilized cellulase

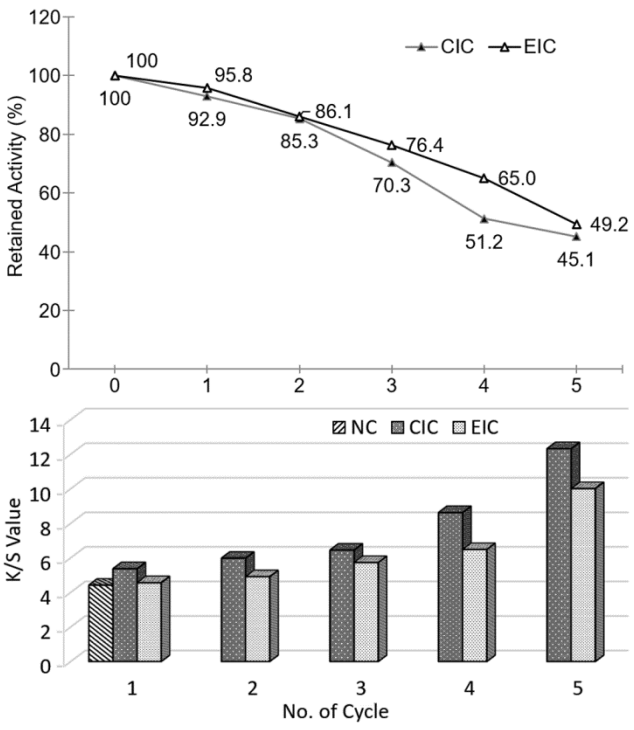

Fig. 7 — (a) Retained activity of immobilized cellulases and (b) *K*/*S* values of denim fabrics after each cycle

Eudragit immobilized cellulases can provide adequate fading effect and can be easily reused for 3 repeated cycles. The Eudragit immobilized cellulase exhibits more decolouration as compared to chitosan immobilized cellulase and can be reused for 4 cycles, while after that the activity of the enzymes gets reduced and results in inadequate fading. Eudragit-immobilized cellulase retains49.2%, whereas chitosan-immobilized cellulase retains 45.1% of its initial activity, thus indicating a significant degree of reusability.

4 Conclusion

The immobilized cellulase demonstrates superior performance, contributing to the sustainable enzymatic washing of denim fabrics. Significant advantages offered by immobilized cellulase enzymes are the reduction of physical damage and back staining issues commonly associated with native enzyme treatment. Moreover, the reusability of immobilized enzymes for consecutive cycles presents an economic and ecological advantage, adding a layer of sustainability to denim fabric treatments. The findings presented herein not only contribute to the scientific understanding of enzyme immobilization but also offer practical solutions for the textile industry to adopt more sustainable practices in denim fabric treatments.

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