

## ORIGINAL ARTICLE

**Effects of adsorption properties and mechanical agitation of two detergent cellulases towards cotton cellulose**CRISTINA CAPARRÓS<sup>1</sup>, NEIL LANT<sup>2</sup>, JOHAN SMETS<sup>3</sup> & ARTUR CAVACO-PAULO<sup>1</sup>

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**Abstract**

The impacts of two hybrid cloned commercial cellulases designed for detergency on cotton fibres were compared. HiCel45 has a family 45 catalytic domain and a fungal cellulose binding module (CBM) from the fungus *Humicola insolens*. BaCel5 has a family 5 catalytic domain and a fungal CBM from *Bacillus* spp. BaCel5 bound irreversibly to cellulose under the buffer conditions tested while HiCel45 was found to bind reversibly to cellulose because it showed low adsorption. BaCel5 seems to yield more activity towards cotton than HiCel45 under mild stirring conditions, but under strong mechanical agitation both enzymes produce similar amount of sugars. HiCel45 had a more progressive production of residual reducing ends on the fabric than BaCel5. These studies seem to indicate that HiCel45 is a more cooperative enzyme with detergent processes where high mechanical agitation is needed.

**Keywords:** cellulase, cellulose fibres, cotton, microfibrils, detergency

**Introduction**

A large number of enzymatic treatments have been applied to industrial processing of cellulosic fibres in order to obtain new finishing effects or to replace harsh chemicals used in conventional cotton processes. In the textile and the detergent industries, cellulases have successfully been used for partial hydrolysis processes (Bhat & Bhat 1997; Buchert et al. 2000; Linder et al. 1999, 1996; Linder & Teeri 1997; Reinikainen et al. 1997). The introduction of biological ingredients permits reduction in the wash temperature and cycle time of domestic laundry (Schroeder et al. 2006). Cellulases from *Humicola insolens* and from *Bacillus* spp are now being used in detergents, whereas cellulases from *Trichoderma* species are widely used in textile treatments (Crutzen & Douglass 2008; Schmid et al. 2002; Tomme et al. 1995).

Cellulases are enzyme complexes that coordinate their activities for the stepwise decomposition of cellulose and derivatives to glucose (Bisaria & Ghose 1981; Schulein 2000). Cellulases have traditionally been classified into three distinct classes,

according to the mode of action: Exoglucanases or cellobiohydrolases (cellulose 1,4- $\beta$ -cellobiosidase, 1,4- $\beta$ -D-glucan cellobiohydrolase, EC 3.2.1.91); Endoglucanases (1,4- $\beta$ -D-glucan-4-glucanohydrolase, EC 3.2.1.4) and  $\beta$ -glucosidases (EC 3.2.1.21) (Knowles et al. 1987; Schulein 1997). Endoglucanases (EG) cut the glucose chains internally. Exoglucanases are capable of degrading cellulose starting from free chain ends. As the main product of the reaction is cellobiose, the exoglucanases are frequently called cellobiohydrolases (CBH).  $\beta$ -Glucosidases degrade cellobiose into glucose.

Fibre hydrolysis with cellulase systems is usually initiated by the interaction between cellulose binding module (CBM) and the cellulose fibre. When the enzyme is adsorbed on the fibre, the hydrolysis mechanisms proceed. During this process, the solid substrate characteristics vary, changing the number of chain ends by reaction with endoglucanase and exoglucanase and changing the cellulose accessibility resulting from substrate consumption and cellulose fragmentation (Boisset et al. 2000; Din et al. 1991; Zhang & Lynd 2006). The complex interactions

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between EG, CBH and the changing substrate characteristics during the hydrolysis are represented in Figure 1. In the first step of the hydrolysis of cotton cellulose, it might be expected that reducing terminals are generated by EG activity without any release of hydrolysis products. The fungus *H. insolens* is one of the most studied cellulolytic micro-organisms. It produces a complete cellulolytic enzyme system and is capable of very efficient degradation of crystalline cellulose (Schulein 1997). The fungal cellulolytic enzymes are normally a mixture of different exo- and endo-glucanases, which can remove mechanically damaged cellulosic material from the textile. The desired anti-pilling benefits can be obtained by degrading an appropriate amount of microfibrils from damaged fibres (short fibres protruding from the surface). However, excessive release of those microfibrils has a destructive effect on the cleaning process of fabrics, that is, appearing as holes. Fungal cellulases have their optimum pH between the acidic to neutral range, although some cellulases produced from *H. insolens* were found to retain good activity and performance in the alkaline range (Boisset et al. 1997; Otzen et al. 1999; Schulein 1997).

The *H. insolens* cellulase used in this work is HiCel45 and belongs to hydrolytic family number 45; its core structure has been described by Davies et al. (1996). The full sequence of the catalytic core, the O-glycosylated linker and the C-terminal family 1 CBM are available. The fungal CBMs are classified as an A CBM from family 1 and have a wedge-like structure (Rasmussen et al. 1991). Endoglucanases (EG) for laundry use constitute a large world market and EG from family 45 from *H. insolens* has dominated this market for several years (Glaser 2000; Koga et al. 2008; Shikata et al. 1990). Novozymes claim that HiCel45 contains a cloned endoglucanase from *H. insolens*, with strong anti-pilling properties and a pH profile that should

be useful in laundry detergent applications. The detergency advantages of HiCel45 claimed are in anti-pilling, fabric softening and colour revival whereas fabric damage is found as a negative effect (Miettinen-Oinonen 2007).

Alkalophilic *Bacillus* species have been known for a long time as producers of alkalophilic cellulases, which show pH optima from neutral to alkaline. The first bacterial alkaline cellulase was produced by KAO Corporation (Japan) and exhibited endo- $\beta$ -1,4-glucanase activity (E.C. 3.2.1.4). The term 'alkaline endoglucanase' indicates an endoglucanase with an optimum pH above 7 and retaining more than 70% of its optimal activity at pH 10 (Ito 1997). In this work, the alkaline endoglucanase BaCel5 was used. BaCel5 contains tandem CBMs consisting of one family 17 CBM and one family 28 CBM, both classified as B CBM, which are supposed to be highly selective towards amorphous cellulose and to have glucan-binding properties (Boraston et al. 2002; McLean et al. 2002). Cellulases from *Bacillus* species generally show good compatibility with subtilisin proteases, which are used in detergent applications (Banik & Prakash 2004; Crutzen & Douglass 2008; Kame et al. 1973; Kottwitz & Upadek 1997; Maase & van Tilburg 1983). These types of enzyme have been described as having excellent detergent effects, as shown on sebum-stained cotton (Ito et al. 1989). The effect is supposed to be based on the weakening of amorphous regions of cellulose, where particles might be immobilized preferentially. In addition, these cellulases cause no damage to the cotton fibre, solving potential problems of tensile strength loss after increased numbers of wash cycles (Boraston et al. 2003; Husum & Friis-Jensen 2007; Otzen et al. 1999; Shaw et al. 2002; Shikata et al. 1990; Suzuki et al. 1988). The reduced anti-pilling properties are probably due to low activity towards crystalline cellulose of the degrading system (Shikata et al. 1990).

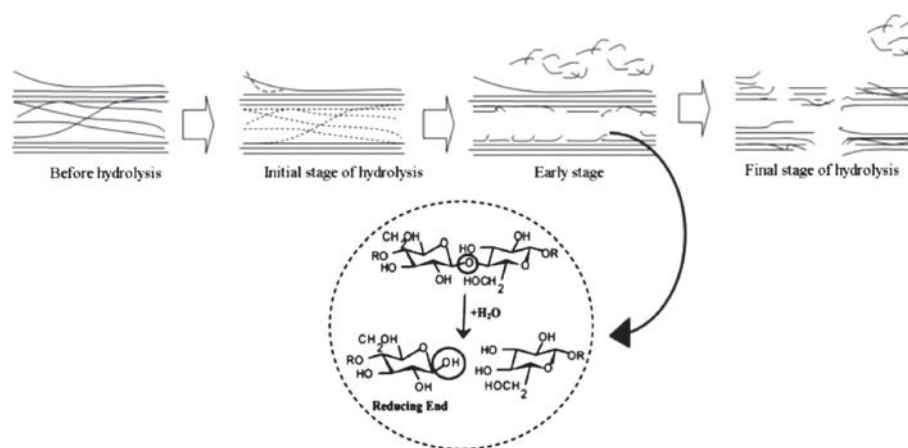


Figure 1. Representation of cellulase interaction during the hydrolysis process, which promotes changes in the substrate characteristics.

Alkaline cellulases can partially cleave in the amorphous regions, without significantly liberating hydrolysis products. Therefore, trapped soils should be easily removable by the action of the ordinary ingredients of detergents without an excess of degradation of cotton fibre (Horikoshi 2006; Hoshino et al. 2000; Husum & Friis-Jensen 2007; Ito 1997).

With both types of cellulase, different levels of mechanical agitation might lead to different levels of protein adsorption and enzymatic hydrolysis. The surface properties have an enormous effect on the mechanism, rate and degree of adsorption. Different impacts of the fabric surface might be expected due to the different cellulase properties, the understanding of which might enhance their potential in laundry detergency.

The purpose of the present work was to provide new insights on the influence of mechanical agitation on cellulase activities towards cotton substrates. The interaction between the activity of two cellulase types and mechanical agitation were studied. In addition, the influence on the adsorption and desorption of two cellulases on cotton cellulose fabrics was evaluated.

## Experimental

### Enzyme and fabrics

The glycoside hydrolase nomenclature suggested by Henrissat et al. (1997) had been adopted in this study, where *H. insolens* cellulases were indicated with the prefix Hi, *HiCel45*, EGV or Carezyme® (Cellulase type 45, EC 3.2.1.4); the *Bacillus* sp. cellulases with the prefix Ba. *BaCel5*, Celluclean® (Cellulase type 5; E.C. 3.2.1.4). The reported activities of *HiCel45* and *BaCel5* were 325.4 and 430 EGU/g, respectively, and they were supplied in a commercial preparation from Novo-Nordisk (Schou et al. 1993).

Unbleached cotton woven fabrics (24/21 ends/picks per cm and area density of 140 g/m<sup>2</sup>) were used as the cellulosic substrate for the enzymatic treatments.

The protein bovine serum albumin (BSA) and glucose were obtained from Sigma. All other reagents used were of analytical grade.

### Enzymatic treatments

For the evaluation of adsorption properties, cotton fabrics (1 g) were treated with the two cellulolytic preparations in 0.1 M sodium phosphate buffer pH 7 or sodium carbonate buffer pH 10 (50 mL) using an enzyme dosage of 150 mg protein L<sup>-1</sup> at 1:50

fabric to liquor ratio, at 40°C. These studies were performed in a shaker bath at 125 rpm, which produces gentle orbital agitation. After the first incubation period (4 h), where the enzyme was adsorbed in equilibrium with the fibre, the protein in solution was measured to calculate the percentage of adsorption. The treatment liquor was then diluted (1:2) with phosphate buffer or carbonate buffer respectively, and the mixture was incubated for at least a further 1 h under the same agitation conditions. After the enzymatic reaction, the supernatant was analysed again for protein determination. Control samples were incubated in the same buffer solution but without enzyme, and samples were removed after each incubation period. Three replicates were carried out for each enzymatic treatment.

A typical adsorption/desorption process is represented in Figure 2. This shows that the free protein concentration returns to a new equilibrium after it has been disturbed by dilution with buffer, demonstrating that the binding of the protein is reversible. The percentage of adsorption and desorption was calculated by the expressions (1) and (2). A continuous line represents reversibility and a discontinuous line, irreversible behaviour.

$$\text{Adsorption(\%)} = 100 \times (A-B)/A \quad (1)$$

$$\text{Desorption(\%)} = 100 \times (C-D)/(A-B) \quad (2)$$

For the evaluation of the effect of mechanical action on cotton fibres, cotton fabrics (1 g) were treated with the two cellulolytic preparations in 0.1 M sodium phosphate buffer pH 7 (50 mL) using an enzyme dosage of 300 mg protein L<sup>-1</sup> at 1:50 fabric to liquor ratio, at 40°C. In order to compare, these studies were performed under two different agitations: (i) in a Rotawash MKII machine (laboratory scale European washing machine) providing vertical agitation of 40 rpm for 5 h and (ii) in 50 mL glass flasks in a shaker bath providing orbital agitation of 125 rpm for 48 h.

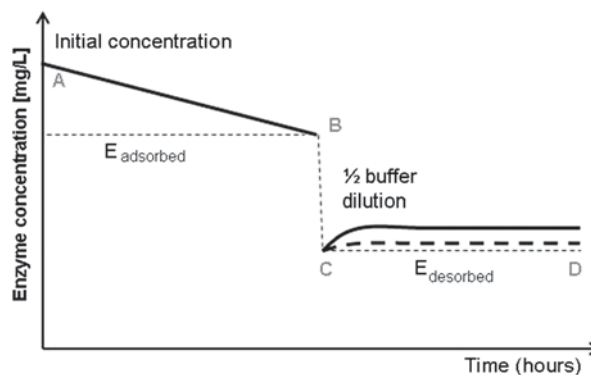


Figure 2. Typical adsorption/desorption curve of protein binding.

For the main incubation periods (0.3, 3, 5 and 48 h), a fabric sample and a supernatant sample with enzymatic solution were taken for further analysis. Control samples were incubated in the same buffer solution but without enzyme, and samples were removed after each incubation period.

After enzymatic treatment, all samples were washed using three cycles of 15-minute duration with deionised water at room temperature and, finally, air dried.

In order to obtain a mechanistic understanding of the effects of BaCel5 and HiCel45 cellulases, it was not possible to use detergent formulations as a reaction medium. However, western Europe washing conditions for liquid detergents were considered such as 20 min of washing, 40°C and basic pH (around 10), in order to approach real washing conditions.

#### *Physical analysis*

Total protein in solution was measured by the Bradford assay (Bradford 1976), with Bovine Serum Albumin (BSA) as standard. For each sample, three determinations were made.

Reducing sugars were measured in treatment liquor baths by the dinitrosalicylic acid method (DNS) (Lopez & Cavaco-Paulo 2008; Sumner & Sisler 1944) using glucose as standard. For the measurement, 0.25 mL of sample was incubated with 0.75 mL of DNS solution containing: 7.5 g/L DNS, 14 g/L sodium hydroxide, 216 g/L potassium sodium tartrate, 0.5% [v/v] phenol and 6 g/L sodium metabisulfite. The mixtures were boiled for 5 min and cooled to room temperature. Absorbance was measured at 640 nm in a Helios Gamma UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA). The concentration of reducing sugars [g/L] was determined against glucose standards (Sumner & Sisler 1944).

Reducing ends of cotton fabric samples after enzymatic treatment were measured by the method described by Cavaco-Paulo (Cavaco-Paulo 1998; Cavaco-Paulo et al. 1996), which depends on complex formed between neocuproine (2,9-dimethyl-1,10-phenanthroline) and reducing groups in the fibre. For the measurement, 0.1 g of sample was incubated with 2 mL of sodium carbonate (2%) and 5 mL of neocuproine reactive aqueous solution containing: 0.2 g/L copper (II) sulphate pentahydrate and 0.4 g/L neocuproine. The mixture was boiled for 5 min and cooled at room temperature. Absorbance was measured at 465 nm in a Helios Gamma UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA). Three analyses were done for each fabric sample. The concentration of reducing sugar [g/L] was determined against glucose standards. In

order to compare the measurement of reducing sugar in solution with reducing ends on the fabric, mg/L of reducing sugar were converted to mg/g of fabric, considering the reaction conditions (50 mL and 1 g of fabric).

The surface morphology of cotton fabrics before and after enzymatic degradation was observed in a JEOL 5310 scanning electron microscope. The samples were previously sputter-coated with gold in an Ion Sputter JEOL JFC 1100 equipment (JEOL LTD., TOKYO) in order to increase their conductivity. Free surface micrographies were taken at various magnifications and their microstructures studied.

## Results and discussion

### *Effect of the adsorption properties on cotton cellulose*

The adsorption of cellulases on insoluble cellulosic substrates is a prerequisite for subsequent hydrolysis and several authors have studied this subject (Kim et al. 1998). Some studies have attempted to understand the driving forces for adsorption of important commercial enzymes such as a detergent protease and a detergent lipase (Duinhoven et al. 1995a, b), highlighting the importance of charge interactions in adsorption (Hilden & Johansson 2004). The first step involves diffusion of the enzyme onto the interface, then a second step takes place when the adsorption and interfacial unfolding occurs. The different structures attained by the protein should be very dependent on the different steps of this mechanism (Maldonado-Valderrama et al. 2005; Miller et al. 2005). Adsorption studies of cellulases are difficult, because of the number of variables involved. Hydrogen bonding, Van der Waals forces and hydrophobic interactions have been reported as dominant interactions, due to the attachment of amino acids from the CBM (Carrard & Linder 2001; Medve et al. 2000).

Adsorption of the enzymes on the cotton fibre was analysed at neutral and basic pH (Figures 3 and 4). BaCel5 bound irreversibly to cellulose under buffered conditions while HiCel45 was found to

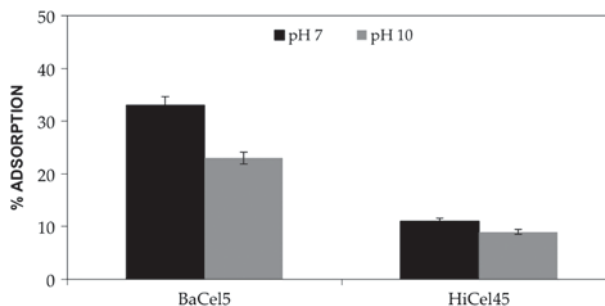


Figure 3. Percentage of protein adsorption for HiCel45 and BaCel5. (150 mg protein/ L; 40°C; 125 rpm; 4 h).



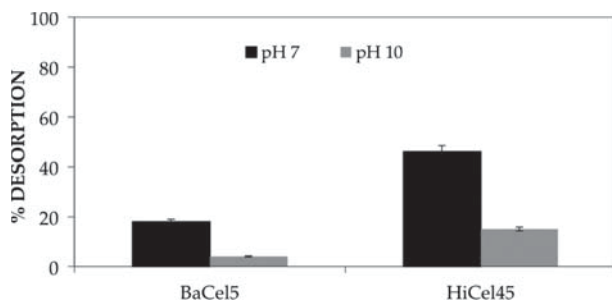


Figure 4. Percentage of protein desorption for HiCel45 and BaCel5. (150 mg protein/L; 40°C; 125 rpm; 1 h).

bind reversibly to cellulose because it showed low adsorption.

The adsorption of HiCel45 was very low at both pH values, suggesting that the action of this EG may be achieved via rapid adsorption/desorption and that its binding to cotton cellulose is highly reversible. Low amounts of protein were adsorbed on the fabric in the first stage of hydrolysis, and significant amounts were desorbed after the dilution step.

CBM from A types have a planar binding site architecture, which is thought to be complementary to the flat surfaces presented by cellulose fibres. Since it is known that CBMs play an important role in cellulase adsorption, this must define the interaction between the cotton surface and the enzyme (Boraston et al. 2004).

The low adsorption levels suggest that the enzyme is acting on the more available cellulose without anchoring to the surface via their CBMs. Recently, CBMs from families 1 and 3 were shown to bind preferentially to the obtuse corners of *Valonia* cellulose microcrystals, which expose the hydrophobic face (Boraston et al. 2007).

With BaCel5, high percentages of adsorption were observed at pH 7 and 10 (33% and 23% respectively). After dilution, about 18% and 4% was desorbed at the respective pH values; therefore, this bacterial enzyme seems to have high levels of adsorption with low reversibility. The adsorption characteristics shown by BaCel5 are consistent with its type B CBM family. This interacts with single polysaccharide chains, binding these substrates for the cognate catalytic module of the enzyme. The depth of these binding sites can vary to accommodate the entire width of a pyranose ring (Boraston et al. 2004). Boraston et al. (Boraston et al. 2003) demonstrated that CBMs 17 and 28 recognised different regions of non-crystalline cellulose, which influences the ability of the enzyme to hydrolyse the polysaccharide (Boraston et al. 2003). By comparison, bacterial cellulase (CenA from *Cellulomonas fimi*) adsorption on crystalline cellulose has been shown to be rapid with no detectable protein desorption

observed over several hours (Boraston et al. 1998; Gilkes et al. 1991; Jervis et al. 1997).

Individual hydrogen bonds are presumed to be weak enough to permit enzyme diffusion across the surface, but an ensemble of bonds maintains the protein at a fixed position. It has been proposed that a CBM–cellulose complex is formed when a number of the hydrophobic residues along the CBM binding face make sufficient contact to dehydrate both the binding face and the underlying sorbent. Dehydration of the interface facilitates the formation of hydrogen-bonds between the protein and the cellulose surface (Gilkes et al. 1991; Jervis et al. 1997).

The irreversible cotton–enzyme interaction requires better understanding because it can negatively affect the enzyme for detergent purposes. High degrees of adsorption must be evaluated since the enzymatic process should only act during the cleaning process and be removed from the fibre surface, after washing (Coutinho et al. 1993; Gilkes et al. 1993). Accumulation of the protein on the fibre surface after washing could occur due to the high degree of adsorption.

#### *Effect of mechanical agitation on cotton cellulose*

The enzymatic degradation of solid cellulose is a complex process that takes place at a solid–liquid interface where the enzymes are the mobile components (Zhang & Lynd 2004). The effect of mechanical action during enzymatic treatments of cotton has been subject of numerous studies since it has implications for delivering the desired finishing effects (Azevedo et al. 2000; Cavaco-Paulo et al. 1996; Rousselle et al. 2003; Timpa & Ramey 1989). Therefore, the influence of different levels of mechanical agitation on the action of HiCel45 and BaCel5 cellulases towards cotton was measured. Low levels of mechanical agitation were reproduced with orbital agitation in a shaker bath and high levels with vertical agitation in a laboratory washing machine using simulated western Europe washing conditions.

A balance between enzyme activity and mechanical agitation is required to achieve the desired effect on textile substrates (Silva et al. 2007). For detergent purposes, the desired anti-pilling benefits can be attained by degrading an appropriate amount of microfibrils protruding from the surface; however, excessive release of microfibrils can be assumed to be destructive. The enzymatic activity was measured as liberation of reducing sugar in the liquor bath and as cuts produced on the fabric surface (reducing sugar on fabric).

Using HiCel45 cellulase, reducing sugar released in the bath treatment increased with higher mechanical agitation. Figures 5 and 6 show that there was

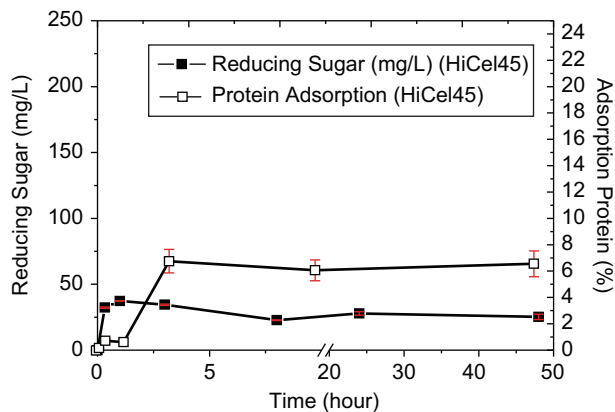


Figure 5. Influence of orbital agitation of HiCel45 and BaCel5 on cotton fabric. Reducing sugar production in the liquid bath and adsorbed protein percentage in the incubation liquor for different incubation periods. (300 mg protein/L; pH 7; 40°C; 125 rpm).

no significant release of reducing sugar, even after 48 h treatment, using orbital agitation. For enzyme adsorption on the cotton fibre, 8 h of treatment was required to observe adsorbed protein. On the other hand, higher amounts of hydrolytic products were

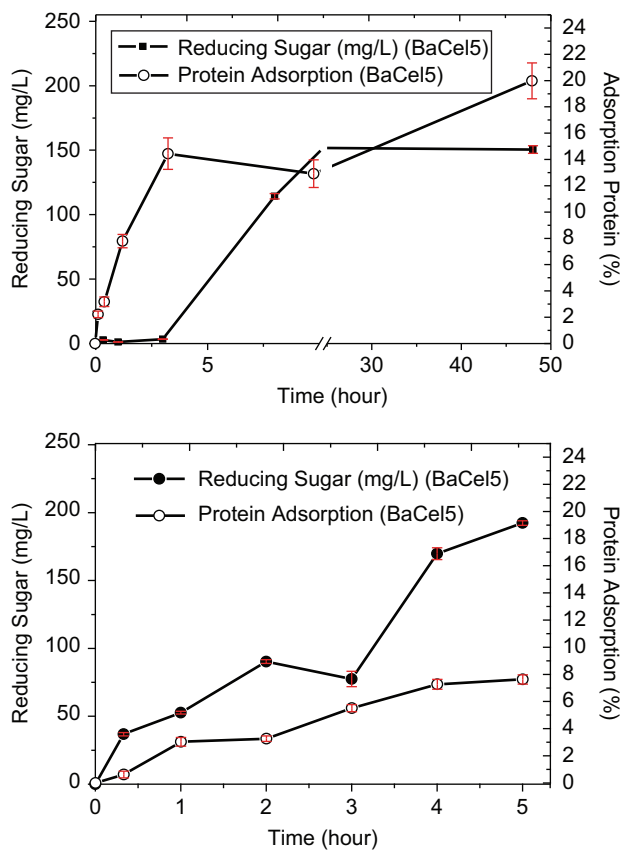


Figure 6. Influence of vertical agitation of HiCel45 and BaCel5 on cotton fabric. Reducing sugar production in the liquid bath and adsorbed protein in the incubation for different incubation periods. (300 mg protein/L; pH 7; 40°C; 40 rpm).

found when vertical agitation was used, with significant amounts of reducing sugars evident after 2 h of treatment. Production increased to almost 250 mg/L at 5 h of treatment.

Figures 7 and 8 show the reducing end groups formed during hydrolysis with HiCel45. Similar amounts of reducing ends were generated for both levels of mechanical agitation after 20 min and 3 h, whereas a maximum of 21–23 mg/g was exhibited at 5 and 48 h of vertical and orbital treatment, respectively. After 20 min, about 11 mg per gram of fabric of reducing ends was produced with both orbital and vertical agitation. By comparison, the measured concentration of reducing sugars solubilized in the liquor bath was only 32 mg/L by using orbital agitation and 7 mg/L using vertical agitation.

These results clearly show that the increase in HiCel45 activity is highly influenced by agitation, with the beating effects promoting enzyme action. Higher mechanical agitation can enhance EG activity by increasing the availability of sites for cellulase adsorption (Cavaco-Paulo 1998). The accessible surface area of cellulose is one of the most important factors in determining initial rates of cellulase

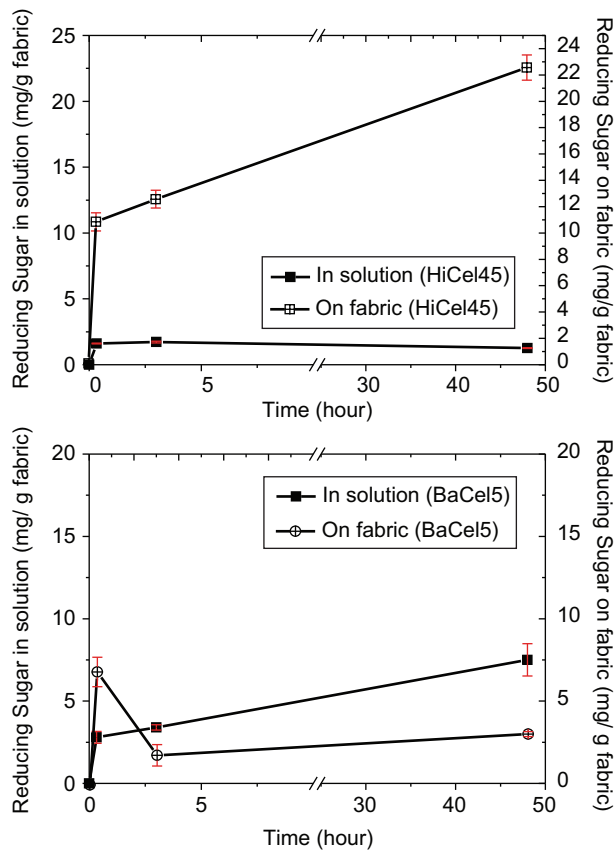


Figure 7. Increase in reducing sugar released in the liquor and reducing ends on cotton fibres produced in orbital agitation by HiCel45 and BaCel5, for indicated incubation periods. (300 mg protein/L; pH 7; 40°C; 125 rpm).

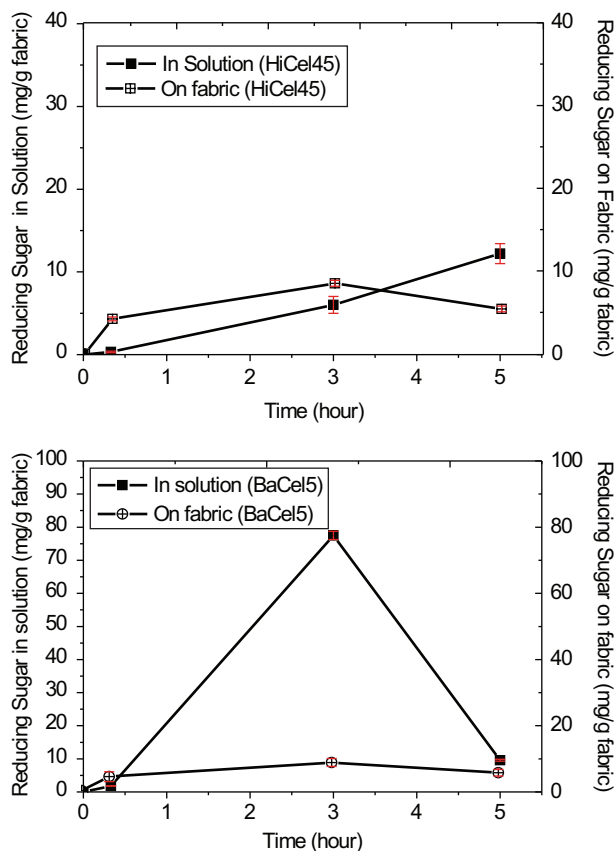


Figure 8. Increase in reducing sugar released in liquor and reducing ends on cotton fibres produced with vertical agitation by the HiCel45 and BaCel5, for indicated incubation periods, both in orbital (A) and vertical agitation (B). (300 mg protein/L; pH 7; 40°C; 40 rpm).

hydrolysis. Mechanical action on these fibres is expected to release protruding fibrils providing more sites on the fibre for enzymatic attack. The loose fibrils present an increased and more exposed area for HiCel45 to be adsorbed, but without solubilizing significant amounts of hydrolysis product into the liquor bath. At that stage of the reaction, HiCel45 might be able to produce cuts on the fabric surface. An increase in reducing sugar production occurred using vertical mechanical agitation.

In the case of BaCel5, Figures 5 and 6 show that more hydrolysis products were obtained (192 mg/L) when vertical agitation was used. Only 20 min of treatment were necessary to produce 37 mg/L of hydrolysis products. For adsorbed protein, although lower adsorption rates were found after 5 h of treatment for vertical agitation, significant amounts of reducing sugar were produced after 4 h of treatment. When orbital agitation was used, 8 h of treatment were required to obtain about 150 mg/L of hydrolysis products, accompanied by significant amounts of adsorbed protein.

The quantification of reducing ends formed on cotton fibre has been used to study the first step in cellulose hydrolysis, as soluble hydrolytic products are difficult to detect. Quantification is based on the reaction of neocuproine with terminal reducing end groups on the surface of treated fabrics. BaCel5 (Figures 7 and 8) exhibited a maximum reducing end concentration on cotton (7 mg/g) after 20 min of enzymatic treatment, but this value was lower after 3 h and 48 h samples (2 and 3 mg/g), when orbital agitation was used. These results confirm that BaCel5 is able to produce reducing ends on the fabric surface even if no significant amounts of reducing sugars were attained in the liquor, in the initial period of incubation. After 8 h incubation, the free cellulose chains were measured as reducing sugar released on the liquor treatment. In principle, the initiation of hydrolysis might be expected when protein adsorption occurs. However, the amount of hydrolysis product depends on specific enzyme characteristics and interaction with the substrate. Hydrolysis products were found when the adsorption reached at least 10% (calculated from Figure 6). After 3 h of enzymatic treatment, 9 mg/g of reducing ends were found, whereas lower values were found for 0.3 h and 5 h samples (4 and 5 mg/g). Reducing sugar released into the liquor increased with treatment time (2 and 9 mg/g, respectively), when a maximum of 8% of protein adsorbed was reached. Vertical agitation using the Rotawash machine was much more effective for hydrolysis (reducing sugar solubilisation) than the lower agitation level (orbital agitation), producing about 37 mg/L at 20 min of enzymatic treatment (Figure 6). This might be due to the beating action of this type of vertical agitation, promoting protein adsorption on the fibre surface.

It is clear that in short treatments, BaCel5 is only able to achieve hydrolysis on the fabric surface; in longer treatments, the enzyme and mechanical agitation action promote release of hydrolysis products into the liquor bath. Therefore, BaCel5 initially promotes the production of reducing ends on the surface, which then makes these exposed microfibrils accessible for solubilisation. Higher levels of mechanical agitation should enhance the EG activity by increasing the availability of the sites for EG adsorption. Mechanical agitation causes more fibrillation and improves access to the fibre surface within the fabric structure.

Figures 9 and 10 show that the surface morphology of cotton fibres physically changed during the enzymatic treatment. Control fabrics, shown in Figures 9 and 10 A and B, revealed the rather smooth surface of raw cotton fibres with characteristic parallel ridges and grooves. With BaCel5, no damage is evident on the cotton



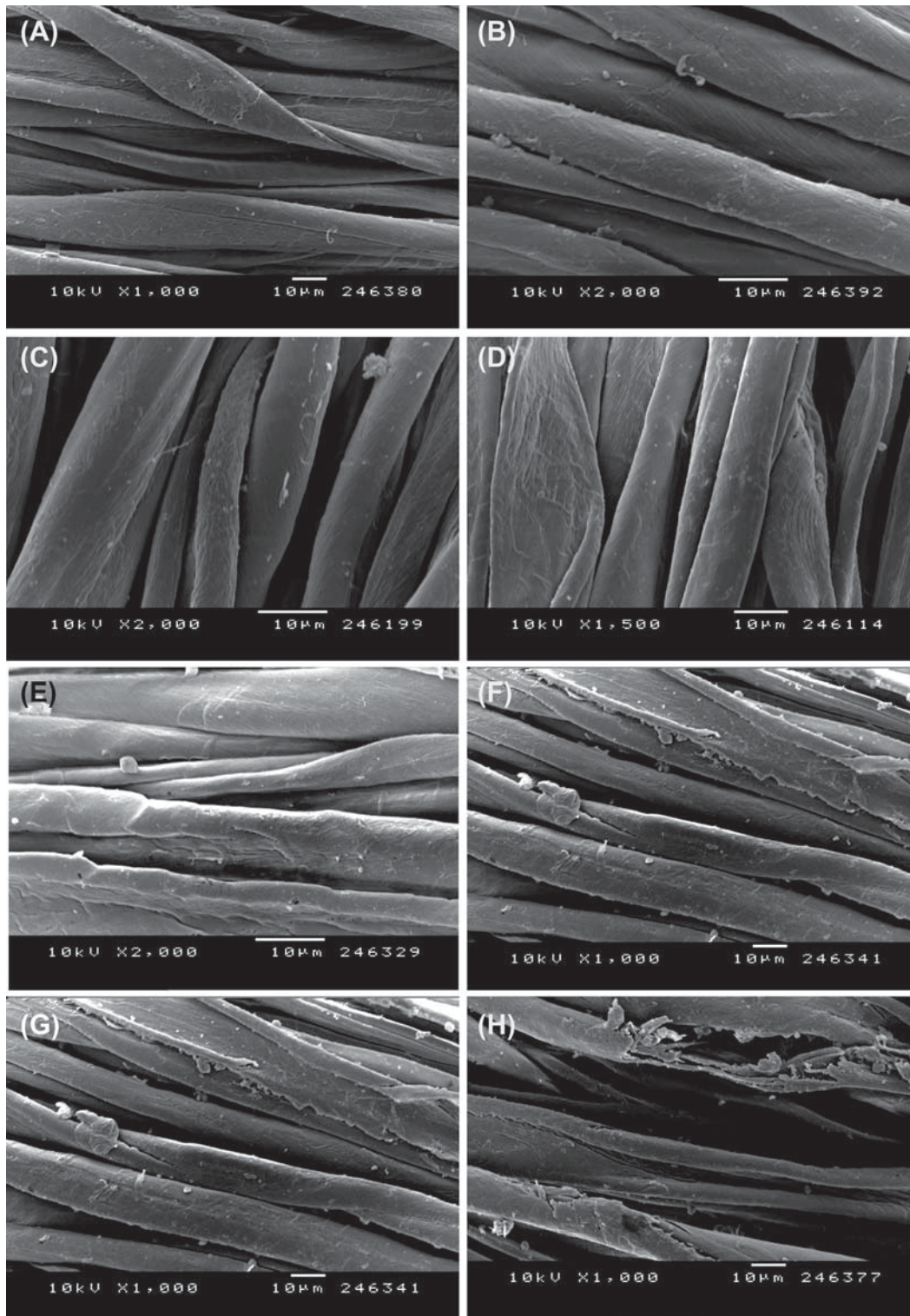


Figure 9. SEM photographs of cotton fabrics for HiCel45 treatments under the following conditions. (A) control for orbital and (B) control vertical agitation, untreated samples; 20 min treated in (C) orbital and (D) vertical agitation; 3 h treated in (E) orbital and (F) vertical agitation; treated for (G) 48 h in orbital and (H) 5 h in vertical agitation. (300 mg protein/L; 40°C; 125 and 40 rpm, respectively).

fabric, while HiCel45 treatment produced holes after 3 h, when vertical agitation was applied (data not shown).

The hydrolytic reaction with HiCel45 caused some changes in cotton surface appearance. The surfaces of specimens subjected to at least 3 h of



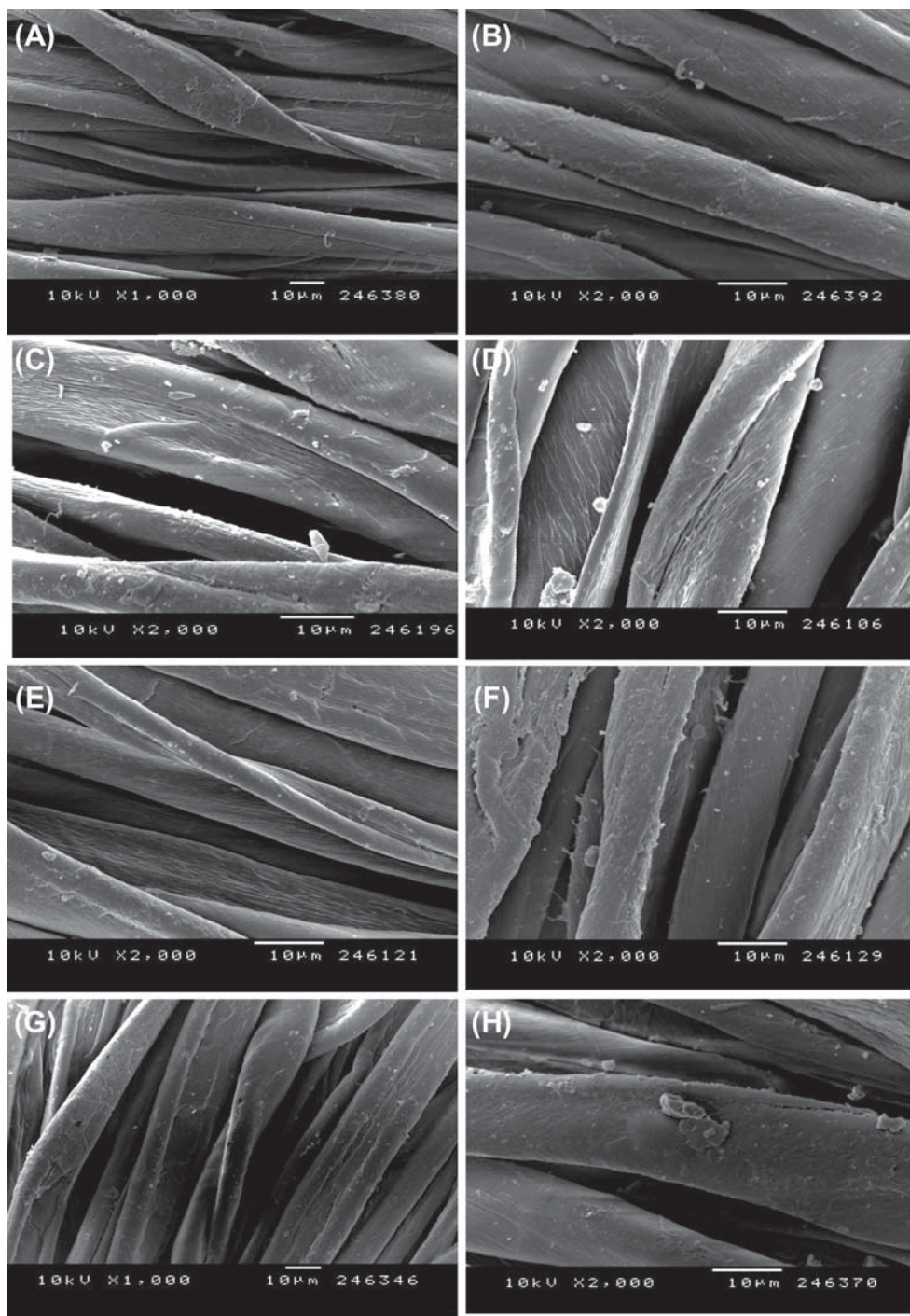


Figure 10. SEM photographs of cotton fabrics: untreated samples (controls) for (A) orbital and (B) vertical agitation; samples treated with BaCel5 for 20 min with (C) orbital and (D) vertical agitation; samples treated with BaCel5 for 3 h with (E) orbital and (F) vertical agitation; samples treated with BaCel5 (G) for 48 h with orbital agitation and (H) for 5 h with vertical agitation. (300 mg protein/L; 40°C; 125 and 40 rpm, respectively).

reaction exhibited polished faces with shallow cavities, using both mechanical agitation forces (Figures 9C and D). However, a 5-hour treatment polished the surface of cotton fibre due to the high-vertical mechanical agitation, which indicated heavy hydrolysis of cotton cellulose (Figure 9H); while a slight

fibrillation of the cotton surface was visible for samples treated for 48 h using orbital agitation (Figure 9G). This implied that the 5-hour reaction with HiCel45 cellulase under the high-vertical agitation conditions used in this study was long enough to cause the hydrolysis of the main cotton body.

Moderate reducing sugar production during 48 h of treatment with low agitation correlated with slight differences on the cotton surface. However, under high agitation, HiCel45 showed high reducing sugar production after 5 h of treatment, which correlates with significant levels of cotton degradation. It is clear that HiCel45 must be limited to conditions producing low degradation rates for detergent application, in order to avoid the destructive impact of fibres.

The hydrolytic reaction with BaCel5 caused changes in morphology of the cotton surface appearance. In the case of orbital mechanical agitation, smooth surfaces were present after 3 h of treatment but polished faces were seen at 48 h (Figure 10C, E G). Using vertical mechanical agitation a slight fibrillation of the cotton surface was seen after 20 min of treatment. The fibres treated for 5 h became less fibrilled and more polished than those treated for 3 h (Figure 10D, F, H). The change of relative fibrillation levels with time of treatment could be explained assuming that the polymer chains are released into solution soon after being detached from the surface of the fibre.

The hydrolysis process on cotton might be controlled by using cellulases under appropriate conditions. BaCel5 releases reducing sugar from the fibre surface at low rates after a certain period of time, mainly from the end terminals on the surface. Fabric damage can be easily avoided by controlling the EG activity, a process, which is highly related with the agitation forces. HiCel45 generates cuts on the surface and, sequentially, releases oligomeric degradation products in the liquor bath. Although high rates of cotton degradation are considered as beneficial for some applications, low rates are required for detergent purposes. Depilling can release dirt from fabrics and produce other benefits (i.e. brightness, softness) but higher levels of those effects can be destructive.

## Conclusions

HiCel45 seems to be an enzyme more fitted for high mechanical agitation processes in detergency while BaCel5 seems to be an enzyme more fitted for milder agitation processes. Both enzymes have been built from a library of cellulase catalytic domain and cellulose binding modules with known properties, leading to improved hybrid clones with dissimilar properties. This is an example how genetic engineering and nature can provide solutions for industry and in particular for detergency.

**Declaration of interest:** The author report no conflicts of interest, The authors alone are responsible for the content and writing of the article.

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