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BIOTECHNOLOGY AND INDUSTRIAL MICROBIOLOGY - RESEARCH PAPER

Biopolishing of denim by the recombinant xylanase II *of Caulobacter crescentus*

Débora Jacomini[1] · Larissa Bussler[2] · José Luis da-Conceição Silva[1] · Alexandre Maller[1] · Marina Kimiko Kadowaki[1] · Rita de Cássia Garcia Simão[1]

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Abstract

Denim, also known as jeans, is a fabric made up of braided cotton threads dyed indigo blue, whose fibers contain approximately 10% of non-cellulosic impurities that reduce its commercial value. Microbial enzymes can act in the cleaning and desizing processes of jeans, improving their color, softness, and covering capacity. The recombinant Xylanase II (XynA2) from the aquatic bacterial *Caulobacter crescentus* (*C. crescentus*), previously characterized in terms of its biochemical features, was applied to the biotreatment of jeans to clean and degum it. The biotreatment performance was evaluated in terms of tissue weight loss, amount of reducing sugars released and analysis of the images obtained by scanning electron microscopy (SEM). Biotreated tissues, at 12 and 24 h, showed a dry weight loss of 4.9 and 6.6%, respectively. The reducing sugars amount released after XynA2 action over the jean's fibers showed statistically significant values when compared with each other and with their respective controls. SEM images clearly shown that the fabric treated for 12 h presented a smooth and polished surface, while the fabric treated for 24 h showed the cotton fibers broken, displaying severe damage to the textile. The best treatment for the jeans was in the presence of 1 U mg^{-1} XynA2 at pH 8 and 60 °C during 12 h. In conclusion, XynA2 of *C. crescentus* was satisfactorily applied for the biopolishing of denim jeans being a more sustainable alternative to the use of chemical and abrasive processes to obtain the same effects.

Keywords Textile applications · Environmental sustainability · Jeans · White biotechnology · Ecological fabric

Introduction

Bioprocessing can be defined as the application of living organisms, their components and/or their products in industrial processes [1]. Among these options, microbial enzymes are attractive due to the development of enzyme technology, which makes them the method of choice for many bioprocesses [2, 3]. The biopolishing of denim jeans refers to a process where enzymes are used to polish the fabric and achieve wear and softening effects. In this process, natural enzymes, usually obtained from microorganisms such as bacteria, are applied to the denim fabric and help break down the fabric's fibers, softening the fabric and creating fraying effects such as fading and streaking. In addition to providing an esthetic appearance, the biopolishing process can also improve comfort and fabric quality [1–3].

The textile industry uses about 75 enzymes of the 7,000 known and made available for biotreatments, the main ones being hydrolases and oxidoreductases [4]. The group of hydrolytic enzymes includes: cellulase, xylanase, amylase, laccase, protease, lipase, esterase, and pectinase, which act directly on native cotton fibers [2, 5]. Xylanases are considered one of the most important microbial enzymes industrially, whose action is essential in the processes of cleaning and degumming (desizing) of denim, a fabric popularly known as jeans [6].

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Débora Jacomini and Larissa Bussler contributed equally to this work.

✉ Rita de Cássia Garcia Simão
rita.simao@unioeste.br

[1] Centro de Ciências Médicas e Farmacêuticas - CCMF, Laboratório de Bioquímica Molecular – LaBioqMol, Universidade Estadual do Oeste do Paraná- UNIOESTE, Rua Universitária, 2069 -, Cascavel, PR 85814-110, Brazil

[2] Centro de Ciências Exatas e Tecnológicas, Laboratório de Bioquímica Molecular– LaBioqMol, Universidade Estadual do Oeste do Paraná, Paraná, Cascavel, Brazil

Denim jeans consist of braided cotton threads and subsequently dyed in indigo blue, whose fibers contain about 10% of non-cellulosic impurities, such as fragments of seed coating, which are strongly linked to the fabric by microfibers hemicellulose [7]. The hydrolysis of these tiny fibers, located in the outer layers of the cuticle and primary cotton wall, keeps the fabric looking new after repeated washes, as it improves its color, smoothness, and coverage capacity [8], in addition to increasing its price market [9].

A relevant fact to consider in an acrylic fabric, including some kind of jeans, is the organization of the fibers, since these can directly affect the pilling property and the appearance of the fabric. Increasing the percentage of regular (non-shrinkable) fibers with high initial modulus in an acrylic fabric directly increases the tendency for these fibers to pull out of the yarn structure and appear on the fabric surface as pills [10].

The textile industries are currently looking for sources that produce xylanolytic enzymes with biotechnological potential, which are stable and have high yields [2]. The bacteria *C. crescentus* Gram-negative, aquatic, ubiquitous, and free-living [11] is an interesting source of these enzymes, as it has seven genes directly involved in the degradation of xylan [12–16]. Among these genes, *xynA2* (CCNA_03137) encodes a xylanase, XynA2 (EC 3.2.1.8) from the 10 family of Glycosyl Hydrolases (GH 10) [17], which acts efficiently on the internal links of the xylan chain of agricultural waste, and it presents promising action in industrial bioprocesses [18].

The use of enzymes with textile applications is an example of white industrial biotechnology, which allows the development of ecologically correct products, with a final quality superior to that of conventional processes [4, 19]. Thus, the objective of this study was to evaluate the enzymatic action of XynA2 of *C. crescentus* in the biotreatment of jeans, through weight loss, release of reducing sugar, and changes in the morphology of the cotton fibers of this fabric.

Materials and methods

Enzyme

The enzyme used was Xylanase II (XynA2) from *C. crescentus* that belongs to the family 10 of Glico-Hydrolases (GH 10) [18]. XynA2 has optimum alkaline pH (8), high optimum temperature (60 °C), thermoresistance at 65 °C and stability at slightly alkaline pH when using 1% beechwood xylan as substrate. In addition, the pure recombinant XynA2 is activated by the cofactor Dithiothreitol (DTT) at 5 mM and presents the follow biochemical features: V_{Max} (maximum velocity) = 54.64 µmol. min^{-1}; K_M (Michaelis-Menten constant) = 5.78 µmol mL^{-1}; and K_{Cat}/K_M (catalytic efficiency) = 1.63 U s^{-1} [18].

Biotreatment of denim fabric

The fabric jeans used has a cotton (100%) composition in both the warp (longitudinal threads) and the weft (transverse threads) in a 3X1 pattern without adding polyester or elastane. The warp is dark indigo blue, and the weft is white. Fabrics were sized in squares (1 × 1 cm) for all the biotreatment tests. For the denim biopolishing process, fabric squares (1 × 1 cm) were immersed in an enzymatic solution containing 1 U mg^{-1} of pure XynA2 (in the absence of dithiothreitol) in sodium phosphate buffer (50 mM pH 8) at 60 °C during 12–24 h. Right away, the tissues were washed twice in boiling distilled water, followed by a wash with cold water to inactivate the enzyme XynA2. Finally, the denim was dried at room temperature for up to 48 h and its weight measured. The control treatments were carried out in the same way, without the addition of the purified XynA2 [20].

Dry weight loss of tissue treated with XynA2

The weight loss of the tissue was determined by gravimetry [21], after drying. Percentage weight loss was determined by Equation 1 [22, 23]:

$$PP\ \% = \frac{P_1 - P_2}{P_1} \times 100$$

Equation 1. Calculation to define the weight loss of denim fabric (PP%), in which P1 and P2 are the dry weights of the tissue before and after treatment, respectively.

Release of reducing sugars from tissue treated with XynA2

The supernatant released after denim biotreatment was quantitatively tested for reducing sugars [7], using the Miller method that uses 3,5-dinitrosalicylic acid (DNS) as a reagent [24].

Scanning electron microscopy (SEM)

The jeans samples treated and not treated with XynA2 from *C. crescentus* were placed in a sample holder (stub) on a double-sided carbon tape and subsequently dried for 24 h in an electric oven at 70 °C and metallized (spray coating) with a thin layer (5nm) of gold on the surface (sputtering). The scanning electron microscope (TESCAN® equipment, model VEGA^3) used has an EDS

system (Energy Dispersive System) coupled, which makes it possible to determine the qualitative and semi-quantitative chemical composition of the samples from the emission of characteristic X-rays. The micrographs were obtained at different magnifications, in secondary electronic mode and the chemical compositions of the microregions of the samples were determined by EDS using an X-ray detector (model X-Act, Oxford Instruments).

The analysis was carried out at the Federal University of Paraná (UFPR, Palotina). The micrographs were obtained in several magnifications (2 and ×5) in module SE (secondary electron) with secondary electron detector. The SEM was used to obtain information about the morphology and fiber structure of denim fabric treated and not treated with *C. crescentus* XynA2.

Statistical analysis

The tests were performed in triplicate, the results of which were subjected to analysis of variance (ANOVA) with Tukey's post hoc test, with 95% significance [25].

Results

Effect of XynA2 on weight loss and reducing sugar release from denim fabric

The percentage of dry tissue weight loss in the different treatments can be seen in Table 1. The denim treated with the XynA2 enzyme, at 12 and 24 h, presented a weight loss higher than that obtained for the controls at the same reaction time, statistically different values $p<0.05$. When comparing the weight reduction of the enzymatic treatments, in the different reaction times, it is observed that the values were also statistically different ($p<0.05$), and that in the time of 24 h, the tissue lost more than 6% of dry weight.

Table 1 Effect of biotreatment using XynA2 (recombinant Xylanase II) on the release of reducing sugar and on the dry weight loss of denim fabric

Samples	Incubation time (h)	Weight loss (%)	Reducing sugar (μmol mL^{-1})
Control	12	2.28^{a*}	1.50^{a}
Control	24	3.17^{b}	2.25^{b}
Jeans + XynA2	12	4.96^{c}	2.85^{c}
Jeans + XynA2	24	6.64^{d}	5.01^{d}

*Different letters indicate weight loss and release of reducing sugar statistically different between samples according to Tukey's post hoc test ($p<0.05$)

The reducing sugars released, after the hydrolysis of the cotton microfibrils of the denim fabric by the action of XynA2, showed statistically different values, when compared with each other and with their respective controls, $p<0.05$ (Table 1). The two control treatments also released significantly different amounts of reducing sugars, $p<0.05$.

Effect of XynA2 on the morphology of denim fabric

The SEM revealed morphological changes in the fiber of the denim fabric after the enzymatic action of XynA2. The untreated denim fibers in the 12 and 24 h reaction times showed impurities in the cotton fibers, as observed in the circles of Fig. 1a and c, respectively. The denim treated for 12 h with XynA2 (Fig. 1b) showed no impurities and hemicellulose threads between the cotton fibers, generating a smooth and polished surface, with a smooth appearance. After 24 h enzymatic treatment with XynA2, the cotton fibers of the fabric were degraded and broken by excessive xylanolytic hydrolysis, causing severe damage to the fabric (Fig. 1d).

In addition, in none of the images analyzed, there was fading or detonation in the treated and untreated tissue.

Discussion

All tests with the denim fabric were done in a stationary system. According to Esfandiari et al. (2014) the agitated system causes significant degradation in the cotton fiber [9]. These hydrolyzed fibers are fragile, and the mechanical treatment is abrupt, which can destroy the surface of the fabric, decrease its strength, in addition to increasing industrial costs [6]. Thus, the fabric submitted to a mechanical treatment is not suitable for luxury consumption, as its appearance is neither excellent nor shiny [9].

The enzymatic reactions were performed at an alkaline buffer (pH 8) and a temperature of 60 °C. The slightly basic pH associated with the elevated temperature has a positive effect on weight loss and the release of reducing sugars from the tissue [7]. Alkaline solutions facilitate the penetration of xylanase into cotton fibers, enhancing the hydrolysis of the non-cellulosic material of the fabric [5]. In addition, temperatures above 50 °C increase the reaction rate, due to the increase in the kinetic energy of the molecules, allowing the enzymes to come into more frequent contact with the tissue [22].

The enzymatic degradation of cotton is generally characterized by the loss of dry tissue weight [5], which cannot exceed 3 to 5%. High percentages such as that obtained in the 24-h treatment with XynA2 (6.64%) indicate severe damage to the cotton fiber, which reflects in a low quality of the fabric, decreasing its commercial value [6, 7]. On the other

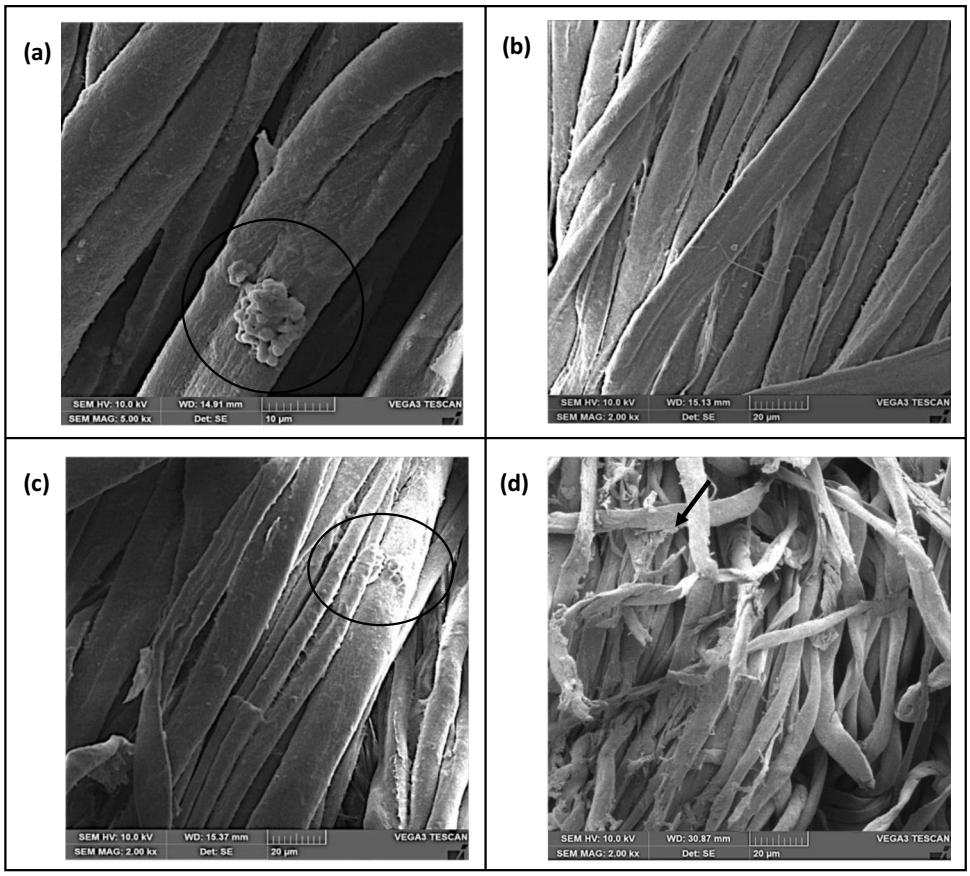

Fig. 1 Scanning electron microscopy of denim fabrics treated in the presence and absence of the recombinant XynA2 from *C. crescentus*. **a** and **c** Denim fabrics treated with sodium phosphate buffer (50 mM, pH 8) at 60 °C, in the order of 12 and 24 h of reaction (controls). **b** and **d** Denim fabrics treated with 1 U mL $^{-1}$ of XynA2 in phosphate buffer (50 mM, pH 8) at 60 °C during 12 and 24 h, respectively. In the circles, impurities adhered to the fabric are highlighted and the degradation of the denim cotton fibers is indicated (arrow)

hand, weight losses of less than 5%, such as that found in the 12-h enzymatic treatment, suggest that, mainly, surface fibers, microfibers, fragments of seed coating, extractable materials with water, waxy substances, gums, and other non-cellulosic impurities were significantly removed, improving the final quality of the fabric [7, 22].

The value of dry tissue weight loss and release of reducing sugars increased with increasing tissue treatment time in the presence of the XynA2 enzyme; similar results were found in other studies [7, 22]. Thus, it is suggested that there is a direct relationship between tissue weight loss and the amount of reducing sugars released in the supernatant [5], whereas while weight reduction characterizes the overall effect of fiber degradation by XynA2, the release of reducing sugars reveals the effectiveness of the enzymatic action [7].

The comparison of Figs. 1a (control) and 1b (treatment) clearly shows the action of XynA2 in cleaning the denim, after 12 h of reaction. Cotton fibers have become smoother and cleaner, reflecting the positive effect of enzymatic treatment in removing impurities deposited on the fabric [10]. In addition, it is suggested that this treated fiber presents an increase in several physical properties, such as whiteness, tensile strength, softness, and smoothness, increasing its commercial value [5].

The 24-h treatment caused severe damage to cellulosic fibers when compared to its control. Similar results have also been obtained by other authors [5, 10, 22], who

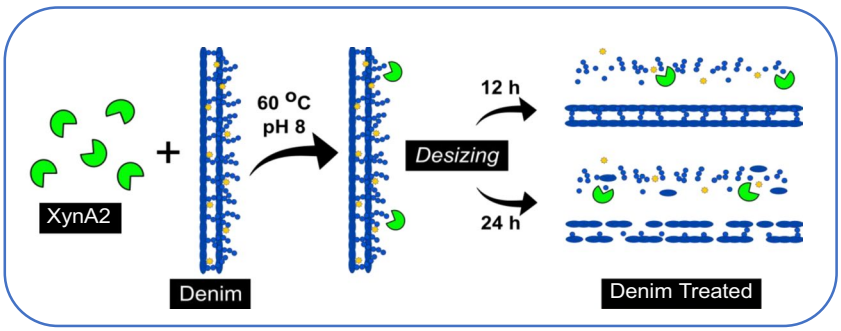

Fig. 2 Biopolishing of jeans by the action of recombinant XynA2 from *C. crescentus*

claim that high enzymatic loads and / or long treatment times can lead to cracks or cavities in the cotton weave, causing ruptures and loss of fabric strength. In a positive way, the tissues treated with XynA2 showed no change in staining, however other studies stated that the treatment with xylanases slightly reduces the intensity of the blue color, due to the partial removal of dye fragments during the enzymatic removal of fibrils from the surface of the cotton [10].

In the present study, the best treatment for denim fabric was at 12 h (Fig. 1c). Aly et al. (2004) also found similar times, and considered them adequate, since each enzyme acts in a different way, due to its kinetic parameters [22]. This bioprocess was able to hydrolyze the impurities in the cotton fibers, making them more accessible to chemicals during the final stages of jeans processing, such as bleaching and dyeing. In addition, the enzymatic treatment improved the quality of the final textile product [6, 8] and helped preserve the environment, by avoiding the production of chemical residues [19, 26].

Conclusion

In the present work, a direct relationship between the weight loss of the tissues analyzed and the amount of reducing sugars released after the action of XynA2 from *C. crescentus* was shown, since the value of the dry weight loss of the tissues and the release of reducing sugars increased in parallel with the treatment time. Scanning electron microscopy analyzes revealed that the most satisfactory treatment for the jeans was for 12 h using 1 U mg^{-1} of XynA2 at pH 8 at 60 °C. Cotton fibers became smoother and cleaner without loss of color, reflecting the positive effect of the enzymatic treatment to removing impurities deposited on the fabric. However, prolonged times (24 h) of XynA2 action on jeans can cause severe damage to the cotton fiber (Fig. 2). The use of XynA2 in textile industrial processes enables products with excellent final quality and the development of advantageous and ecologically correct actions face to environmental sustainability goals agreed between different countries in the globe for the coming decades.

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Data Availability The authors declare that the data obtained in this work are available to anyone interested and statement that all scientific data published will be used exclusively for the services provided by this publication, not being available for other purposes or to third parties.

Declarations

Conflict of interest The authors declare no competing interests.

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