

Technology for Denim Finishing Using Laccase Enzymes: A Review

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Abstract: *Enzymatic technologies applied to the textile industry are viable in environmental parameters. Laccases and peroxidases are of special interest because they are able to initiate oxidation of indigo in the finishing process of jeans. Only two authors have applied these enzymes to denim fading, although there are studies that prove the bioactivity of the microorganisms and their metabolites on the indigo dye. Therefore, inefficient scaling limits the implementing of laccases to the production process of jeans.*

Keywords: *denim, finishing, laccase enzymes*

I. Introduction

Today, the textile industry is made up of different, though interrelated, subsectors, which produce everything from fibers to home fashion assortments. Each subsector may be considered a separate industry [1]. The textile industry in Ecuador has a high socioeconomic impact, as it generates 50,000 direct and 200,000 indirect jobs. It is the sector that employs the second largest number of people, after only the food, beverages and tobacco sector. The provinces with the largest number of businesses dedicated to this activity are: Pichincha, Imbabura, Tungurahua, Azuay y Guayas [2].

The textile industry's production is classified in three categories: cellulose fibers (cotton, rayon, linen, ramie, hemp, and lyocell), protein fibers (wool, angora, mohair, cashmere, and silk), and synthetic fibers (polyester, nylon, spandex, acetate, acrylic, Ingeo, and polypropylene) (Ghaly, [3]). The various stages in each category employ a wide variety of dyes and other chemicals such as acids, bases, salts, wetting agents, fixing agents, degumming agents, among others, whose residues are discharged as wastewater, negatively impacting the environment [1], [4], [2], [5]. The types of dyes and chemicals vary according to the fabric to be manufactured. Some of the dyes used to color cellulose fibers are reactive dyes (Remazol, Procion MX, Cibacron F), direct dyes (Congo Red, Direct Yellow 50, Direct Brown 116), naphthol dyes (fast yellow GC, fast scarlet R, fast blue B), and indigo dyes (indigo white, Tyrian purple, indigo carmine) [6], [7]. Indigo dyes are commonly used to dye jeans and denim derivatives, with a yearly production of 80,000 tons [8], which corresponds to about 11% of the global market for textile dyes [3].

Jeans and denim products are generally subjected to an ageing process, which consists of stone-washing the fabric, and fading of the indigo color [9]. The conventional fading processes generally employ chemicals such as chlorine and hydrogen peroxide, which become persistent contaminants when discharged as wastewater into water bodies. Modern textile treatments offer alternatives for fabric fading through the use of enzymes, which are environmentally friendly (as they are biodegradable) and act on specific molecules [1]. However, the use of this enzymatic technology is not very widespread in the industry. It is therefore necessary to analyze the sector in detail to identify the limitations that prevent its large-scale implementation. The objective of this study is to describe progress made in enzymatic engineering applied to textile fading through a broad bibliographic search that will uncover technological limitations that prevent its implementation by the industry.

Denim

Jeans are a universal, timeless and fashionable garment, worn by most people, without distinction by gender, age, or socioeconomic status. Its not only a textile product, but a social phenomenon which has withstood social revolutions, economic crises and world wars. Furthermore, it has different connotations, from being a symbol of worker exploitation or of hippie protest, through a fetish of the fashion industry, to being the

most accessible, basic and common clothing item in all societies. Jeans are made from a sturdy cotton textile called denim [10]. Denim was initially used to manufacture camping tents and wagon covers. Levi Strauss and his brother-in-law opened their first clothing store in 1853, where they started to sell denim trousers made to order. At first, jeans were simple and brown in color; subsequently, blue jeans were introduced and rivets were added in the areas of greatest stress. By 1873 they received patent N° 139.121, giving birth then to the first brand of jeans, Levi Strauss & Co. From then on, jeans' production technologies, styles (skinny jeans, bootcuts, and so forth), and colors continued to be developed. This fabric then started to be used to make other garments such as jackets, hats, and blouses [11], [12].

Indigo dyes

Indigo (2,2'-bis-indigo), (CI Vat Blue 1) or vat indigo, with a chemical formula of $C_{16}H_{10}O_2N_2$, shown on figure 1, is a crystalline, dark blue powder [13], [14], whose melting point is between 390 and 392°C. It is soluble in chloroform, nitrobenzene, and concentrated sulphuric acid, producing a blue solution, but it is insoluble in water, alcohol or ether. It behaves as a monomer [15]. Together with its sulfonated derivatives, it accounts for 31% of the global market for industrial dyes [16]. It has an aromatic structure with aryl rings, which have displaced electrons capable of absorbing electromagnetic radiation of different wavelengths. This is responsible for indigo's characteristic coloration. These aryl rings are called chromophores, and consist of a simple double bond between carbons replaced by two N-H donors and two C-O acceptors [17], [18].

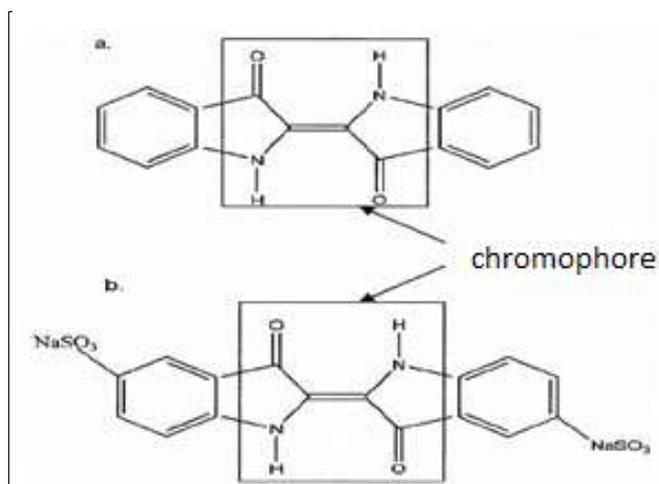


Figure 1. (a) Indigo molecule and (b) indigo carmine molecule [18]

Natural indigo is obtained from the sap of the *Indigofera tinctoria* shrub. Commercial production of synthetic indigo began in 1897 with phthalic anhydride as the starting material. In 1901, however, aniline began to be used as the raw material, as it was more economical. The initial process to obtain indigo from aniline was to make the latter react with chloroacetic acid at a temperature of 100°C to form N-phenylglycine. By 1920, the approach was changed, having aniline react instead with formaldehyde (CH_2O), hydrogen cyanide (HCN), caustic alkali (KOH/NaOH), and water [19]. The N-phenylglycine fuses with sodamide ($NaNH_2$) and caustic alkali (KOH/NaOH) at 220°C during 5-6 hours in an inert atmosphere to form indoxyl. Finally, indoxyl oxidizes in air at 80-90°C to form indigo, which is subsequently purified [18]. When indigo is sulfonated, indigo carmine is produced (see figure 1), which is another widely used vat dye [20].

Mechanisms to fix indigo to fabrics

Indigo is fixed to fabrics during dyeing through a complex reduction-oxidation reaction due to its insolubility in water and its non-affinity to cellulose fibers. It can be reduced by agents such as sodium dithionite ($Na_2S_2O_4$), hydroxyacetone, hydrogen, or by means of electro-chemical methods in a high alkaline medium (pH 11-14) [21]. The reducing agent donates hydrogens to extract the oxygen or to add electrons to indigo, thus becoming oxidized. The reduced indigo, as anion leuco enolate, dissolves in water and the solution becomes clearer; its affinity for cellulose increases, and penetrates the gaps in the textile's fibers. When the textile dries up, indigo is oxidized by the air's oxygen, and returns to its insoluble form, but without separating from the fibers due to the mechanical bonds that were established. Only the dye that was not fixed will be dislodged [18]. After the dyeing, an oxidation reaction with hydrogen peroxide or atmospheric oxygen is carried out under a high pH to remove the excess of reactants. After the oxidation, vat dyes are subjected to a thermal treatment in an alkaline solution supplied by detergents in order to obtain the final textile, as shown in figure 2 [21].

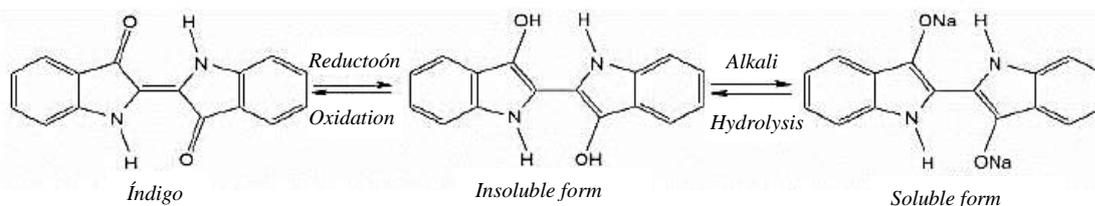


Figure 2. Indigo's redox reaction in the textile dyeing process[21].

Indigo degradation in denim

Innovative decolorization methods have been reported such as the use of low-temperature plasmas and cathodes in magnetic fields[9], [22]; contact with an oxidizing gas or vapor in the presence of humidity [23]; wash cycles using a detergent and an emulsifier, clarified with water, chlorine bleaching and a fabric softener of the quaternary-ammonium type, clarified, and with softener added [24]; contact with a solution of a reducing agent or immobilized on absorbent stones[25]; immersion in hypochlorous acid [26]; wash cycles with potassium permanganate, clarified with water, and neutralization with a monodentate or polydentate chelating agent of carboxylic acid or salt or a combination of them with hydrogen peroxide [27]; among others, which are costly and contaminate and degrade the fabrics.

The enzymatic degradation of the excess dye is more favourable in terms of its environmental impact and fabric degradation, given that enzymes are biodegradable, act in a specific way, and can be easily deactivated to avoid a prolonged action on denim. Laccases are enzymes capable of decolorizing indigo. They are, therefore, suitable for the finishing processes of denim, accompanied by a mediator in a water medium. The laccase oxidizes the mediator, generating free radicals, which in turn oxidize the indigo [28], [29]. It has been determined through a scanning electron microscope that the combination of laccases with cellulases help to improve luminosity and to reduce staining[29]. Peroxidases such as manganese peroxidase and lignin peroxidase are also enzymes capable of degrading indigo, due to their non-specific activity on compounds with a polyphenolic substructure. Unlike laccases, they require hydrogen peroxide to start the oxidation reaction[30].

Additional studies have demonstrated that these enzymes that decolorize denim can be produced from bacteria such as *Bacillus* spp[31], *Paenibacillus* larvae[32], *Scytalidium thermophilum*[33], *γ-Proteobacterium* and white-rot fungus such as *Trametes hirsuta*, *Sclerotium rolfsii* [34], *Coprinopsis cinérea*, *Gongronella* sp [35], *Corilopsis rigida* [36], *Ceriporiopsis subvermispora* [37], *Pleurotus* spp [38], *Phanerochaete chrysosporium* [39], *Piptoporus betulinus*, *Trametes versicolor* [40].

Mechanisms for indigo degradation

Studies have demonstrated that the main indigo degradation metabolites are isatin and isatin 5-sulfonic acid, generated after the breaking of the C=C double bond and the forming of the C=O ketone group through oxidizing means. However, bacteria such as *Bacillus* spp. generate a contrary effect through the reduction of the C=C and C=O bonds to -CH for the production of 5-indolinesulfonic acid as a degradation metabolite as shown in figure 3[31]. Indoline and its derivatives such as indoline-2-carboxylic acid, pentopril and sonicotinamide have been the object of interest as synthetic organic chemicals for their potential use in the pharmaceutical industry[41].

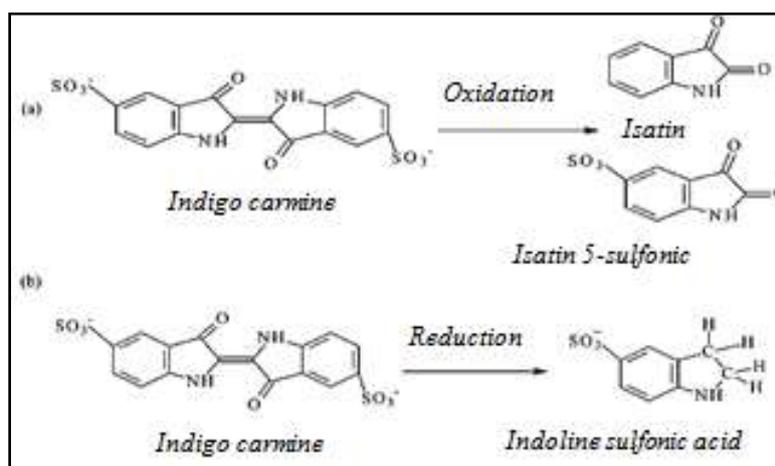


Figure 3. Mechanisms for indigo carmine degradation[31].

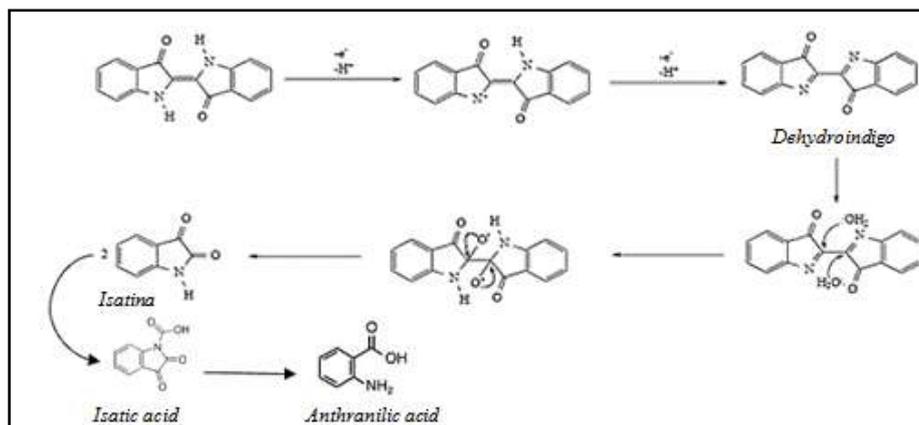


Figure 4. Step-by-step indigo degradation through oxidation [34]

The molar relation between indigo and molecular oxygen is 1. According to figure 4, the first product that is formed is dehydroindigo (visible at 440 nm), a compound easily attacked by nucleophiles such as water that provides oxygen atoms [34]. Laccases' catalytic activity triggers oxidation through the drawing out of four electrons from the substrate to reduce molecular oxygen to water and oxidize indigo into isatin. From this point on, a hydrolysis results without the mediation of the enzyme. As an intermediate product, isatic acid is formed, which is unstable and spontaneously breaks down through decarboxylation producing anthranilic acid as the final degradation product [34], [41]. Various factors may influence the overall speed indigo's enzymatic degradation, such as adsorption phenomena, transport limitations and the accessibility and potential redox of the enzyme and substrate [34].

II. Conclusion

The use of ligninolytic enzymes in denim's finishing processes constitute an environmentally friendly and efficient alternative, which has been insufficiently exploited. Only two studies were found that talk about the use of laccases in the denim industry. The rest of the research is more basic, that is, only the microorganism or its enzymes are employed to act on an indigo-colored solution, or on the pure dye. The research is, therefore, not sufficiently applied, remaining mostly in the laboratory. It would appear that the limitations of the enzymatic technology for denim decolorization in the fading process is the lack of scaling of the bioprospecting for potential microorganism.

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