Dyeing of textile fibres with enzyme reduced indigo: effect of reaction conditions on indigo reduction and dyeing quality

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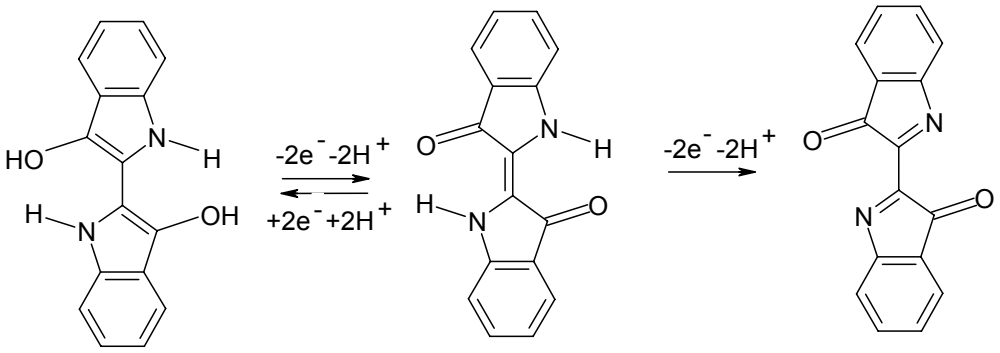
Abstract

Increasing eco-efficiency of textile wet processing has become a key factor to provide sustainability in the textile industry. The conventional reducing agent used in vat (indigo) dyeing (the sodium dithionite) is not recyclable and as a consequence causes enormous environmental problems. Although, a lot of efforts have been dedicated to develop more ecologically attractive methods for indigo dyeing, there is still no satisfactory alternative to the chemical reducing agents.

This paper reports on the application of enzymatic indigo reduction as a viable alternative to the conventional reducing chemistry at indigo dyeing. The potential of oxidoreductases from newly isolated Bacillus subtilis for indigo dyeing was studied in the presence of anthraquinone mediators. Dyeing with different textile fibre-forming polymers was investigating using UV-Vis spectroscopy and cyclic voltammetry to follow reaction. The dye reduction process was optimised in terms of redox potential, pH and temperature conditions and compared to the chemically (sodium dithionite based) approach to obtain the reduced leuco dye form. The colour and colour fastness properties (to wash, light and perspiration) of enzymatically indigo-dyed fibres were evaluated.

1. Introduction

Indigo (C.I. Vat Blue 1) is one of the oldest and still the most consumed colouring vat agents due to the popularity of the blue jeans. Indigo is insoluble in water, but the reduction in alkaline medium generates a soluble form known as leuco dye. The reduced dyestuff penetrates into the fibre and after re-oxidation (in fiber) back to the insoluble form, remains fixed on the fabric (Scheme 1).



Leuco indigo Indigo blue Dehydro indigo

Scheme 1. Mechanistic scheme for the oxidation/reduction of indigo

Nowadays in most industrial processes, indigo is still reduced by sodium dithionite ($Na_2S_2O_4$) in highly alkaline medium (pH 11-13). This procedure causes several environmental problems by sulphur containing by-products (i.e. sulphites, sulphates, sulphur, etc.) formed in the decomposition of sodium dithionite and very high pH generated [1]. The other problems arise from the parameters control of the reduction process and the storage of reducing agent. The presently used dosing control techniques based mainly on photometric or titrimetry discontinuous methods [2] require relatively long response time for determination of the dye concentration. A method which allows continuous dosing of indigo into “existing reducing system” in industrial textile dyeing would drastically reduce the response time, eliminate or minimize the production of inorganic waste from chemical reducing agents, thereby responding to range of requirements related to uniformity and quality of the dyed fabric at lower expenses.

An elegant alternative to the hazardous reducing agents could be the use of enzymes capable to specifically reduce the keto groups and thereby solubilize redox (vat) dyes. Recently, we have shown that azoreductases from *B. cereus* and *B. subtilis* were able to reduce keto groups and sulphide bonds in various redox dyes (C.I. Acid Blue 74, C.I. Natural Orange 6 and C.I. Leuco Sulphur Black 1) [3,4]. In this work the reduction of indigo with mediated NADH-dependent reductases isolated from *Bacillus subtilis* is quantitatively studied. The parameters affecting the mediated enzymatic process were determined continuously by elctrochemical measurements and the dyeing properties of different type of fibres were investigated. Anthraquinone derivatives were used as mediators in both bacterial and electrochemical reduction of indigo, and particularly 1,8-dihydroxyanthraquinone was introduced as effective redox mediator for indigo reduction [5,6].

2. Experimental

2.1 Chemicals and materials

In this study indigo (Fluka), mediator 1,8-dihydroxy-9,10-anthraquinone (Aldrich), cofactor NADH (β-Nicotinamide adenine dinucleotide disodium salt) (Aldrich), NaOH (Aldrich), $Na_2S_2O_4$ (Aldrich) as reducing agent and H_2O_2 (Aldrich) were used as obtained from the providers. Indigo reductases (42.18 mg ml^{-1} of protein content

determined by Lowry method [7]) were isolated from *Bacillus subtilis* as previously described [8]. Nitrogen gas was employed for de-aeration.
Industrially prepared for dyeing polyamide (knitted), cotton (woven) and polyester (woven) fabrics were used. A fabric were previously washed in the presence of surfactant for 30 min at 40°C and after washing the surfactant was removed from the fabric first with tap water, followed with distilled water.

2.2 pH and temperature dependent indigo-reductases activity=

The pH and temperature activity profile of indigo reductases was determined according to the efficiency of indigo reduction in the range between pH 4 – 12 at 60°C (optimal temperature for indigo reduction using reductases) and at pH 7 and pH 11 in the range between 25 - 85°C for 60 min. The activity assays were carried out in cuvettes of 10 ml. The enzymatic reaction mixtures contained 0.03 mM indigo, 50 mM phosphate buffer (pH 4 - 12), 0.5 ml reductases and 12.5 µM mediator 1,8-dihydroxy-9,10-anthraquinone. The reaction was initiated by the addition of 1 ml NADH (14.18 mg ml^{-1}; final concentration 2 mM). The reactions were followed spectrophotometrically measuring the change of absorbance at the leuko indigo absorption maximum (at 420 nm) as a results of the colour change from dark blue (610 nm) to yellowish-brown after 15 min of pre-incubation using Carry 50 UV-VIS spectrophotometer (Varian); the spectrum reverted to the original spectrum of indigo when the solution was exposed to H_2O_2 or air.

2.3 Electrochemical experiments

The cyclic voltammetry experiments were performed using a µAutolab Type III (EcoChemie) potentiostat/galvanostat controlled by Autolab GPES software version 4.9. All the experiments were carried out in a 20 ml Methohm cell with a three-electrode configuration. The working electrode was a glassy carbon with a surface diameter of 3 mm (Metrohm). The counter and reference electrodes were platinum (Metrohm) and Ag/AgCl (Metrohm) electrode, respectively. The renewal of the glassy carbon surface was achieved by polishing with 1.0 and 0.3 µm alpha-alumina (Micropolish, Buehler) on a micro cloth polishing pads (Buehler), followed by washing in an ultrasonic Selecta bath for 2 min.
The experiments of conventional reducing agent $Na_2S_2O_4$ (1 g l^{-1}) with and without indigo (0.03 mM) dye were performed at a scan rate 50 mV s^{-1} in a thermostated cylindrical reactor (volume 20 ml) at 60°C at pH 11 (50 mM Tris-NaOH buffer). The same experiments were performed for NADH-dependent oxidoreductases (50 ml l^{-1} enzyme solution, 2 mM NADH) purified from *Bacillus subtilis* with anthraquinone mediator (12.5 µM) as reducing system for indigo dye at pH 11 (50 mM Tris-NaOH buffer). Ultra pure water obtained with a Milli-R3 plus/Milli-Qplus 185 purification system from Milipore Iberica S.A was used throughout this work. The tasted solutions were carefully purged with nitrogen for 5 min and the voltametric curves were recorded under a nitrogen atmosphere on the surface.

2.4 Dyeing

Pre-wetted polyamide fabrics were dyed in sealed, stainless steel dye pots of 200 cm^2 capacity. Samples were placed in 80:1 liquor ratio (dyeing bath volume (l):fabric weight (kg)) dyebath at 25°C containing 0.16% owf indigo, 50 ml l^{-1} enzyme solution from *Bacillus subtilis*, 2 mM cofactor NADH and 12.5 µM mediator 1,8-dihydroxy-9,10-anthraquinone in Tris-NaOH buffer (pH 11). Dyebath was stirred and heated up to 60°C under nitrogen atmosphere and the vatting/dyeing was continued for 60 min

at the same temperature. At the end of the dyeing, the dyed samples were removed, rinsed thoroughly in tap water and allowed to oxidize in open air. Samples were further soaped with 2 g l^{-1} sodium metasilicate-5-hydrate (Cotoblanc) at boiling for 15 min using the same liquid ratio and rinsed with water for 15 min. The fabrics were finally hot rinsed for 1 min at 60°C, cold rinsed at 20°C and air dried. Comparatively, the conventional/chemical dyeing of polyamides was carried out in Tris-NaOH, pH 11 using 2 g l^{-1} $Na_2S_2O_4$ as reducing agent.

2.5 Colour measurements

Colour strength of the dyed fabrics was estimated from the reflectance measurements using Spectraflash SF 600 PLUS spectrophotometer (Datacolour) at standard illuminant D65 (LAV/Spec. Incl., d/8, D65/10°). The colour was evaluated by CIELAB colour values (L*a*b*) and colour strength (K/S) was calculated using the Kubelka–Munk equation:

$$K/S = \frac{(1-R)^2}{2R} \quad (1)$$

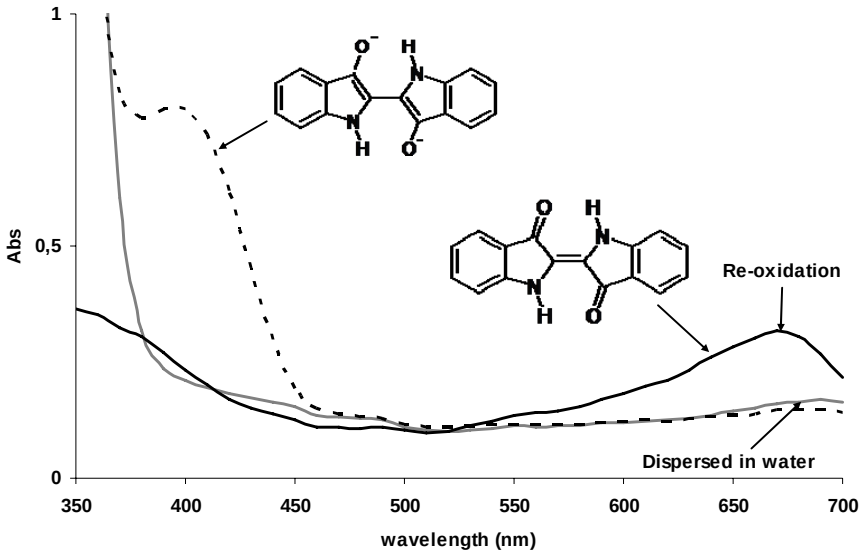
where *R* is the reflectance of the fibre at the wavelength of maximum absorption (610 nm).

Each sample was folded once to give two thicknesses and average of three readings was taken each time. Colour fastness to washing, light, alkaline and acid perspiration were determined using standards ISO 105-C04 (Part C04), ISO 150 B04, and ISO 105-E04 (Part E04), respectively.

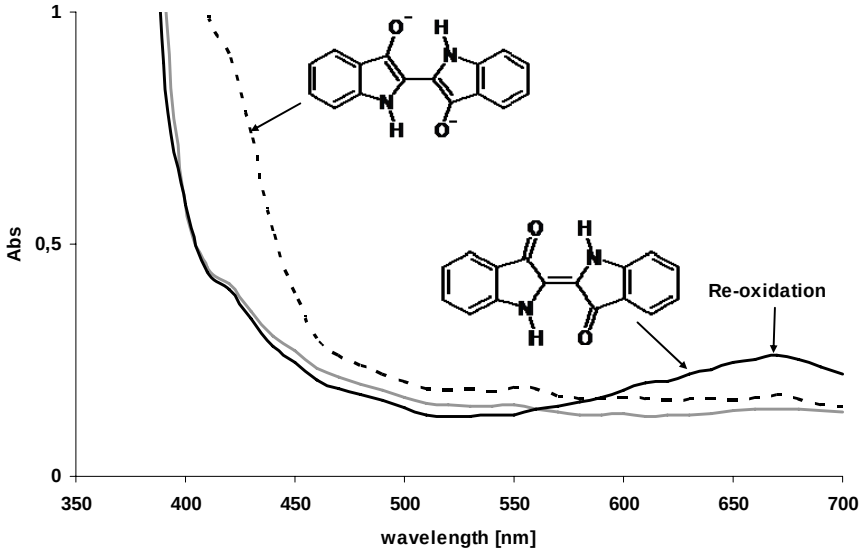
3. Results and discussion

3.1 Indigo-reductases activity

The redox state of indigo can be monitored by changes in of absorbance between 400 and 450 nm with a maximum at 420 nm as a results of the colour changes from dark blue to yellowish-brown. While the insoluble oxidised form of indigo shows low absorbance at 400 to 450 nm, the reduced forms shows absorption maxima at ca. 420 nm (Figure 1).



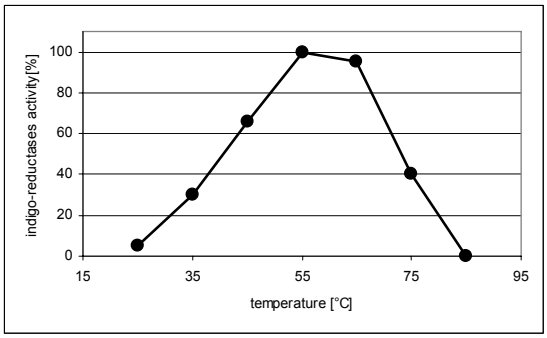
(a)



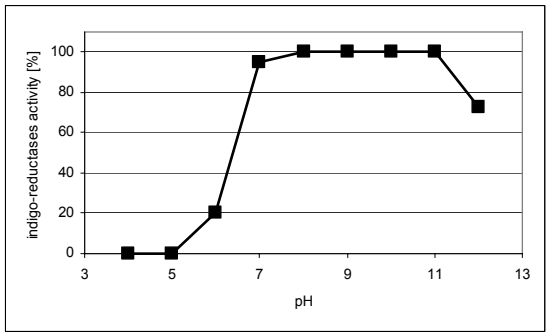
(b)

Figure 1. Absorption spectra of oxidised forms before reduction and after re-oxidation, and reduced leuco form of indigo using $Na_2S_2O_4$/NaOH (a) and mediated enzymes (b) as reducing agents and H_2O_2 as oxidizing agent

Before the dyeing, the reaction conditions for enzymatic indigo reduction were optimized. As it can be seen from Figure 2(a) reductases can fully reduce indigo between 55 and 60°C at pH 11. Because of thermal inactivation of the enzymes above 65°C, the pH optimum for indigo-reductases activity was determined at 60°C. Figure 2(b) shows a total reduction of indigo in the range of pH 7 - 11, while in acid conditions the reductases activity significantly decreased due to denaturation of the enzyme proteins.



(a)

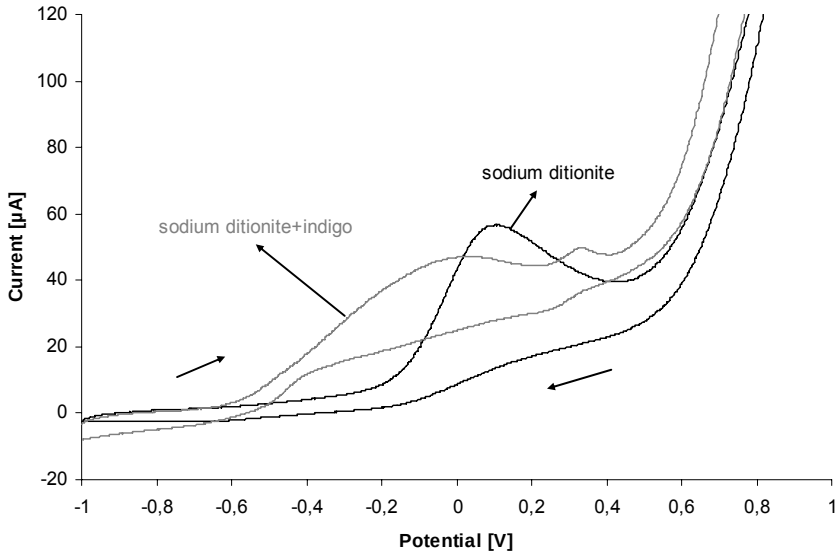


(b)

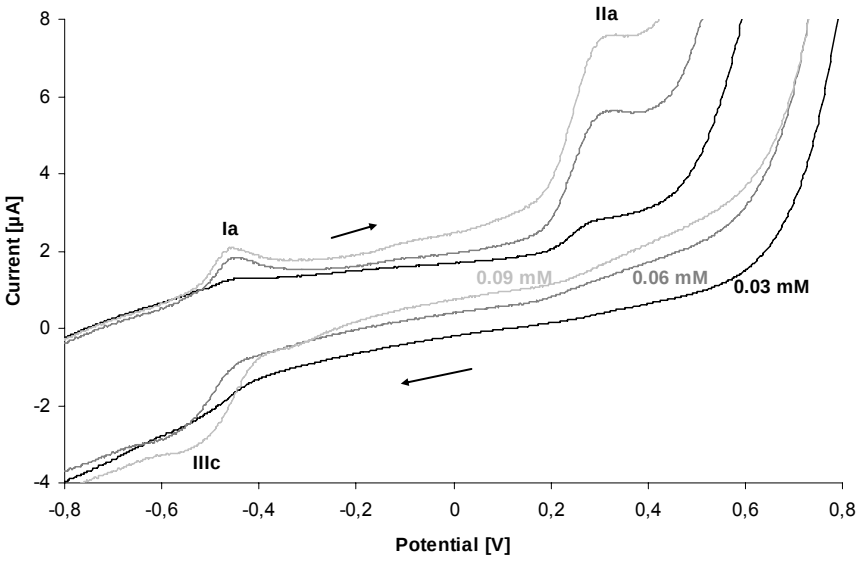
Figure 2. Indigo-reductase activity (a) at pH 11 vs. different temperatures and (b) at 60°C in different pH

3.2 Cyclic voltammetry of chemical and enzymatic reduction of indigo

With sodium dithionite in solution electrochemical detection of leuco indigo was impossible due to the interference caused by sodium dithionite (high anodic peak), but an oxidation peak, at potential of 340 mV (vs. Ag/AgCl), that can be related to the oxidation of indigo to dehydro indigo molecule (Scheme 1), was observed. When experiments were performed with NADH-dependent oxireductases (50 ml l^{-1} enzyme solution, 2 mM NADH) with anthraquinone mediator (12.5 μM) as reducing system for indigo molecule, two electrodic processes one reversible at potential around -550 mV (Figure 3 (b); Ia/IIIc) and one irreversible at 300 mV (Figure 3 (b); IIa); were recorded after 30 minutes at 60°C.



(a)



(b)

Figure 3. Cyclic voltammograms of (a) sodium dithionite (1 g l^{-1}) with and without indigo; and (b) of 0.03, 0.06 and 0.09 mM of indigo dye in a mediated enzymatic reducing system at 60°C, pH 11 after 30 min. Scan rate = 50 mV s^{-1}.

First process (Ia/IIIc) corresponds with the oxidation/reduction of leuco indigo/indigo species, being leuco indigo generated by means of the application of mediated enzymatic reducing system. The second (IIa) oxidation process involves the oxidation of indigo through the –NH groups of the indol structures to yield dehydro indigo.

Previous to textile dyeing, the electrochemical detection of the different dye forms was carried out also in the presence of three different textile fibres. As can been seen in Figure 4, the presence of polyamide and cotton decreased the intensity of the leuco indigo anodic peak, a fact that was not observed in the case of polyester after 30 min processing at 60°C. After removal of the fibres from the dyeing solution no colouration was observed on polyester and cotton, while the polyamide was blue coloured.

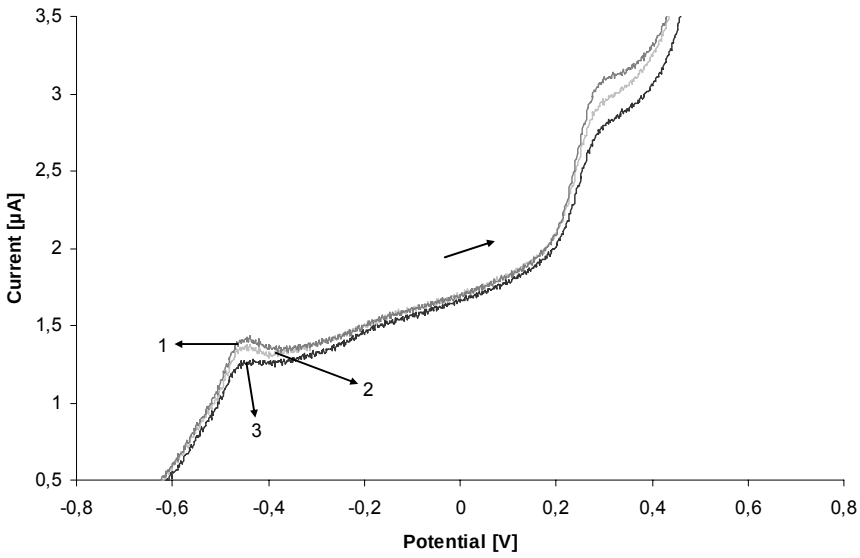


Figure 4. Linear scan voltammograms of 0.03 mM of indigo dye in a mediated enzymatic reducing Tris-NaOH buffer (pH 11) system at 60°C after 30 min in presence of different textile fabrics (1-polyester; 2-cotton and 3-polyamide). Scan rate = 50 mV s^{-1}.

3.3 Dyeing properties of enzymatically indigo-dyed fabrics

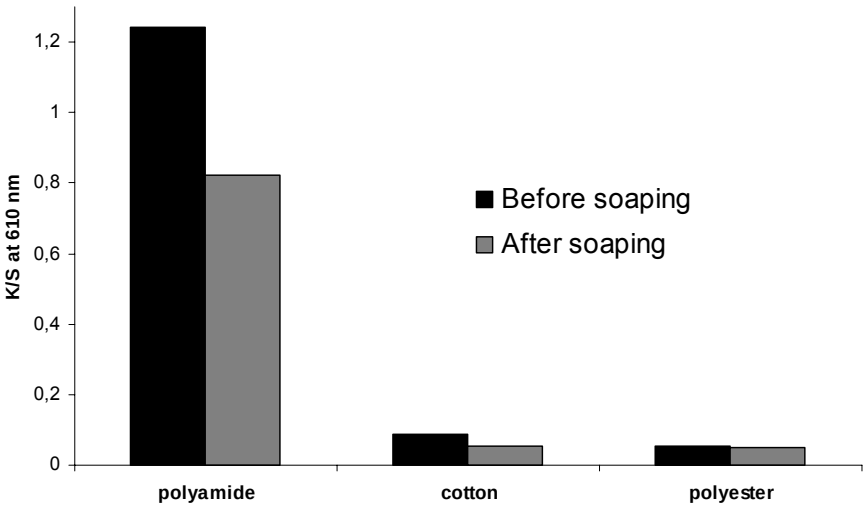


Figure 5. K/S values of polyamide, cotton and polyester indigo mediated enzymatic dyed at 60°C, pH 11 for 60 min before and after soaping.

As can been seen from Figure 5 different colour strength was achieved on the different fabrics. Surprisingly, the enzymatically reduced indigo showed substantivity towards polyamide and not towards cotton as expected. Conventional indigo dyeing is carried out under highly alkaline medium where the cellulose hydroxyl groups are dissociated and the leuco indigo form has substantivity towards cellulose fibres.

However, in the case of indigo enzymatic reduction some adsorption of denaturated enzyme proteins on cellulose fibres surface could be the reason for “limited” colouration effect on cotton. Polyester is normally dyed at very high temperatures, thus no colouration was expected. After soaping the K/S values for all fabrics decreased, indicating removal of surface bound dye molecules and consecutively brighter colours. During soaping isolated molecules of indigo pigment reorient and associate into a more crystalline form, often producing a significantly different shade along with improved fastness to light and washing. In order to confirm that polyamide and cotton colouration is due to the enzymatically reduced indigo, blank experiments at 60°C for 60 min with only indigo were performed. As expected, there was no colouration on polyamide and cotton.

3.4 Fastness properties of dyed polyamides

Colour fastness to washing, perspiration and light of both, chemically and enzymatically indigo (0.16% owf) dyed polyamide was determined. The results showed comparable fastness properties of the dyed samples using both dyeing processes (Table 1).

Table 1. Wash, perspiration and light fastness of chemically and enzymatically indigo-dyed polyamide after 60 min of dyeing (0.16% owf) at 60°C and pH 11 after soaping

samples	wash fastness	perspiration fastness		light fastness
		alkaline	acid	
Chemical dyed polyamide	3-4	5	5	4
Enzymatic dyed polyamide	3-4	5	5	3

4. Conclusions

This works clearly shows the potential of novel indigo reductase enzymes from a *Bacillus subtilis* strain in indigo dyeing. In the presence of redox mediator 1,8-dihydroxy-9,10-anthraquinone, the isolated reducing enzymes appear as an efficient reduction substitute for the environmentally harmful sodium dithionite. Electrochemical measurements indicated that the mediated enzymes were capable to reduce indigo to its soluble leuko form at pH 11 and 60°C. The indigo adsorption on textiles from enzymatically reduced dyebaths largely depends on the fibre type. Unexpectedly, high colour strength was obtained on polyamide fabrics, while in the case of cellulose the colouration was limited.

The enzymatic indigo reduction process has the advantage over the currently used chemical indigo-reducing systems as an economically an ecologically competitive dyeing technology. Nevertheless, further work is required to optimise the dyeing process of cellulose materials.

Acknowledgements

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5. References

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