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Dyeing of Textile Fabrics with Bio-dyes

Abstract

EU directives define in detail guidelines which modern industrial technologies should meet in order to eliminate or minimise their share in contaminating the natural environment. Reducing energy and water consumption, and restricting the use of hazardous substances in the environment are the basic criteria that should characterise new technologies. Due to restrictions in the use of azo dyes, especially of those which can be reduced to amino products (Directive 2002/61/EC), the dyestuff industry is constantly looking for new colouring substances. Particular emphasis is placed on the implementation of new technologies of synthesis which have a minimal impact on the environment and at the same time decrease manufacturing costs by reducing the consumption of raw materials and energy and reduce waste. Industrial processes belonging to white biotechnology successfully exploit the potential of natural biological systems in industrial processes, making industrial activity more environmentally friendly. Bio-dyes obtained in this way are durable, environmentally friendly, and have interesting and fast colours. The process of bio-dye synthesis occurs during processes applying enzymes or fungi with precursors that are used to synthesise chemical dyes. This paper presents newly synthesised bio-dyes. In their synthesis enzymes or fungi were used. During the research work, the dyeing properties of the bio-dyes were tested as well as the colour fastness of textiles made of protein and synthetic fibres. The resulting colours obtained after bio-dye application are more uniform than in the case of chemical dyes and have good colour fastness.

Key words: *bio-dyes, dyes synthesis, dyeing, textile materials, laccase, fungi, colour fastness.*

Introduction

At present new methods of obtaining coloring substances using enzymatic or fungal processes for their synthesis are arousing a lot of interest among researchers. Biological preparations used for the synthesis often replace toxic chemicals. Industrial technologies based on biological processes belong to the so-called "white biotechnologies", which allow to minimise the consumption of raw materials, energy and water, and reduce the cost of dye manufacture as well as the use of toxic substances dangerous for the environment. Bio-dyes thus obtained are environmentally friendly and have interesting (attractive), durable colours [1 - 5]. The bio-dye synthesis process itself takes place in bioreactors containing enzymes or fungi which act as natural biocatalysts with the use of precursors for the preparation of synthetic dyes. Some of the precursors used in the dye preparation process are characterised by medium toxicity and the bio-dye obtained is non-toxic, as in the case of phenoxazin dye synthesised by fungal laccase from a simple phenolic precursor [6]. Fungal laccase, produced by many species of the fungi *Basidiomycota*, is a very effective biocatalyst that oxidises colourless precursors to colour bio-dyes. The main advantages of this enzyme as a biocatalyst are its very low substrate specificity and the fact that it is an extracellular enzyme, hence its preparation from fungal cultures is very simple and quite cheap. Fungal biomass which secretes laccase to the culture medium may be used instead of pure enzyme as well [7, 8].

Both wool and natural silk are protein fibres. The chemical material of wool fibres is keratin - fibre-forming protein, produced in a process of biosynthesis of α-amino acids (H_2N-CHR-COOH), which, like amino groups of basic character, have acid carboxyl groups. With the formation of keratin in the process of polycondensation taking place in the cavity of the dermis of an animal, there are 19 α-amino acid residues involved which differ from each other in chemical nature and affinity to water. These amino acids form amide bonds CO-NH- with one another, called peptide bonds. Due to the presence of both basic and acidic groups in the chains of protein fibres, fibres exhibit amphoteric properties and may form ion bonds with dyes of both acidic and basic character.

Keratin can undergo hydrolysis under the influence of both acids and alkali. The resistance to acid hydrolysis is much higher than that to alkaline hydrolysis. Keratin can undergo oxidation and reduction reactions and is generally resistant to organic solvents. The process of dyeing protein fibres with anionic dyes can be divided into the following stages:
1) adsorption of dye molecules on the surface of the fibre,
2) diffusion of dye molecules through the fibre surface to its inside to alkali reactive groups,
3) bonding inside fibres of colorless acids (e.g. H_2SO_4),
4) displacing of colourless acid ions by colored ions,
5) bonding of coloured anions with wool reactive groups [9].

Fibroin macromolecules, from which natural silk fibres are formed, contain both amino and carboxyl groups, such as in keratin-wool, but in a four-fold less amount. Due to the smaller number of $-NH_2$, -COOH groups, natural silk has a lower affinity to dyes than wool. Silk fibres in a base can be dyed with water-soluble dyes (anionic, cationic). However, anionic dyes are most widely used; their bonding with a silk fibre is the same as in the case of wool. A silk fibre swells greatly in water, which facilitates the diffusion of dyes inside therein. Natural silk fibroin is subject to the same reactions as keratin, apart from oxidation and reduction reactions (due to a large number of cystine residues in keratin, which are ab-

sent in fibroin). It is also more resistant to alkaline hydrolysis than keratin [10-16].

Synthetic fibres are formed from a polymer produced in a synthesis reaction programmed by a human. The group of basic synthetic fibres in the clothing industry includes polyester and polyamide fibres. Polyamide fibres are characterised by a compact structure and strong hydrophobicity, which reduces the adsorption of dyes on the fibre surface and their diffusion into the fibre. These fibres are resistant to alkalis, reducing agents and most solvents.

Polyamide has dyeing properties most similar to natural fibres, which is connected with the presence of amino, carboxyl and imino groups in the fibre. The structure of polyamide fibres is most similar to protein fibres, and these fibres can be dyed with groups of dyes used for natural fibres and also with dispersed dyes.

The process of dyeing synthetic fibres can be divided into the same stages as in the case of natural fibres [14, 15].

The aim of the study was to examine the dyeing properties of new bio-dyes obtained through biosynthesis using a fungal biocatalyst, in comparison with conventional organic dyes. The biocatalyst of the bio-dye synthesis reaction was laccase obtained from the culture fluid of *Cerrena unicolor* fungus grown under shaking conditions in a minimum mineral medium. As precursors, two naphtalene compounds were used (precursor I and precursor II), containing also sulfonic derivatives with amino-, hydroxy-, and methoxy- substituents.

Materials and methods

Materials used in tests

For the studies products with natural fibres were used - wool, natural silk and synthetic fibres - polyamide. These fibres can form ionic bonds with dyes. Woven fabric of wool (100%), raw, mass per unit area 158 g/m^2. Woven fabric of natural silk (100%), mass per unit area 70 g/m^2. Polyamide knitted fabric (100%), mass per unit area 144 g/m^2

Dyes

Classical organic dyes (commercial)
Acid dye – C.I. ***Acid Blue 260*** (Reax, Poland); *CAS 62168-86-9; $\lambda_{max} = 590$ nm*

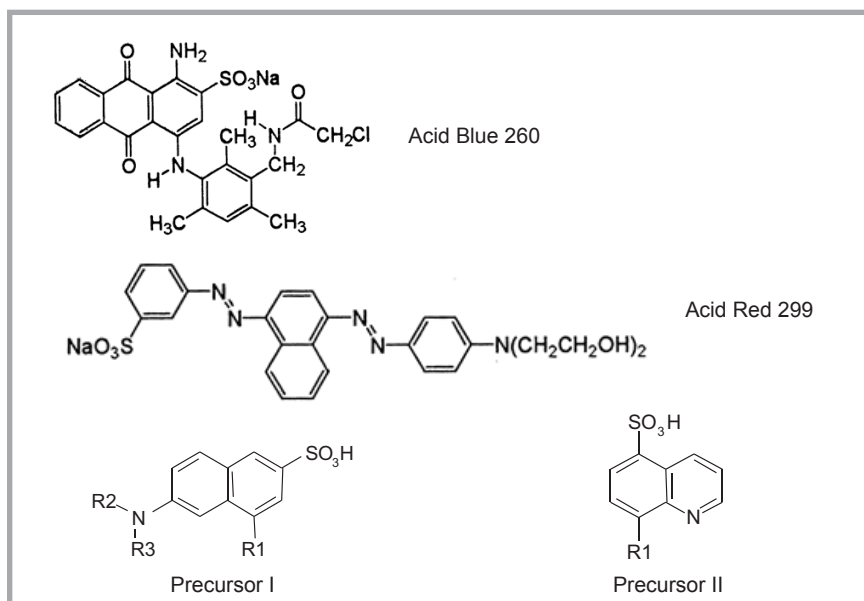This dye used in aqueous solution was characterised by maximum absorption at

Figure 1. *Dyes used in this work.*

a wavelength λ_{max} of 590 nm, at which the absorbance measurements were made.

Acid dye – C.I. ***Acid Red 299*** (Reax, Poland); *CAS 12220-29-0; $\lambda_{max} = 525$ nm*

Dyes obtained by synthesis using a fungal biocatalyst (UMCS) (bio-dyes)
NCN 9 (UMCS, Poland); $\lambda_{max} = 550$ nm, (precursor I)

NCN 9+14 (UMCS, Poland); $\lambda_{max} = 560$ nm, (precursor I + precursor II)

The dyes used in this work are presented in **Figure 1**.

Chemical additives
Formic acid 80%

The process of dyeing woven fabrics of wool, natural silk and polyamide knitted fabric

The products selected, fabric from wool and natural silk fibres as well as polyamide knitted fabric, were dyed with the above dyes in a bath at a concentration of 0.2% and 0.5% relative to the fibre mass. Formic acid was added to the dyeing bath in an amount of 1% (pH 3.1 - 3.4), at a dyeing temperature of 100°C, for 115 minutes. Before the dyeing process the polyamide knitted fabric and woven wool fabric were washed in a water bath at 60 °C for 60 min.

The dyeing process, carried out in a laboratory dyeing apparatus Redkrome (Ugolini, Italy) at a bath of ratio 1:20, proceeded according to the diagram shown
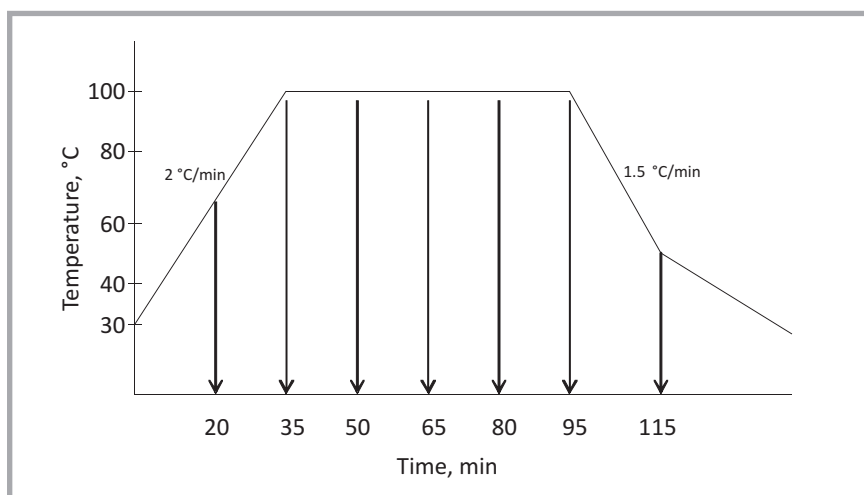

Figure 2. *Diagram of the dyeing process for woven fabrics made of wool and natural silk as well as polyamide knitted fabric with selected dyes.*

Table 1. λ_{max} values of wavelength corresponding to the absorption maximum for the dyes tested in aqueous solution, at which measurements of absorbance and wavelength λ_{max} were made corresponding to the maximum absorption for each dye at which K/S values were read.

DYE		Acid Blue 260	Acid Red 299	NCN 9	NCN 9+14
λ_{max}, for dyes in water solution		590	525	550	560
λ_{max} at which values of K/S factor were read	wool	630	540	520	550
	polyamide	630	550	480	530
	natural silk	630	540	520	540

in *Figure 2*. After dyeing the samples were rinsed in water at 70 °C for 10 minutes and then in cold running water.

Methods of evaluation of the dyeing process and textile products after dyeing with selected dyes

The measurement of absorbance was performed on a UV-VIS spectrophotometer - Jasco V 600, (Jasco, Japan). The dyes used in the aqueous solution were characterised by the maximum wavelength λmax given in *Table 1*, at which maximum absorption measurements were made. For each of the resulting dyeing (for wool, silk and polyamide), values of the K/S factor were determined at a wavelength corresponding to the maximum absorption for dyeing with each dye (*Table 1*).

The degree of dye exhaustion in bath *E* was calculated according to formula (1) after prior analysis of the calibration curves for each dye.

$$E = \frac{DFZ_0 - DFZ_1}{DFZ_0} \, 100\% \qquad (1)$$

where: DFZ_0 - parameter which characterises the amount of dye in a bath before dyeing in m^{-1}, DFZ_1 - parameter which characterises the amount of dye in a bath after dyeing in m^{-1}.

Spectrophotometeric evaluation of the changes in colour intensity of fibres
The concentration of dye on the fibre during and after the dyeing process was assessed indirectly by determination of the coefficient of *K/S* using the Kubelka-Munk formula (2) [17], proportional to the dye concentration on the fibre.

$$\frac{K}{S} = \frac{(1 - R)^2}{2R} = k \cdot c_w \qquad (2)$$

where: *K* – light absorption coefficient, *S* – light scattering coefficient, *R* – light remission rate, c_w – dye concentration in the fibre, *k* – coefficient of proportionality.

The dyed fabric samples were dried and the coefficient of remission (R) was measured. Measurement of the remission coefficient with calculations of the value of the relative dye intensity (K/S) were performed on a spectrophotometer - Spectraflash 500, Datacolor Int. (USA) in accordance with PN-EN ISO 105 J01: 2002.

Evaluation of dyeing quality
The dyed textiles were tested for colour fastness to:
■ washing at 40 °C according to Standard PN ISO 105-C06:2010 Method A1S
■ alkaline sweat according to Standard PN-EN ISO 105-E04:2011
■ acidic sweat according to Standard PN-EN ISO 105-E04:2011
■ distilled water according to Standard PN-EN ISO 105-E01:2010
■ dry friction according to Standard PN-EN ISO 105-X12:2005
■ wet friction according to Standard PN-EN ISO 105-X12:2005

Biocatalyst and biotransformation
Extracellular laccase (LAC) from the fungal strain *Cerrena unicolor* (Fungal Collection of the Department of Biochemistry, UMCS, Lublin, Poland) was purified using a procedure previously described [18]. LAC was stored frozen at a temperature of -18 °C prior to use. After thawing, the LAC was diluted with a tartrate buffer solution to prepare a working LAC solution with known activity. The biotransformation of a single precursor (precursor I) or mixture of two precursors (precursor I and precursor II, at a 1 : 1 ratio) was conducted in 1000-mL Erlenmayer flasks with reaction mixtures containing the appropriate activity of LAC, with 100 mM of Na-tartrate buffer pH 4.5 and 1 mM of each precursor. The flasks were incubated at a temperature of 28 °C under shaking conditions (140 r.p.m.) for a maximum of 48 hours, in a state where no increase in colour formation was observed. The evolution of colour formation as a function of time was recorded by visual observation and using a UV–vis spectrophotometer. The reaction was stopped after 48 hours, and colour products were freeze-dried by lyophilization

and used without purification as bio-dyes (NCN 9; NCN 9+14). Within the framework of this paper the dyeing of different fabrics using the bio-dyes obtained was tested and their structures will be analysed within the framework of further experiments.

■ Results and discussion

Figures 3 - 5 show in the form of graphs the relationships between the degree of dye exhaustion in a dye bath and the dyeing time for different textile materials dyed with bio-dyes and conventional organic dyes.

Analysis of the results of the degree of dye exhaustion in bath E in % leads to the conclusion that for polyamide fibres the exhaustion rate E in % for the dyes tested at a concentration of 0.2% in a bath amounts to from 98.9% to 99% (*Figure 3*). For bio-dyes in the first stages of the dyeing process (20, 35 & 50 minutes) the degree of exhaustion is lower compared to conventional organic dyes. A lower degree of exhaustion indicates a slower exhaustion of dye from the dye bath, which from the point of view of dyeing technology promotes a more uniform dyeing of textile products. The final dyeing stage - after 115 minutes - indicates that the amount of dye remaining in the bath after the completed dyeing process is at the same level for all dyes tested (*Figure 4*). It should be noted that for Acid Blue 260 the exhaustion rate of dye from the bath is at the level of 99% already after 20 minutes of dyeing.

For fabrics made of wool fibres (*Figure 4*) in the case of bio-dyes and classical organic dyes used at a concentration of 0.2% in a dye bath after stage IV of the dyeing process (after 65 min.), the degree of exhaustion of the dye is reduced compared to the earlier stages of dyeing, which may be connected with a partial desorption of the dye from the fabric sample to the dye bath. This phenomenon occurs in a conventional dyeing process of wool fibres with acid dyes. The reduction in the degree of dye exhaustion after 65 minutes of the dyeing process is confirmed by a small reduction in the relative dyeing intensity [K/S] of wool fibres after this dyeing stage (*Figure 6*). In the case of wool dyeing in the first stages of the dyeing process, the dye exhaustion rate in the bath is also lower for bio-dyes (after 20, 35 & 50 minutes) in comparison with conventional organic dyes. Uneven dyeing due to the rapid exhaustion of
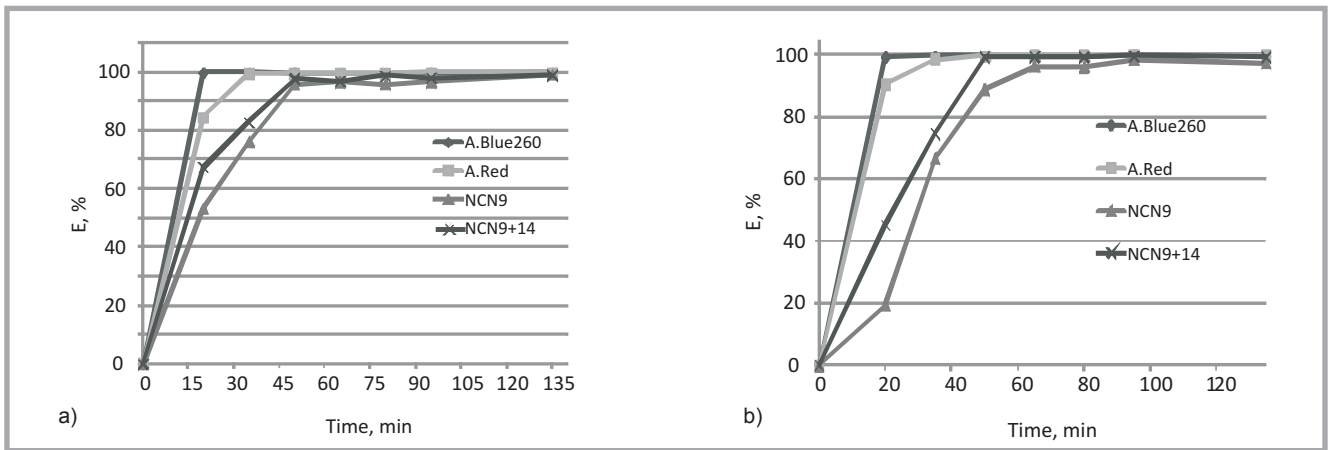
Figure 3. *Degree of dye exhaustion in a dye bath in the dyeing process of polyamide fibres - dye concentration: a) 0.2%, b) 0.5%.*
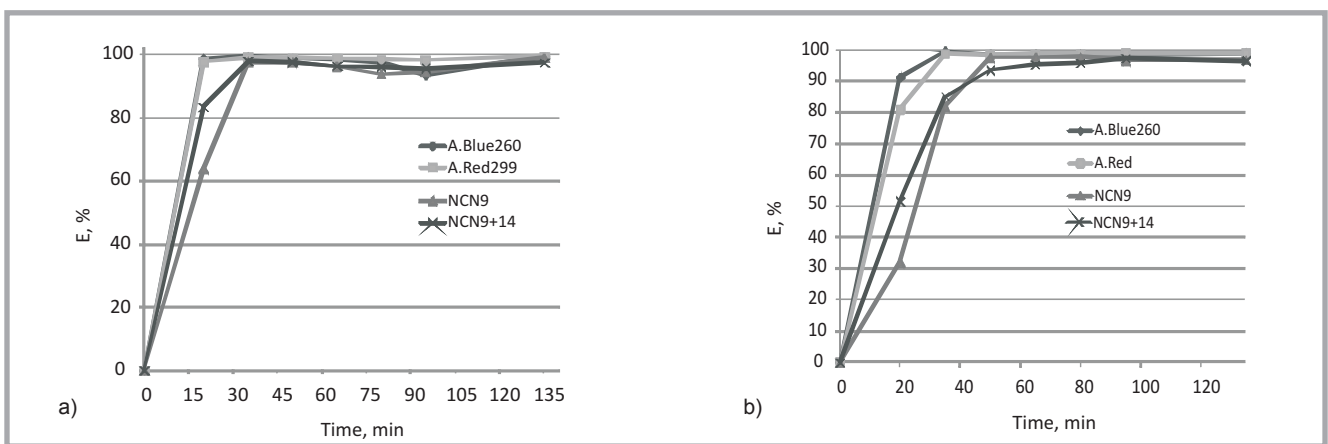

Figure 4. *Degree of dye exhaustion in a dye bath in the dyeing process of wool fibres - dye concentration: a) 0.2%, b) 0.5%.*
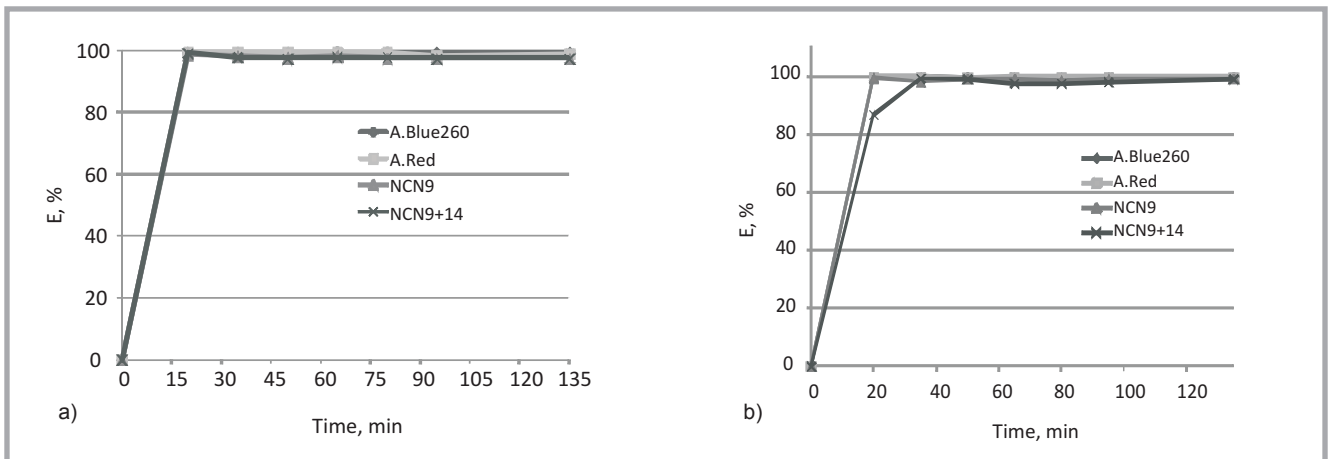

Figure 5. *Degree of dye exhaustion in a dye bath in the dyeing process of natural silk fibres - dye concentration: a) 0.2%, b) 0.5%.*

organic dye in the bath in a given process can be eliminated by the use of leveling agents or by changing the pH of the dyeing process. However, in the subsequent stages, at a temperature close to 100 °C, the dye penetrates the fibre again.

The comparative analysis of the dyeing processes of natural silk fabric in baths containing 0.2% and 0.5% of the bio-

dyes or conventional organic dyes tested also showed a high degree of exhaustion in the dye bath, which was at the level of 97 - 99%, as shown in *Figure 5*.

For the textile products of wool, silk or polyamide fibres tested in the first stage of the dyeing process, the bio-dye exhaustion in the bath was lower than in the case of classical organic dyes

(*Figures 3 - 5*). In consequence it contributed to the improved uniformity of dyeing for textile products dyed with bio-dyes.

The relative color intensity for wool fabric dyed with Acid Red 299 achieves the highest value at a concentration of 0.2% - 7.22 and amounts to 0.5% - 16.4. For Acid Blue 260, colour intensity values
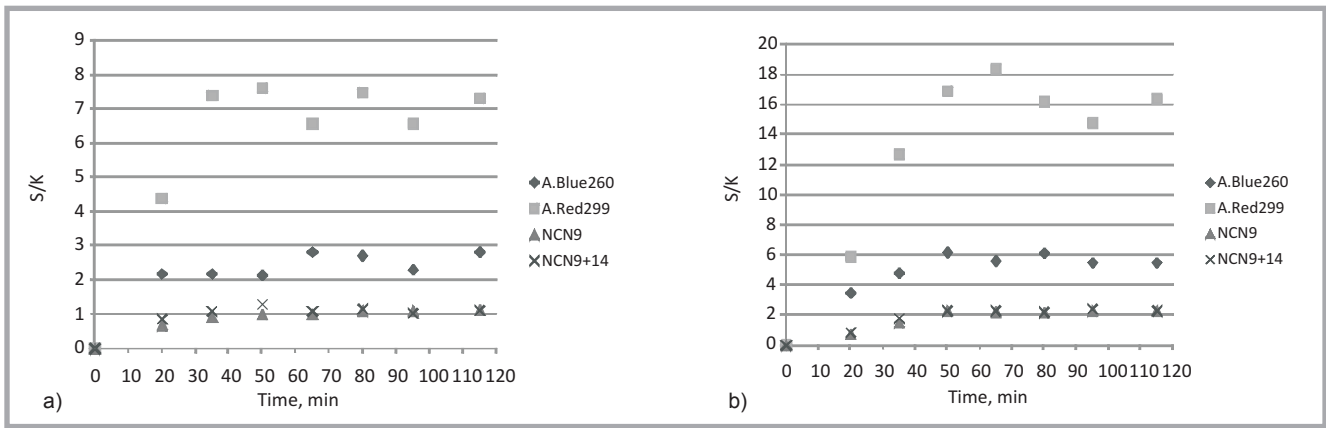
Figure 6. *Changes in colour intensity of wool fibres during the dyeing process - dye concentration: a) 0.2%, b) 0.5%.*
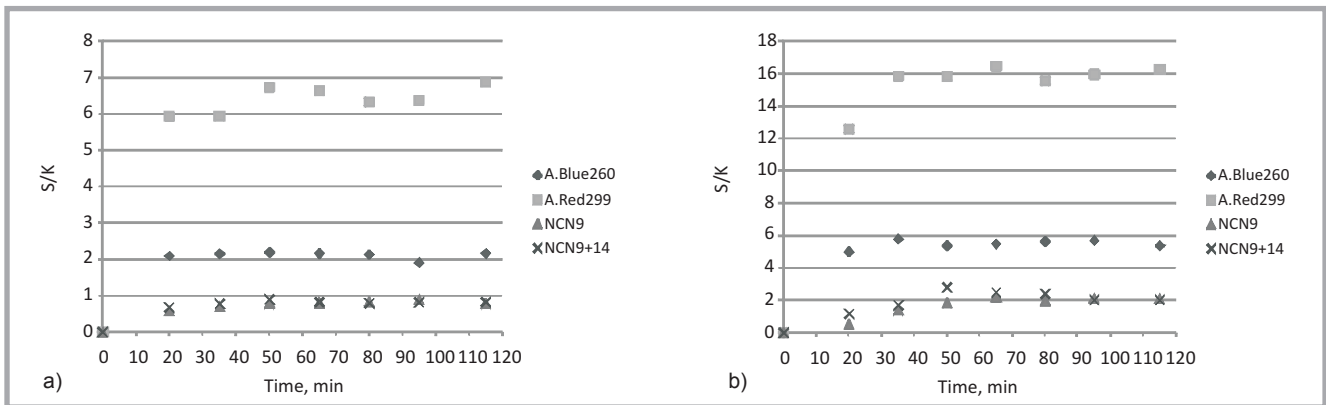

Figure 7. *Changes in colour intensity of polyamide fibres during the dyeing process - dye concentration: a) 0.2%, b) 0.5%.*
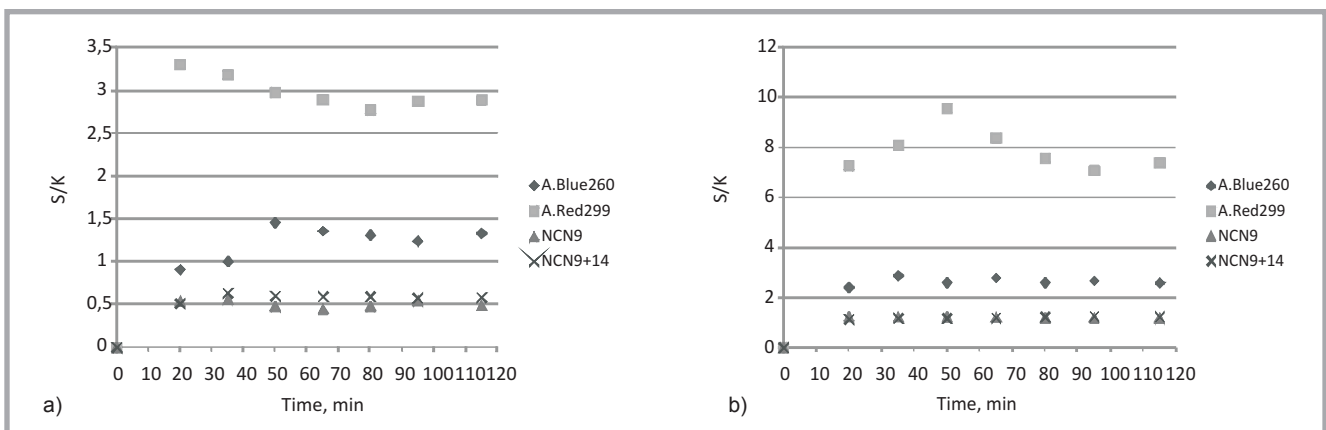

Figure 8. *Changes in colour intensity of natural silk fibres during the dyeing process - dye concentration: a) 0.2%, b) 0.5%.*

are relative lower, for 0.2% the amount is 2.80 and for 0.5 - 5.50 (***Figure 6***). Lower values of K/S were obtained for products dyed with bio-dyes.

In the case of polyamide, K/S results are comparable to those for wool (there are slight differences in the values). For natural silk, K/S values are lower in comparison with wool or polyamide (***Figures 6 - 8***). Table presents test results for the colour fastness of textile products dyed with conventional organic dyes and bio-dyes.

In the case of Acid Blue 260 dye and bio-dye NCN 9 used for wool, natural silk and polyamide, the colour fastness values tested are comparable. For Acid Blue 260, the colour fastness of the fabric made of wool fibres to acidic sweat is 4-5 and for dyeing with bio-dyes NCN 9 it is 5. For the silk fabric dyed with Acid Blue 260, colour fastness to washing is 4-5, while for NCN 9 it is 5, which indicates no colour change after the test (***Table 2***).

For products dyed with bio-dyes lower light resistance values were obtained (3-4) in comparison with those dyed with conventional organic dyes (4-5) (***Table 2***).

◼ **Conclusions**

◼ The bio-dyes tested - NCN 9, NCN 9+14 are easily dissolved in water

◼ Slower exhaustion of dye in a dye bath in the early stages of the dyeing

Table 2. *Colour fastness of textile products dyed with natural dyes (dye concentration in the bath - 0.2%). 1) Colour fastness index acc. to grey scale, in which index '5' means no change and index '1' - great change; 2) Colour fastness index acc. to blue scale, in which index '8' means no change and index '1' - great change, a) changed colour of the sample tested; b) soiled whiteness of the accompanying fabric - cotton, c) soiled whiteness of the accompanying fabric – wool; d) soiled whiteness of the friction cotton fabric.*

Parameter tested	Test results					
	Natural silk		Wool		Polyamide knitted	
Color fastness	fabric dyed with					
	A. Blue 260	NCN 9	A. Blue 260	NCN 9	A. Blue 260	NCN 9
washing 40°C [1] a/ b/ c/	4-5 4-5 5	4-5 5 5	4-5 5 5	4-5 5 5	4-5 5 5	4 5 5
Alkaline sweat [1] a/ b/ c/	5 5 5	5 5 5	5 5 5	5 5 5	4-5 5 5	4 5 5
Acidic sweat [1] a/ b/ c/	5 5 5	5 5 5	4-5 5 5	5 5 5	4-5 5 5	4 5 5
Distilled water [1] a/ b/ c/	4-5 5 5	4-5 5 5	4-5 5 5	4-5 5 5	4-5 5 5	4-5 5 5
Dry rubbing [1] *warp* d/ *thread* d/	5 5	5 5	5 5	4-5 4-5	4-5	4-5
Wet rubbing [1] *warp* d/ *thread* d/	4-5 4-5	4-5 4-5	5 5	4-5 4-5	4-5	4
Artificial light [2] a/	4	3-4	4-5	3-4	4-5	3

process of polyamide, wool and natural silk fibre (lower degree of dye exhaustion in a bath) promotes a more even fibre dyeing. The resulting colouring of textile materials by bio-dyes is characterised by greater uniformity than in the case of conventional organic dyes

■ The relative intensity of fibre colouring, expressed by the K/S parameter, is higher for conventional organic dyes than for bio-dyes

■ The use of higher dye concentrations in a dye bath allows to get darker colouring; dye exhaustion in a dye bath, for both conventional organic dyes and bio-dyes, is at the same level as in the case of natural silk. Slightly lower values of dye exhaustion in a dye bath (97 - 98%) were noted in the case of bio-dyes used for polyamide and wool.

■ Values of the colour fastness (washing, alkaline and acidic sweat, distilled water, dry and wet rubbing) of wool, silk and polyamide knitted fabrics tested after dyeing with bio-dyes are very good, for the most part there is no colour change of the samples of textile products tested

■ Colour fastness to artificial light is lower (by 1 degree) for bio-dyes than conventional organic dyes

■ The bio-dyes developed may be an alternative to dyeing products of wool and natural silk fibres as well as polyamide with classical organic dyes

■ The bio-dyes synthesis method using fungal laccase as an oxidant may be an alternative for the preparation of this type of dye. The use of enzyme biocatalysts allows to reduce the energy, water and reagents used during the synthesis and the improvement of biotechnological processes leads to reducing costs associated with the bioprocess itself as well as the enzyme preparation process. Biocatalytic alternatives are particularly needed in oxidation process (for example, dye synthesis), which are still carried out with different organic and inorganic oxidants (for example, chromium compound, permanganate, periodate). There is clearly a definite need for catalytic alternatives employing clean primary oxidants such as oxygen and environmentally compatible catalysts such as enzymes (for example, fungal laccase).

Acknowledgements

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