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Wastewater effluents analysis from sustainable algae-based blue dyeing with phycocyanin

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Abstract

Sustainability in the textile industry is a challenge with an imminent need to be tackled. One approach can be focused on replacing fossil-based dyes with renewable and less polluting alternatives. In this sense, this study focuses on validating the suitability of an innovative natural-based blue dye, phycocyanin, sourced from *Spirulina platensis* microalgae. The laboratory-based experimental approach envisages the exhaustion dyeing of pre-mordanted cotton and bleached wool with phycocyanin-rich extract, representing the sustainable blue dye. The color characterization of naturally blue-dyed fabrics was performed via CIE*Lab* coordinates, depth of color by color stregth values (*K*/*S*), values of dye exhaustion, and colorfastness to laundering and light. The results indicate suitable dyeability with the natural blue dye, with process improvements a possibility. The main environmental character of this process was analyzed from the dyeing effluent characterization perspective. Measurements of chemical oxygen demand, biochemical oxygen demand, and metal content were performed on effluents resulting from dyeing processes with variable parameters, to analyze the influence of mordant use, process temperature, time, and pH. Findings indicate that the application of optimum dyeing process conditions results in the lowest oxygen demand values, suitable for further wastewater reuse, according to international industrial effluent limitations. The biological fungi wastewater treatment resulted in the reduction of biochemical oxygen demand and chemical oxygen demand values by around 80%, comparable with the industrial process, validating the sustainable character of using algae-based phycocyanin in the bath exhaustion dyeing process.

Keywords

Wastewater analysis, natural blue dyeing, phycocyanin, microalgae, biochemical oxygen demand, chemical oxygen demand, metals

The environmental impact of the textiles industry is of great importance, as the quantity of clothes bought in the EU has increased by 40% in the last decades, generating a proportional evolution of the dyeing process.[1] This process requires approximately 150 l of water for each kilogram of dyed fabrics and discards a corresponding amount of wastewater effluents rich in process auxiliaries.[2] One of the main hazard creators for environmental safety is represented by untreated dyes reaching water bodies. They are demonstrated to generate, coloration, which further impedes photosynthetic processes and is finally indirectly linked to the development of various types of cancers.[3] Water coloration is one of the visible side effects of the textile dyeing industry generating a decrease in water transparency, thus obstructing light penetration in water bodies and affecting the ecosystem as a whole.[4] A vast amount of research has been performed in the analysis and characterization of wastewater effluents and defines some of the most important parameters to be measured, such as chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), metals content, coloration, and others.[5] These define the

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quantity of organic and inorganic matter, metals, and auxiliary elements, that decrease the possibility of contaminated wastewater reuse. These effluents are qualitatively categorized in correspondence to the types of colorant matter used in the process,[6] thus giving great importance to the initial raw materials and auxiliaries used in the dyeing process.

The blue color of textiles can be derived from different chemical structures depending on the fiber nature[7] from both natural[8,9] and synthetic sources,[10] and the interest is not only in dyeing but also in the discolouration.[11] Phycocyanin is a water-soluble accessory photosynthetic pigment.[12] This main blue chromoprotein found in cyanobacteria is characterized by a structure of open chain tetrapyrroles,[13] as can be observed in Figure 1.

Spirulina platensis is characterized by unicellular blue-green algae, due to the main chromophore contained, phycocyanin. These cyanobacteria grow mainly in tropical areas, in aquatic ecosystems, such as lakes, ponds, or tanks. Industrial cultivation is performed in open tanks or photobioreactors with fresh water or wastewater.[14] A series of cultivation parameters may be varied and optimized for enhancing the yield of the compounds of interest, and these include the nutrient medium,[15] carbon source,[14] temperature,[16] water quality,[17] light,[18] pH[19], and many more.[14]

This highly characterized colorant matter is used in a variety of industrial applications such as in food, cosmetics, nutraceuticals, pharmaceuticals, medical, and many more contexts.[11] Nevertheless, there is no evidence of phycocyanin, blue colorant matter being used in the textile industry.

The textile industry is one of the main consumers of potable water and, implicitly, the main producer of wastewater.[20] In this sense, it is of great importance to give consideration to the treatments applied to these effluents and, if possible in the most favorable case, to the type of compounds used in the textile processes.

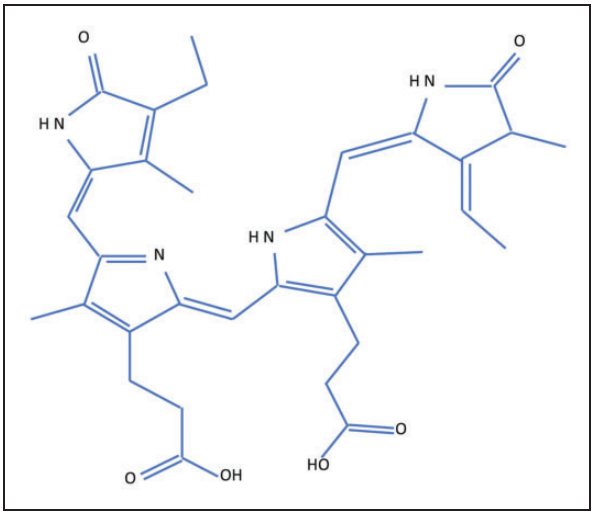

Figure 1. Phycocyanobilin chemical structure.

International regulations limit discharges into wastewater treatment plants,[21] therefore the sector dedicated to the treatment of various wastewater effluents has demonstrated and applied successful types of water treatments, aimed at further water reuse. These methods can be physico-chemical, biological, or a combination.[3] The physico-chemical wastewater treatments, on many occasions, are not considered environmentally friendly.[22] As an alternative, the category corresponding to biological methods has been successfully applied for textile effluent degradation.[23] Various microorganisms, like fungi, bacteria, yeasts, and algae, are acknowledged to be capable of mineralizing dyes and decolorizing effluents, under specific environmental conditions.[24] Analysis of the efficiency of the microorganisms involved in biological wastewater treatment tends to reveal favorable characteristics of the fungi's effectiveness when compared to bacteria. With the generation of high-added-value by-products throughout the process, there is a possibility of treated water reuse in animal and human food production and additional increased capability of degrading recalcitrant compounds.[25,26] Yeasts are used in highly contaminated wastewater effluents, in acidic conditions.[27] Meanwhile, microalgae are used in the treatment of wastewater effluents in the tertiary and quinary treatments, based on their capability of using nitrogen and phosphorus for their growth.[28]

Considering the scarcity of naturally occurring blue dyes, this study proposes the validation of the application of a sustainable algae-based blue dye, phycocyanin, in the textile industry. As previously mentioned, phycocyanin is widely used in industrial applications in various industries, but very few publications are available focusing on algae-based dyes applied in the textile industry. In this sense, the dyeability of cotton and wool fabrics with phycocyanin was assessed in this study, via the conventional textile exhaustion dyeing process. The influence of various parameters, such as dyeing temperature, time of the process, pH, and mordants employed in the process, on parameters such as COD, BOD_5, and metals were analyzed. Biological fungi wastewater treatment was applied for the identification of BOD_5 and COD parameters in reduction efficiency, to confirm the treatment possibility, and thus sustainable character, of these effluents.

Materials and methods

Materials

Standardized natural fabrics such as commercial wool (James Heal, England) and bleached cotton, of 200 g/m^2, EMPA221 (Intexter UPC, Spain), in compliance with ISO 105-F01, were purchased for the dyeing experiments. Distilled water was used as the aqueous base for the experimental dyeing liquors.

A series of eight mordants (Table 1), synthetic and biomordants, were selected to be applied in the study for the analysis of their influence on dyeing wastewater parameters. Table 1 contains the concentration of mordant employed, indicated as a percentage reported to the weight of the fabric to be treated (w.o.f.). In this sense, the [%] w.o.f. indicates that for every 100 g of fabric 1 g of mordant is employed. Every mordant was used separately, one mordant and one individual piece of fabric.

The dye used in the dyeing processes was phycocyanin-rich liquid extract sourced from *Spirulina platensis biomass* (tailored production by Banco Espãnol de Algas (BEA), Spain). Standard solutions used for inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis were supplied from Merck. The experimental wastewater biological treatment was performed with a fungi mixture provided by a local wastewater treatment plant (Muro de Alcoy, Spain.

Methods

Spirulina platensis cultivation. The *Spirulina platensis* microalgae was cultivated by BEA (Spain), using strains from their collection. Open raceway pond-tailored cultivation, for increased yield of phycocyanin synthesis, was performed under stress conditions (nitrogen depletion) for the cyanobacteria. This was completed with the optimized parameters of CO_2 and light. The biomass was then harvested and filtered, for separation from the culture medium and stored in –20°C conditions.

Extraction and quantification of phycocyanin, blue colorant matter

Extraction of the blue colorant matter. The fresh *Spirulina platensis* biomass was used for the extraction of phycocyanin, the blue dye. A simple extraction process was applied to the cyanobacteria biomass which, due to the weak cellular wall of this microalgae, is easily breakable. In this sense, a cellular wall weakening was performed through the subjection of the biomass to three freeze-defrost cycles, at temperatures of –20°C and 4°C respectively, for a duration of 12 h for each step, representing the preparation step before the mechanical colorant extraction. Protection from light was applied to the complete process. Further, mechanical cell rupture was performed via mortar and pestle on the biomass, with the addition of water adjusted at pH 7, and glass spheres, for increased extraction yield. The final separation process was carried out by centrifugation for 30 min at 4000 rpm, followed by filtration, with filtration paper. The resulting extract was improved with the addition of 20% of ammonium sulphate (supplied by Sigma-Aldrich, Spain), for stabilizing properties.

Quantification of the extracted blue dye. The extraction approach of phycocyanin considers the intimate connection of the three chromophores forming the phycobilisome structure in the cellular structure, and therefore their interconnection in terms of quantification. These chromophores present clear fluorescence and light absorption capacity, allowing their quantification with the use of spectrophotometrical methods.[29]

The colorant of interest in this specific study is phycocyanin, with the maximum absorbance wavelength of 615 nm (indicated in the corresponding equation). Further, the intimately connected allophycocyanin's maximum absorbance wavelength is 652 nm, generating

Table 1. Mordants used individually for wool and cotton pretreatment in the phycocyanin dyeing process

Mordant	Chemical formula	Supplier	(%) w.o.f.
Cream of tartar (potassium acid tartar or potassium bitartrate)	$KC_4H_5O_6$	Gran Velada (Zaragoza, Spain) Sigma Aldrich (Madrid, Spain)	6
Alum (potassium alum)	$AlK(SO_4)_2$	Gran Velada (Zaragoza, Spain) Sigma Aldrich (Madrid, Spain)	20
Ferrous sulphate	$FeSO_4$	Sigma Aldrich (Madrid, Spain)	3
Tartaric acid	$C_4H_6O_6$	Sigma Aldrich (Madrid, Spain)	6
Tannic acid	$C_{76}H_{52}O_{46}$	Sigma Aldrich (Madrid, Spain)	2
Aluminum triformate	$C_3H_3AlO_6$	Sigma Aldrich (Madrid, Spain)	10
Myrobalan-tannin	$C_{76}H_{52}O_{46}$	Greening (Bédarieux, France)	25
Oak gall-tannin	$C_{76}H_{52}O_{46}$	Greening (Bédarieux, France)	25

the need for the employment of both absorbance values for the quantification of phycocyanin.[30]

The liquid phycocyanin-rich extract, the blue dye in this study, was quantified through the analysis of ultraviolet (UV) spectral absorbance, due to the wavelength-based absorbance capacity of this chromophore. The spectral investigation was performed by molecular spectroscopy using a Thermo Scientific Evolution 60S UV-Visible Spectrophotometer (Thermo Fisher Scientific, Waltham, USA). The calculation of the amount of mg/ml of phycocyanin in solution, via Bennett and Bogorad's[31] equation and applying the extinction coefficients defined by Bryant et al.[32]

$$PC = \frac{(A615–0.474(A652))}{5.34}$$

where PC represents phycocyanin, and A is the value of absorbance at the indicated wavelength.

Fabrics pretreatment. In order to observe the influence of different factors in the wastewater parameters, wool fabrics were bleached, and both wool and cotton textiles were pre-mordanted.

Wool fabrics were subjected to a bleaching pretreatment at 55°C, for 60 min. The bleaching solution, characterized by a bath ratio, M:L = 1:15 (for each gram of untreated wool fabric 15 ml of bleaching solution was added), comprised of a mixture of decalcified water with 20 ml/l of 30% of hydrogen peroxide (Fisher Scientific), 2 g/l of pyrophosphate tetrasodium (Sigma Aldrich), 2 g/l nonionic Clarite detergent (Huntsman) and the solution pH was adjusted to a level of 8.5–9 with ammonia 25% (PanReac). Further, the fabrics were rinsed and dried at room temperature.

A pre-mordanting process was applied to both fabrics, bleached wool and cotton, with a mordant solution, as indicated in Table 1, at 85°C for 45 min, at material to liquor ratio of 1:40.

Dyeing process and wastewater sampling

Dyeing process. Phycocyanin is a natural colorant and is considered a mordant dye, due to the well-known lack of affinity between natural colorants and textile fibers. In this sense, pre-mordanted fabrics were used for the conventional dyeing process.

The exhaustion dyeing process was applied to the pre-mordanted cotton and bleached wool fabrics, where various parameters, such as temperature and time, were modified for the identification of their influence on the quality of the dyeing effluents resulting from the process. Table 2 details the variations of the dyeing process parameters, compared to the optimum combination (identified in previous studies), marked in Table 2. The different temperature values selected for the dyeing process follow the limitations, with slight variations, imposed by the sensitivity of the colorant matter, stable up to 65°C.[13,33] The optimum conditions were defined in previous studies through mordants, pH, time, and temperature variations, considering the degradation of the colorant matter and the fastness of the obtained dyed fabrics.

A particular experimental case involving cotton metamordanting with cream of tartar was performed for comparison purposes. The metamordanting process involved the application of the mordant directly into the dyeing liquor meanwhile subjected to the conditions applied in the dyeing process.

All of the textile dyeing processes were performed with the laboratory exhaustion dyeing device, Ugolini Redkrome (Italy), which consists of an insulated chamber with a stainless steel interior and a varnished exterior, where the rotating disk to which the tinting glasses are attached is placed. The temperature increase is provided with infrared lamps. Cooling of temperature is done via air/water interchange.

Subsequent to the dyeing process, the samples were cold-water rinsed, and further washed with non-ionic textile detergent (Clarite, Huntsman) for 30 min at a temperature of 50°C, with continuous shaking, and finally dried at room temperature.

Table 2. Dyeing process parameter variables

	Parameter					
Fabric	Bath ratio	Time (min)	Temperature (°C)	pH	Temperature raise	Phycocyanin concentration (% w.o.f.)
Cotton	40:1	60[a]	65[a]	7[b]	2°C per min	2
		90	50			
Wool	40:1	60[a]	65[a]	5[b]		
		90	50			
		60	75			

[a]Optimum dyeing conditions.
[b]Value of pH, adjusted with buffer by Hach-Lange.

Wastewater sampling. The resulting effluent of the dyeing process was cooled at room temperature, in the initial recipients, after the removal of the dyed fabric. Immediately after cooling the wastewater was transferred to a container and kept without ligth. a and stored in an obscure environment at a constant temperature of 4°C.

Dyed fabrics characterization. CIE*Lab* color space was measured to objectively determine the color coordinates of the dyed cotton and wool fabrics. The DC 650 spectrophotometer (supplied by Datacolor, Spain), with 10° observer and D65 illuminant, was employed. The measurement was performed according to the UNE-EN ISO 105-J01:2000 European standard for textiles characterization. The color differences were calculated following the equation proposed by the International Commission on Illumination (CIE).[34]

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

$$\Delta L^* = L^*_{sample} - L^*_{reference\ sample}$$

$$\Delta a^* = a^*_{sample} - a^*_{reference\ sample}$$

$$\Delta b^* = b^*_{sample} - b^*_{reference\ sample}$$

The reference sample in this study is represented by the non-mordanted sample dyed at optimum conditions (65°C for 60 min).

Color strength (K/S) assessment was performed via the measurement of the dyed fabrics' reflectance values with a Perkin Elmer Lambda 950 UV-VIS spectrophotometer (Spain) and the calculation with the Kubelka-Munk equation.[35,36]

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

where K is the absorption coefficient, S is the scattering coefficient and R reflectance.

Dye exhaustion value assessment was based on the plot of the calibration curve, by spectrophotometric measurements of the original dyeing liquor, with the UV Thermo Scientific Spectrophotometer (Evolution 60S). The main dyeing liquor was used as a stock solution, from which various dilutions were performed and measured. The maximum absorbance wavelength of the dye $\lambda_{\text{max-phycocyanin}} = 620$ nm represented the basis for plotting the calibration curves. The following step was represented by the absorbance spectrum of the wastewater effluents obtained from the dyeing process.

The Lambert-Beer equation[37] was used for the quantification of the dye in the analyzed samples.

$$A = \varepsilon c l$$

where A is absorbance, ε is molar absorption coefficient, c is molar concentration and l is the optical path length.

The difference between the initial dye concentration (2% w.o.f.) and the concentration of dye measured in the dyeing wastewater effluents, represents the dye absorption coefficient.

Colorfastness to laundering and light. European standards were used for the assessment of the fastness to laundering and light of the dyed samples, following the corresponding standardized methodologies UNE-EN ISO 105-C06:2010[38] and UNE-EN ISO 105-B02:2014[39] respectively.

Laundering fastness assessment was performed with a Gyrowash apparatus (James Heal, UK), which involves immersing the dyed fabrics into canisters containing 150 ml water and 0.6 g of detergent, and 10 steel balls, for 45 min at 40°C. Color change was tested against a grey scale in accordance with UNE-EN ISO 105-A02, the staining on white fabrics was also evaluated by means of greyscale according to UNE-EN ISO 105-A03.

Lightfastness assessment was realized in accordance with UNE-EN ISO 105-B02, color fastness to artificial light, with a Xenon arc fading lamp (James Heal, UK), where the samples were pre-conditioned via spraying with water and then exposed to the lamp for cycles of 16 h. Samples were evaluated by means of a blue scale.

Wastewater analysis. Wastewater effluents obtained from the different dyeing experiments were subjected to quality parameters analysis, such as BOD_5, COD, and metal content, throughout standardized international methods.

Table 3 summarizes the correspondence between the fabric treatments and the assessment performed.

BOD_5 was determined via the pressure gauge according to the Standard Method ISO 5210-D, a procedure based on standardized international wastewater analysis. COD was determined via spectrophotometry according to the adapted procedure, and based on normalized international methods.

Metal content, focused on the identification of aluminium and iron content, by employing the ICP AES S.M. 3120-B (ed.22), refers to the measurement of metals in water by plasma emission spectroscopy. Sample preparation for analysis implies the appropriate addition of nitric acid, which is further diluted to a predetermined volume and mixed before the analysis.

Table 3. Different mordant textile process-wastewater effluent assessment relationship and rationale

Mordant	Process	Wastewater assessment	Justification
No mordant	Dyeing	BOD_5, COD	Influence of the organic colorant matter on the dyeing effluents quality
Cream of tartar (potassium acid tartar or potassium bitartrate)	Pre-mordanting and metamordanting	BOD_5, COD, metal content	Influence of the organic colorant matter and mordant in the dyeing effluents quality
Alum (potassium alum)	Pre-mordanting	Metal content	Validation of effective use of synthetic auxiliary with the generation of quality dyeing effluents
Ferrous sulphate	Pre-mordanting	Metal content	
Tartaric acid	Pre-mordanting	Metal content	
Tannic acid	Pre-mordanting	Metal content	
Aluminum triformate	Pre-mordanting	Metal content	
Myrobalan-tannin	Pre-mordanting	Metal content	
Oak gall-tannin	Pre-mordanting	Metal content	

BOD_5: biochemical oxygen demand; COD: chemical oxygen demand.

The method includes the sample ionization at a high temperature, for the generation of an emission spectrum, which further allows the identification of the content of metals. Samples were tested in triplicate for each measurement. The limit of detection (LOD) was 30,000 ug/l for iron and 20,000 ug/l for aluminum and RSD% was lower than 5% for iron and lower than 7% for aluminum. The limit of quantification was 0.02 mg/l for both of the metals tested.

Wastewater fungi treatment experiments. The wastewater effluents obtained from the cotton and wool dyeing process were subjected to fungi treatment experiments, to assess the efficacy of this biological treatment when phycocyanin dye is employed in the textile finishing process. The fungi solution is represented by a combination of different anaerobic microorganisms such as heterotrophic bacteria and protozoan species capable of degrading organic matter. This process involves the use of these nutrients for their reproduction without the need for solar energy, being bio-reducers.

The fungi solution was combined with the dyeing wastewater and maintained at a stable temperature ($20 \pm 1°C$), in a closed recipient for a duration of 24 h, performing the anaerobic digestion phase. A number of types of samples were considered when COD and BOD_5 comparative analyses were envisaged: fungi solution, centrifuged fungi solution, treated wastewater with fungi, and centrifuged treated wastewater.

Results and discussion

Phycocyanin characterization

The blue dye used in this study, the *Spirulina platensis*-based phycocyanin, is characterized by fluorescence emission peaks, in the wavelength range 635–648 nm.[40]

The phycocyanin concentration value was calculated according to a calibration curve. Results show that the concentration obtained from the extract was 0.738 mg/ml. This concentration was used to define the percentage of colorant used in the dyeing process.

Dyed fabrics color characterization and fastness assessment

Figure 2 shows cotton and wool dyed samples. This image shows the fibers in order to get an idea of the color obtained. The mordanted treatment and repetitions show slight differences which are objectively quantified by color characterization.

*CIE*Lab *color characterization.* Phycocyanin-dyed mordanted and non-mordanted cotton and wool fabrics were subjected to color characterization by measuring the CIE*Lab* color coordinates. The complete set of results is presented in Table 4. The results of the color characterization of the dyed samples, are defined by L^* referring to the luminosity and intensity of a color (where $L^* = 100$ is pure white), and the color coordinates a^* and b^*, which define the color space. In this sense, $a^* < 0$ indicates green, $a^* > 0$ indicates red shades; $b^* < 0$ indicates blue color space, and $b^* > 0$ specifies yellow.

According to Table 4, it can be observed that, among cotton experiments, the most intense color is obtained with the higher temperature applied (60°C). It must be noted that the least blue color shades achieved, as indicated by the b^* coordinate are at 50°C where $b^* > 0$, which means a slight tendency to yellowish. The bluest samples were obtained with the use of the optimum conditions, with b^* comprised between –1.55 and –0.15. On the other hand, the

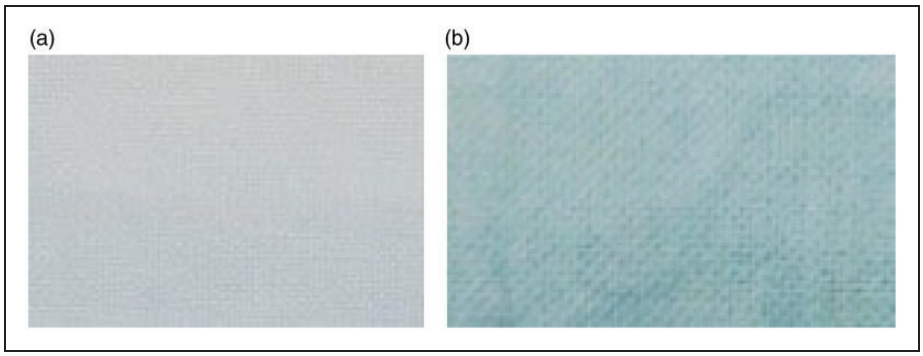

Figure 2. Samples dyed with phycocyanin: (a) cotton and (b) wool.

Table 4. CIELab coordinates of the phycocyanin dyed samples and *K/S*

Reference	Sample	Color coordinates			
		*L**	*a**	*b**	*K/S*
M1	Cotton no mordant (50°C, 90 min)	79.15	–0.99	13.36	29.81
M2	Cotton no mordant (65°C, 60 min) (repetition 1)	83–69	–1–76	–1–55	33–47
M3	Cotton no mordant (65°C, 60 min) (repetition 2)	85–98	–1–17	–0–99	34–70
M4	Cotton no mordant (65°C, 60 min) (repetition 3)	83–87	–0–83	–0–15	35–11
M5	Cotton Cream of tartar 3% (premordant)	85–94	1–11	0–94	34–18
M6	Cotton Cream of tartar 3% (metamordant)	84–07	–1–03	–0–04	33–59
M7	Wool no mordant (50°C, 90 min)	73–73	–2–54	19–19	21–39
M8	Wool no mordant (65°C, 60 min) (repetition 1)	76–31	–3–43	3–56	20–71
M9	Wool no mordant (65°C, 60 min) (repetition 2)	75–64	–5–11	4–49	21–46
M10	Wool no mordant (65°C, 60 min) (repetition 3)	73–61	–4–73	2–51	21–69
M11	Wool no mordant (75°C, 60 min)	70–04	–3–36	4–46	19–38

K/S: depth of color.

wool samples present a darker coloration than the cotton samples, with the bluest tones defined by the optimum dyeing conditions (60°C), considering that higher temperatures will degrade phytocomponents in natural dye. Nevertheless, these values are validated, in terms of quality, referring to colorfastness in Table 5.

Regarding the color strength (K/S) values, indicated in Table 4, it can be observed that similar values were obtained among the optimum dyeing conditions, for cotton and respectively wool experimental cases. Nevertheless, similarity among the results is observed, even though there is a variation in processing temperature and time, the values obtained must be correlated with the fastness results indicated in Table 5, confirming the better performance of the wool dyeing with phycocyanin, at optimum conditions.

Figure 3 locates the samples in the color space, according to coordinates a^* and b^*, and positions most of the samples in a similar color area, a blueish area, with green/yellow influence due to the residual chlorophyll existing in the colorant-rich extract.

Finally, Figure 3 validates the similar color (showed in Figure 1) obtained via exhaustion dyeing of cotton and wool fabrics with specific influences provided by parameters variations.

Dye exhaustion values. The percentage of the remaining dye in the dyeing effluents was calculated as the dye absorption coefficient and represents the difference between the initial dye used in the process (2%) and the remaining dye in the wastewater effluent. The obtained results are presented in Figure 4. Acceptable coefficients are found with better results obtained in the experimental case of wool dyeing. Nevertheless, similar results are obtained among all the analyzed samples, characterized by dye absorption coefficient of approximately 60%.

Laundering and lightfastness. The dyeing process quality was assessed through laundering and light fastness measurements, according to ISO standards, with the complete set of results indicated in Table 5. Dyed cotton fabrics show poor behavior with regard to

Table 5. Laundering and lightfastness of the dyed fabrics with phycocyanin

Sample	Color change	Staining						Light fastness
		Wool	Acrylic	Polyester	Polyamide	Cotton	Acetate	
Cotton no mordant (50°C, 90 min)	1–2	4–5	4–5	4–5	4–5	4–5	4–5	1
Cotton no mordant (65°C, 60 min) (repetition 1)	1	4–5	4–5	4–5	4–5	4–5	4–5	1–2
Cotton no mordant (65°C, 60 min) (repetition 2)	1	5	5	5	5	5	5	1–2
Cotton no mordant (65°C, 60 min) (repetition 3)	1	4–5	4–5	4–5	4–5	4–5	4–5	1–2
Cotton cream of tartar 3% (premordant)	2	4–5	4–5	4–5	4–5	4–5	4–5	4
Cotton cream of tartar 3% (metamordant)	1	4–5	4–5	4–5	4–5	4–5	4–5	4–5
Wool no mordant (50°C, 90 min)	1	4–5	4–5	4–5	4–5	4–5	4–5	1
Wool no mordant (65°C, 60 min) (repetition 1)	3–4	4–5	4–5	4–5	4–5	4–5	4–5	4
Wool no mordant (65°C, 60 min) (repetition 2)	4	4–5	4–5	4–5	4–5	4–5	4–5	3
Wool no mordant (65°C, 60 min) (repetition 3)	4	4–5	4–5	4–5	4–5	4–5	4–5	3
Wool no mordant (75°C, 60 min)	3	4–5	4–5	4–5	4–5	4–5	4–5	1

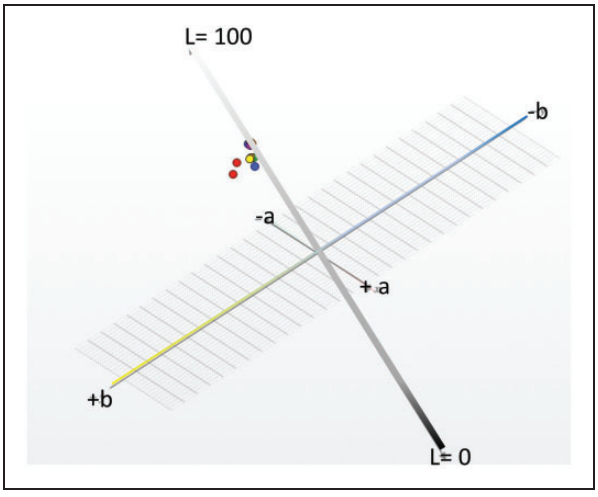

Figure 3. Color coordinates plot of the blue dyed fabrics with phycocyanin.

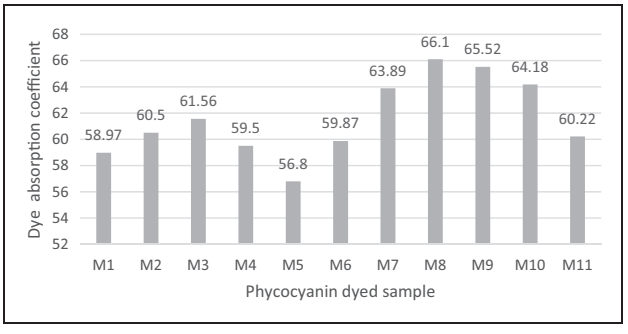

Figure 4. Dye absorption coefficient of the phycocyanin dyed cotton and wool fabrics.

color change and light, with slight improvements obtained with the pre-mordanting process, indicating upgrading possibilities. Table 5 shows results from the cream of tartar mordant, although the rest of the mordants did not show better results. Further studies on the concentration of mordants should be conducted. On the other hand, better results are obtained for the wool samples dyed with phycocyanin, with boosts revealed with the application of optimum dyeing conditions.

From Table 5, it can be observed that wool shows higher color fastness than cotton. This can be explained due to the amphoteric behavior of wool. The carboxyl groups on the phycocyanin can react with amino groups on wool establishing amide binding, whereas on cotton only hydrogen bonds occur with hydroxyl groups on cellulose, which are weaker giving lower fastness.

COD and BOD_5 of dyeing wastewater results

COD and BOD_5 analyses were performed on wastewater effluents obtained from the exhaustion dyeing processes with phycocyanin blue dye applied on cotton and wool fabrics. Parameter variations were performed for the identification of the influence of temperature, time, pH of the process, and use of mordants for comparison with previously defined optimum conditions. This analysis focused on validating the optimum conditions as the most sustainable ones from an effluent quality characteristics point of view.

Cotton dyeing process wastewater effluents analysis. Initial dyeing experiments were performed, on fabrics without pre-mordanting treatment, for the assessment of the influence of time and temperature variation of the wastewater effluent parameters. Figure 5 illustrates a comparative analysis among different process temperatures (50°C and 65°C) and process times (90 min and 60 min), for the identification of their influence on the values of COD and BOD_5.

Higher values of COD or BOD_5 would be directly related to a higher quantity of organic matter in

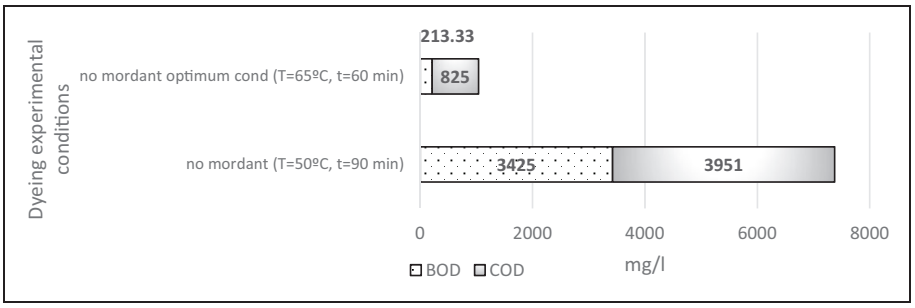

Figure 5. Influence of dyeing process temperature on chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) wastewater effluent values.

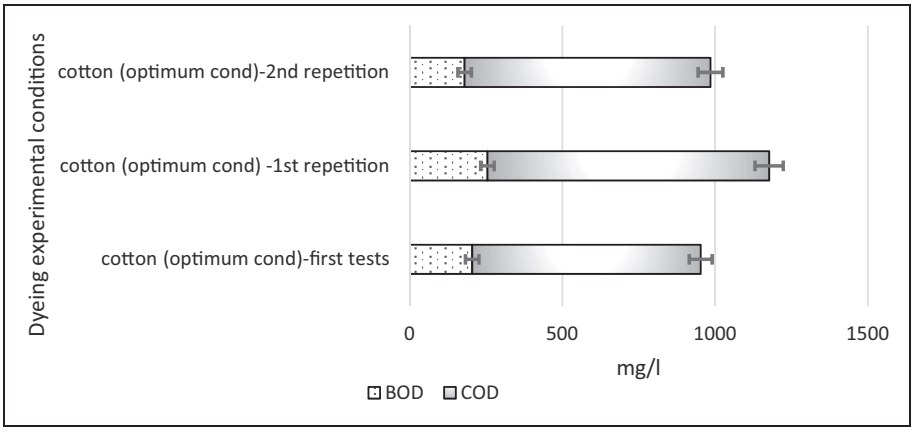

Figure 6. Validation of cotton dyeing process repeatability considering optimum conditions.

wastewater due to the lower efficiency of the dyeing process. As can be observed in Figure 5, lower quantities of organic matter were present in the obtained wastewater with the application of optimum dyeing conditions.

The final validation of the process temperature and its impact on the quality of the dyeing wastewater effluent is analyzed in Figure 5 in the experimental setup where no mordant is applied. It reveals lower values of BOD_5 and COD when the process temperature is 65°C in contrast to dyeing at 50°C. This can be justified by the fact that these conditions influence a higher absorption of the colorant matter, the phycocyanin, by the cotton fabric.

At the same time, the presumable protein chromophore degradation should not be disregarded. It is confirmed by the kinetics degradation studies that phycocyanin stability is valid up to 65°C at pH 5.[20,21] But, interference with this situation, in this study, may be occurring, during the cotton dyeing process performed at pH 7, destabilizing the chromoprotein. In this sense, not only the applied temperature and pH may have a slight influence on the degradation of the colorant material during the dyeing process, but also this highlights the need for mordant use due to the need for dye-textile substrate affinity.

Further experiments confirm the repeatability of the dyeing process and validate the optimum dyeing conditions, temperature 65°C and process time of 60 min. The obtained values present very slight variations, as observed in Figure 6. This is confirmed by the standard error bars which slightly overlap, indicating the fact that the difference is not statistically significant.

Further to the selection and validation of the optimum dyeing condition, defined by 65°C during 60 min, the influence of the various momentums (pre-mordanting and metamordanting) of mordanting with cream of tartar was analyzed. In this sense, and according to Figure 7 the lowest values (the most favorable scenario) were obtained with the application of the mordant during the dyeing process in the dyeing bath (referred to as metamordanting). Considering the cream of tartar is organic matter, this reduction implies that despite having included organic matter in the process, there is a clear reduction in the presence of dye in wastewater, thus confirming process efficiency. This is a possible indication of an increased affinity of the dye with the cotton fabric due to the presence of cream of

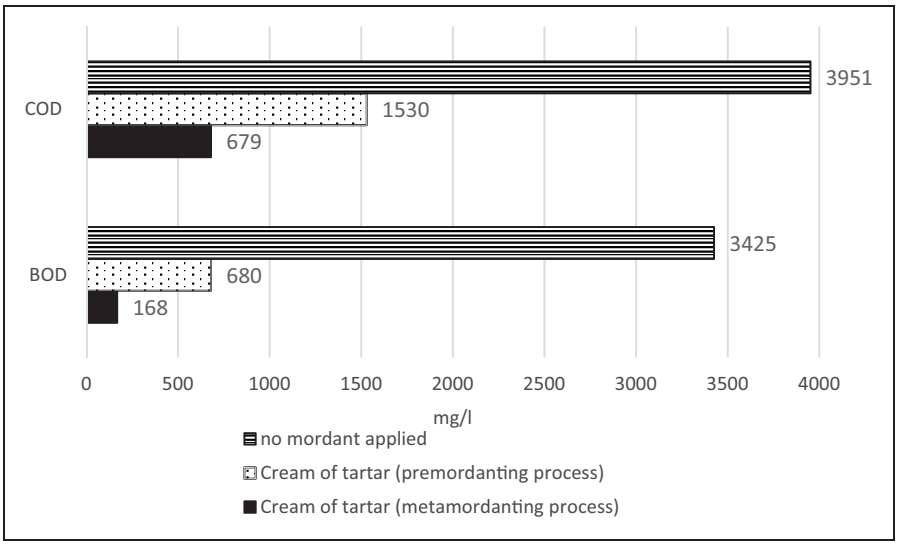

Figure 7. Influence of inclusion of cream of tartar mordant in chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) levels in cotton dyeing.

tartar as a mordant, as has been demonstrated with different dyes.[19] Basically, it must be due to the chemical structure of the cream of tartar, potassium bitartrate. Its hydroxyl groups will react with both hydroxyl groups from cotton and the dye. Further, this indicates a lower yield for cotton reaction with phycocyanin when there is no mordant.

Furthermore, when the mordanting processes are compared, there is a considerable difference between pre-mordanting and metamordanting. The reduction in organic matter in the metamordanting case highlights the higher effectiveness of this process in comparison to the pre-mordanting. However, it must be remarked that metamordanting is reducing the quantity of dye in wastewater by more than 50%, compared with the situation where the dyeing process is performed without mordant involvement. It should be noted that the lowest values for COD and BOD_5 confirm that the concentrations used for mordant and dye during the metamordanting process are optimal.

Wool dyeing process wastewater effluents analysis. In the context of validation of optimum process conditions for wool dyeing with phycocyanin, the influence of process temperature in COD and BOD_5 values in the resulting effluent, and three different temperature variations were assessed (50°C, 65°C, and 75°C), and process length (50 min and 60 min). Figure 8 illustrates the obtained results and it can be observed that the lowest values were obtained when 75°C was applied during the process. These results do not reflect the most favorable conditions, due to exceeding the protein stability temperature.[20,21] Nevertheless, with reduced oxygen demand and favorable values, the optimum conditions are justified, as it has been demonstrated that there is high stability of phycocyanin at 65°C, in pH = 5 conditions.[20,21] Thus, the optimum conditions, do reveal the lowest negative impact on wastewater effluents resulting from wool dyeing with phycocyanin but discard the option of protein denaturation and validate a more efficient dyeing process.

Considering wool dyeing process wastewater effluent analysis, the validation of the repeatability of the selected optimum exhaustion dyeing conditions, without the use of mordants was performed. Figure 9 confirms the dyeing process validity, by the slight overlapping sections of calculated standard error between the BOD_5 and COD values, corresponding to various repetitions of the dyeing process.

Wastewater biological fungi treatment

An experimental study was performed on both non-mordanted wool and cotton dyeing process wastewater effluents, to identify the effectiveness of conventional biological wastewater treatment with the employment of fungi solution. For comparison purposes, effluents containing only fungi, no treatment wastewater, and treated wastewater, subjected to centrifugation process also, for sedimentation purposes, were analyzed. Table 6 reflects the obtained values of BOD_5 and COD for the analyzed wastewater effluents. It can be observed that the addition of the fungi increases the values of the parameters, but the centrifugation applied generated separable sedimentation from the wastewater effluent. In terms of BOD_5, EPA regulations for wastewater effluent discharge of 45 mg/l is an accepted value for average weekly limitation,[24] and according to

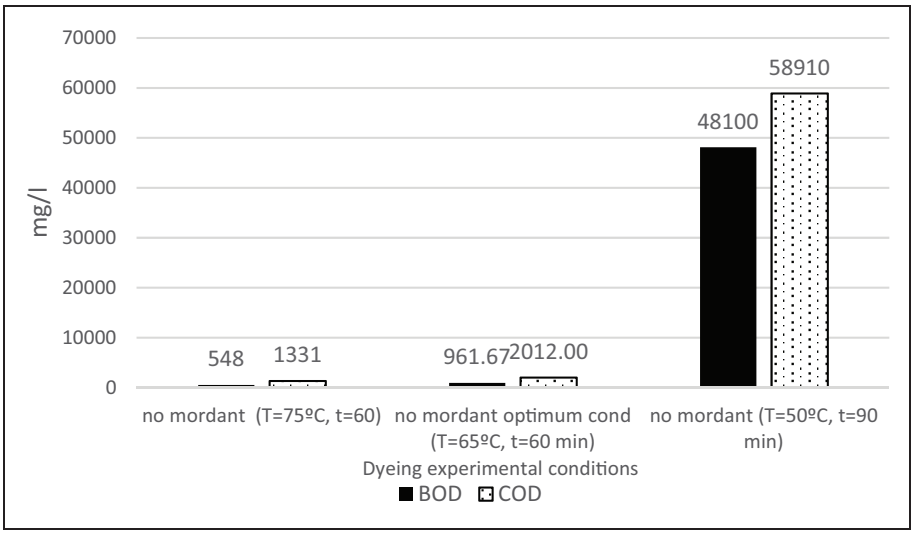

Figure 8. Wool dyeing process temperature influence on wastewater parameters (chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5)).

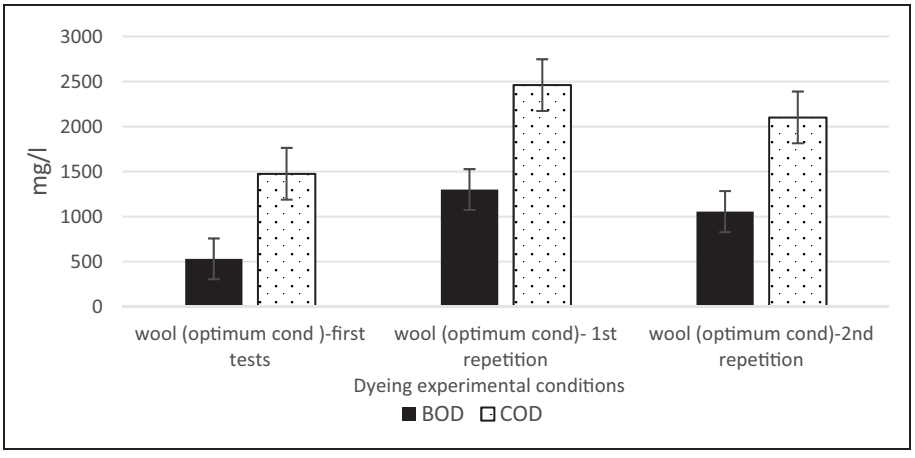

Figure 9. Validation of wool dyeing process repeatability considering optimum conditions. BOD_5: biochemical oxygen demand; COD: chemical oxygen demand.

Table 6, the cotton and wool wastewater from biological treatment is below the limits for wastewater. It is of great importance to highlight that the results obtained in this study are obtained at a laboratory scale, meaning the concentration of effluents is highly increased, as opposed to industrial wastewater effluents, which are mixed with effluents from different types of textile processes, generating an increased dilution level of the effluents. The limitations indicated are referring to industrial wastewater effluents limitations. In this sense, the laboratory scale results are acceptable.

The positive effect of biological wastewater treatment with fungi is reflected in the high percentage reduction of BOD_5 and COD parameters of the wastewater effluents (Figure 10) resulting from the dyeing of cotton (80.2%, respectively 66.87%) and wool

Table 6. Assessment of the wastewater effluent treatment with fungi

Wastewater effluent	BOD_5 (mg/l)	COD (mg O_2/l)
Original fungi solution	600	10,248
Fungi solution centrifuged	20	430
Wool wastewater	243	908
Wool wastewater treated with fungi	500	5728
Wool wastewater treated with fungi centrifuged	60	244
Cotton wastewater	202	664
Cotton wastewater treated with fungi	500	5234
Cotton wastewater treated with fungi centrifuged	40	220

BOD_5: biochemical oxygen demand; COD: chemical oxygen demand.

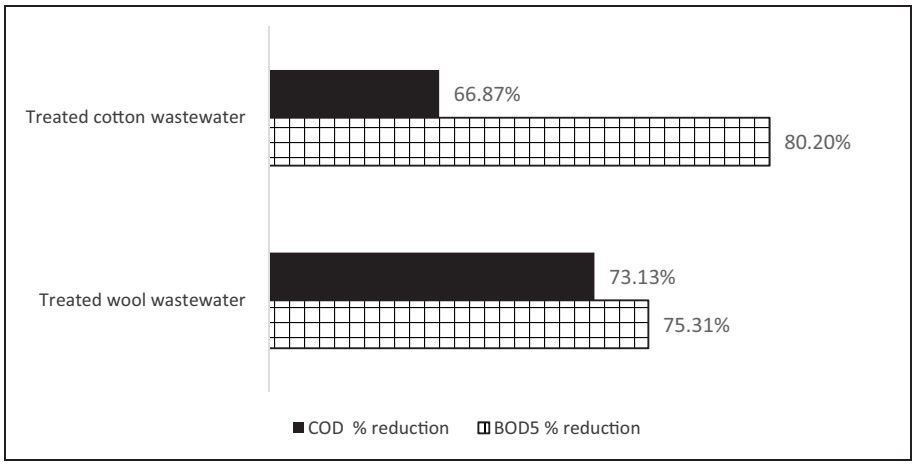

Figure 10. Chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) percentage reduction with fungi wastewater treatment.

(75.31%, respectively 73.31%). These results are comparable with the scientific literature and industrial fungi wastewater treatment applications.[23] Such an important reduction can be attributed to the effectiveness of fungi with the natural dye. Regarding the fungi treatment conditions, at the industrial wastewater treatment plant scale, the process involves the sedimentation of the fungi, and that is the justification of the sedimentation applied via centrifugation at the laboratory scale, for time-saving purposes.

Metal content analysis in cotton and wool dyeing wastewater effluents

Considering the existing regulations of metal content discharge limitation generated by dyeing wastewater effluents, the influence of mordants applied in the pre-mordanting of fabrics, on the metal content was analyzed. Spanish national limitations,[24] for aluminium and iron daily discharge limits, were used as a reference in these experiments.

Regarding cotton dyeing wastewater effluents, Figure 11 illustrates a comparison among the aluminum and iron concentrations assessed in the dyeing effluents, with the application of eight mordants in the pre-mordanting of cotton and bleached wool, prior to the dyeing process. It can be observed that the use of alum exceeds the daily limit of aluminum by approximately 11%, whereas the use of ferrous sulphate exceeds the iron limit by approximately 60%. The mordants with the highest values indicate that they do not represent the most efficient auxiliaries selection for this process, due to the high quantity of metals remaining in the effluent, thus they could be discarded from use in this specific dyeing process and validate the use of cream of tartar.

Wool dyeing wastewater effluents were analyzed in terms of aluminum and iron content, for compliance with Spanish national dyeing effluents daily limits discharge,[24] to compare the influence of the use of eight different mordants employed on these limiting ranges. In this case, as revealed in Figure 11, the aluminum daily limit is surpassed by the use of alum and aluminum triformate with over 90% and 60% respectively. In terms of iron content, ferrous sulphate tops the daily limitation with approximately 90%. The results reveal that the most compatible mordants with the wool fabrics are represented by the cream of tartar, tartaric acid, tannic acid, and the biomordants myrobalan and oak gall, due to the low quantities of the residual mordant present in the effluent. This residual mordant represents the chemical that did not exert its function in the finishing process.

Nevertheless, it should be considered that these aluminum and iron discharge limits, of 10 mg/l and 5 mg/l, are imposed for industrial discharges, and the results presented in this study are performed at a laboratory scale, thus a scale interference should be considered. Industrial discharges are always diluted, due to the common collection of all the complementary textile processes effluents, such as rinsing, reducing in this way the final metal or organic matter concentration of the final resulting wastewater effluent. This does not occur at a laboratory scale. In this sense, the obtained results can be considered successful, as even in their concentrated state, they are generally in compliance or easily corrected to comply with regulations. On the other hand, in future experiments, the percentage of exceeding mordants must be revised and correlated with performance, or consider the employment of the less contaminant ones.

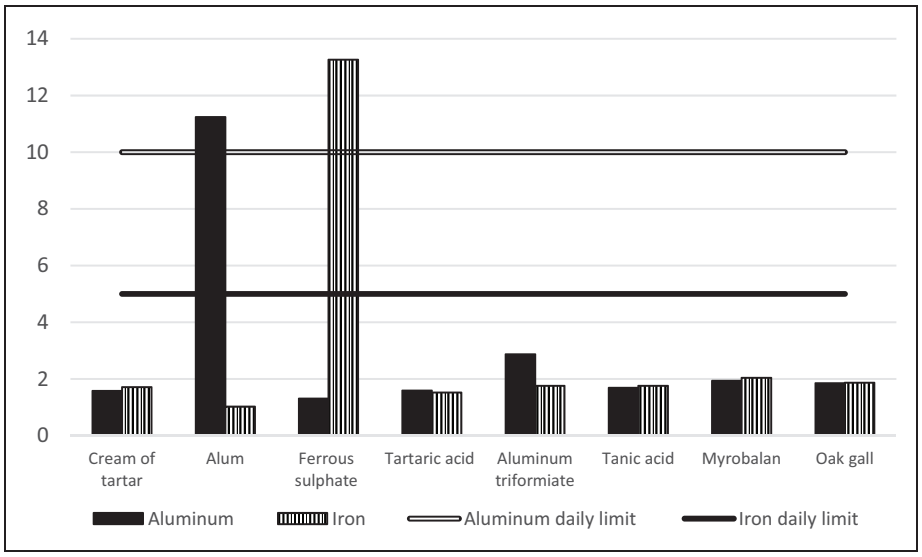

Figure 11. Iron and aluminum content of effluents resulting from pre-mordanted cotton fabrics dyed with phycocyanin.

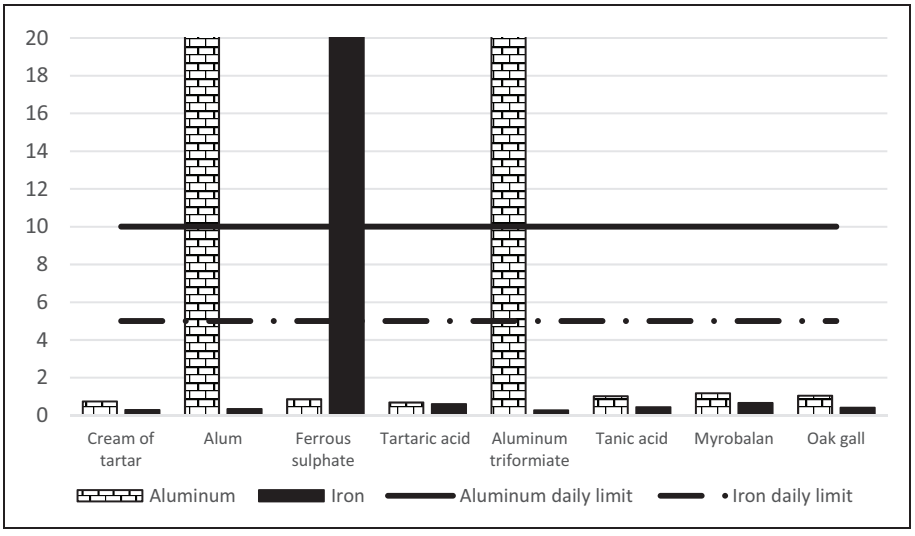

Figure 12. Iron and aluminum content of effluents resulting from pre-mordanted wool fabrics dyed with phycocyanin.

A comparison between Figure 11 and Figure 12 allows the comparison of cotton and wool wastewaters. There is a strong coincidence in the high presence of iron or aluminum for both wool and cotton for both alum and ferrous sulphate mordant applications, however, aluminum triformate shows a lower presence for cotton samples than for wool ones.

Conclusions

In the sustainability context, applied to the textile industry, the conscious selection of raw materials has driven research towards the conscious selection of the materials used in corresponding processes. In this sense, environmentally friendly dyes are the subject of interest for industrialization.

Blue natural and renewable dye, sourced from an innovative source, algae-based feedstock represents the focus of this study. The experimental approach confirms the possibility of dyeing cotton and bleached wool fabrics with blue dye, phycocyanin, sourced from microalgae *Spirulina platensis*. Limitations in process temperature, due to dye sensitivity, have facilitated the identification of optimum dyeing conditions, such as 65°C for 60 min. Textile substrates pre-mordanting experiments have revealed fastness improvements, indicating the possibility of further investigations to reach more competitive results for this natural dye.

Apart from the sourcing of this natural dye, the sustainability of the dyeing effluents represents the complete validation of the suitability of phycocyanin for a less polluting blue textile coloration process.

The dyeing effluents characterization results validated, in terms of parameters like COD and BOD_5, and metal content, the possibility of safe discharge, and reuse of these effluents for further activities.

Regarding the cotton dyeing wastewater effluents, it can be confirmed that the optimum process conditions, combined with the cream of tartar metamordanting process, reveal lower quantities of organic matter contained in the dyeing wastewater, thus possibly indicating an increase in the affinity of the dye with the cotton fabric. Nevertheless, according to the scientific literature, even though the selected process temperature (65°C) does not represent a negative factor, the pH 7 of this process may cause protein instability and therefore degrade the phycocyanin used as colorant matter.

Regarding the wool dyeing process wastewater analysis, the optimum conditions are confirmed, and the low values of organic matter are obtained due to phycocyanin-fabric affinity, considering that pH 5, used in this process, increases the phycocyanin protein structure stability.

Fungi wastewater treatment of wool and cotton dyeing effluents was confirmed as efficient, and the BOD_5 and COD reductions of up to 80% are comparable with industrial treatment plants' efficiency levels.

The metal content influence due to the use of mordants was analyzed by comparing eight different auxiliaries, and results exceeding Spanish national daily limitations were obtained when alum (for aluminum limitations) and ferrous sulphate (for iron) were used in the cotton pre-mordanting process. Nevertheless, in the case of the wool pre-mordanting process, this exceeding of the daily limit was obtained for alum and aluminum triformate (for aluminum) daily limit and ferrous sulphate for Iron. This generates the need for a new set of testing for the reduction of these mordant concentrations and comparison with their efficiency in the dyeing process.

As a whole, this study demonstrates the applicability of a sustainable and innovative natural blue dye in textile coloration with good quality wastewater effluents obtained in the exhaustion dyeing process of wool and cotton, with the use of mordants, and the possibility of successful conventional fungi biological wastewater treatment, for further reuse of these effluents.

Declaration of conflicting interests

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