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Note from the field

Dyeing wet blue goat nappa skin with a microbial colorant obtained from *Penicillium minioluteum*

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ABSTRACT

A colorant from *Penicillium minioluteum* behaving like an acid dye was identified, extracted, purified, and characterized. It was used for dyeing wet blue goat nappa skin at different pH, time, and temperature to standardize these parameters. Highest color depth and percentage dye exhaustion was achieved at pH 2.0 (altered after 30 min of dyeing), temperature 80 °C, and time 60 min. Study of dyeing mechanism revealed adsorption phenomenon similar to the Langmuir distribution. Interestingly, color diffusion was found to be uniform, faster, and attained equilibrium in a shorter span of time unlike conventional leather dyeing process. It was due to the presence of proteases; organic polyphenols; functional amphoteric auxiliaries; and alcohols and amines as per Fourier transform infrared spectroscopy in the colorant. Final sample dyed using standardized dyeing conditions showed good rub fastness and marked to moderate perspiration fastness. However, light fastness was found to be very poor. Furthermore, no change in tear and tensile strength was recorded in the dyed samples as compared to the undyed sample.

1. Introduction

Synthetic dyes hold a major share in the dyeing of leather. In general, about 70% and 20% dyestuffs used today belong to acid and direct class (Hunger, 2002; Zengin et al., 2012). Chemically, all these dyes belong to azo, anthraquinone, and triphenylmethane dyes (Hudson and Britten, 2000; Puntener, 2000). Unfortunately, most of these dyes have been reported to cause cancer in healthy humans. Recently, 22 carcinogenic amines have been identified and restricted for use by EU and REACH regulations (Sivakumar et al., 2009). In fact, according to some studies, the number of such amines is likely to increase if further toxicological tests are conducted (Rao et al., 2002; Velmurugan et al., 2009). Moreover, these dyes have poor biodegradability due to higher biological and chemical oxygen demands. The conventional leather dyeing process is also very cumbersome and employs numerous chemicals and auxiliaries. Due to the number of pollutants involved in the wet processing of leather, this industry is striving to find natural and eco-friendly dyestuffs and auxiliaries to reduce the environmental pollution (Dave et al., 2015).

Because of these issues and regulations concerning synthetic dyes, an increase in demand for eco-benign dyestuffs for leather has been reported in many studies (Onem et al., 2011; Selvi et al., 2012; Sivakumar et al., 2009). In a course of time, several natural dyes have been explored to overcome the disadvantages of synthetic dyes (Onem et al., 2011; Selvi et al., 2012). These natural agents are non-toxic, non-allergic, non-carcinogenic, biodegradable, and are highly compatible with the environment (Rao et al., 2002). Several earthy colors have been screened for application on leather from sources such as onion peel (Onal, 1996); lac, pomegranate, logwood, Indian madder, Indian gum Arabic (Rao, 2010); henna (Musa et al., 2009); and *Bixa orellana* seeds (Selvi et al., 2012). Apart from eco-friendly dyeing, application of some natural dyes and agents can also enhance the after dyeing characteristics of leather. Recently, wool which is similar to leather in the structure has been dyed using Juglone extracted from *Pterocarya fraxinifolia*. Interestingly, wool showed antifungal and ultraviolet protection properties after dyeing with Juglone (Ebrahimi and Parvinzadeh, 2015). Moreover, improvement in several dyeing properties and color strength was also reported when natural agents like bentonite-type clay and protease enzyme were used in wool dyeing with madder (Parvinzadeh et al., 2014; Parvinzadeh, 2007). It is evident from these studies that natural dyes and agents are far better than synthetic colorants and auxiliaries.

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However, due to certain inherent drawbacks, plant-based natural dyes are considered unsuitable for industrial use. The first drawback is their limited availability and instability, due to dependency on the season, environmental conditions, and restricted flora and fauna (Shahid et al., 2013). Secondly, their extraction procedures are tedious, time-consuming, and produce less quantity of dye. Thirdly, their cost is very high i.e. about USD 1/g because of which these dyes are largely used for making high value added products (Siva, 2007; Velmurugan et al., 2009).

Nowadays, microbial colorants are gaining importance because they can overcome the limitation of synthetic and natural dyes. These colorants are not dependent on season, geographical conditions, and land. Moreover, they can be produced under controlled conditions with predictable yield (Shirata et al., 2000). They are biodegradable, easy to dispose of, and have a wide color palette (Joshi et al., 2003; Poorniammal et al., 2013; Sengupta and Singh, 2003). Industries like food, pharmaceutical, cosmetic, and textile are also exploring the potential of these colorants (Dharmaraj et al., 2009; Venil et al., 2013). However, their application has not been realized much for leather. Therefore, in this study, efforts have been made to dye wet blue goat nappa with a colorant from a non-pathogenic fungus namely *Penicillium minioluteum* (Fapohunda et al., 2012; Pitt, 1981).

2. Materials and methods

2.1. Fungal colorant, leather, and wool

P. minioluteum was grown on Sabouraud dextrose broth [dextrose 20 g/L (Merck, India); peptone 10 g/L (Fischer Scientific, India); pH 5.6] at 15 °C ± 2 °C in a static incubator (Metrex Scientific Instruments, New Delhi, India) for 28 days. For dyeing, chrome tanned wet blue goat nappa skin of 0.8 mm thickness was sourced from GB Leathers, Karol Bagh, New Delhi, India. For dye identification, 100 % scoured wool fabric having a thread count of 118 was sourced from Nehru Place, New Delhi, India.

2.2. Identification of colorant

The color was used as dye liquor for dyeing wool at 80 °C for 45 min. It was then subjected to dye identification as per IS: 4472 (Part II) – 1968. Based on the finding that the colorant belongs to acid dye class, a method for purifying the anionic dyestuff adopted by Hall and Perkins (1971) was employed. Here, 100 mL of the crude colorant was added to a separating funnel (Borosil, India) with 5 mL of hydrochloric acid (10% w/w) (Molychem, India) and shaken vigorously. Subsequently, 150 mL of *n*-butanol (Merck, India) was added in the same funnel and agitated again. After some time, the organic layer of *n*-butanol having color was separated and washed six times with 50 mL distilled water containing few drops of hydrochloric acid. The purified colorant was characterized using Perkin–Elmer Fourier transform infrared spectrometer (FTIR) over a scanning range of 650–4000 cm^{-1}.

2.3. Leather dyeing and its standardization

Leather samples (2.54 × 2.54 cm^2) were dyed in a static water shaker bath (NSW, India) using 50 mL of the crude colorant. Before dyeing, all samples were neutralized with 1 % borax (Molychem, India) solution and washed for 5 min at 60 °C. For standardizing the pH, dyeing was commenced at 60 °C for 30 min without any pH change. After the stipulated time, pH of all the three dye baths was altered differently as 2.0, 3.0, and 4.0 using 85 % of formic acid (Molychem, India). Subsequently, dyeing was continued at the maintained pH for another 20 min. Standardized pH was later used to standardize the time (20, 40, and 60 min) and temperature (60 °C, 70 °C, and 80 °C) of dyeing.

All samples were assessed for color depth (*K/S*) and color values (L^*, a^*, b^*, C^*, *and* H^*) recorded via computer color matching system (Macbeth, Color Eye 3100, USA), and percentage dye exhaustion (1). Dyeing parameters i.e. pH, time, and temperature showing highest *K/S* and percentage dye exhaustion were considered as standardized for final dyeing of leather.

$$E(\%) = \frac{O.D^1 - O.D^2}{O.D^1} \times 100 \quad (1)$$

Here, $O.D^1$ represent the optical density of the dye liquor before dyeing, and $O.D^2$ represent the optical density of the spent dye liquor after dyeing recorded using spectrophotometer (Visible spectro 105, Systronic, India) at λ_{max} 490 ± 5 nm.

2.4. Mechanism of dyeing

Using the standardized dyeing parameters (pH, time, and temperature), samples were dyed with different percentages of crude lyophilized colorant viz. 0.25, 0.5, 0.75, 1, 1.25, and 1.5. Subsequently, color depth (*K/S*) of each percentage was recorded and plotted for analyzing the adsorption isotherm. For understanding

Table 1
Functional groups of purified colorant according to FTIR.

Wavenumber (cm^{-1})	Spectral observation	Functional group	Reference
3410.71	A broad peak assigned to OH stretching with overlapping of N–H bond	Hydroxyl (O–H)	Ahmad et al. (2012); Hewedy and Ashour (2009); Neshati (2010)
	Likely to be unsaturated i.e. contains C=C Either hydroxyl or amino group	Amino (N–H)	Hill and Holman (2000); John (2006); McMurry (2012)
2846.98, 2880.24, 2908.01, 2943.52, 2965.93	Peaks related to alkane or alkyl	Alkyl (C–H)	Fox and Whitesell (2003); Hewedy and Ashour (2009); Hill and Holman (2000); John (2006); McMurry (2012)
2144.13	Very weak absorption peak indicative of unsaturated carbon–carbon multiple bonding	Alkyne (C≡C)	Field et al. (2013); John (2006); McMurry (2012)
1644.75	Improved absorption indicative of unsaturated hydrocarbons with attached hydrogen	Alkenyl (C=C)	John (2006)
1404.73	Phenol or tertiary alcohol bend	Alcohol (O–H)	John (2006)
1467.93	Weak bending vibration of alkyl group	Alkane/Alkyl (C–H)	John (2006)
1064.69	Sharp peak indicates presence of sulfur compounds	Sulfonic acid (S=O)	Carraher and Swift (2002); Stuart (2004)
926.06, 872.18, 818.21, 761, 720.35	Aromatic C–H bending vibrations	Aryl group (C–H)	John (2006)