Studies on the Application of Biocolorant for Leather Dyeing Using *Monascus Purpureus*

by

A. Tamil Selvi, G.C. Jayakumar, N. Usha Rani, A. Yasothai and Swarna V. Kanth*

Centre for Human and Organizational Resources Development

Council of Scientific and Industrial Research-Central Leather Research Institute,

Adyar, Chennai 600 020, India

Abstract

World-wide requirement for colorants from natural origins have been rapidly increasing in the leather sector due to environmental effluent concerns. Biocolorant obtained from *Monascus purpureus,* which yields red colored extract, has been studied for the dyeing process of leather. The effect of varying conditions like pH, concentration, time and temperature on the levelness of the dyeing, shade brightness, color intensity and exhaustion of the dye have been studied herein, and the conditions optimized. The change in color shade obtained has been quantified by reflectance measurements and compared with the visual assessment data. The bulk properties viz., softness and grain smoothness have been found to be marginally improved, versus control, in biocolorant treated leathers. There is no significant change in strength properties by the use of these natural colorants in the post tanning process.

Introduction

The global leather industry is currently undergoing transformation due to pollution and discharge legislations. Thus, the leather industry is under pressure to look for natural materials as replacements for conventional dyeing, which results in colored effluent with high pollution load and toxic substances.[1] Moreover, in the recent past there has been a growing interest in the revival of natural dyes in leather dyeing for improved eco-balances and developing niche products for special markets.[2,3] With recent advances in gene technology, attempts have been made to create cell factories for the production of colorants through the heterologous expression of biosynthetic pathways from either already known or novel color producers.[4] Several microbial species have been studied, which include common madder (*Rubiatinctorum*L),[5] woad (*Isatistinctoria*L)[6] and weld (*Reseda luteola* L)[7] that proved to be interesting sources of red (alizarin), indigo (indigotin) and yellow (luteolin) for their dyeing properties. Furthermore, all the three dyes were extensively studied until the commercial success of their synthetic analogues.[8] The main disadvantage of these natural dyes lies in the order of magnitude of their extraction yield factors (a few grams of colorant per kg of dried raw material). This does not make the current market cost effective, limiting their application to high-value-added products. To overcome this limitation, appropriate use of fermentation physiology together with metabolic engineering could allow the efficient mass production of colorants from microbial sources.[9]

Microalgae and several classes of fungi are known to produce a wide range of excreted water-soluble biocolorants.[10] Among, the several biocolorant producing microorganisms described in the literature,[11,12] the fungus *Monascus purpureus* (*M. purpureus*) has been thoroughly studied. It has been traditionally used for manufacturing food colorants, fermented foods, textile dyeing, biomedical applications and beverages.[13] Even though, use of such fungi to color foodstuffs is not a novel practice,[14] an attempt to the use of *M. purpureus* for coloring of leather is latest to the leather sector. Considering the apparent heat and pH stability of the *M. purpureus* derivatives along with the socio-climatic independence of such a readily available raw material, these fungi seem to be well worth for further exploration as an alternative source of natural colorants for leather. The several biocolorants produced by *M. purpureus* are oligotides and have been subdivided into three groups.[15] Rubropunctatin and monascorubrin are orange colorants, presenting different side chains on the ozolactone ring.[16] Their two azoto analogues are the red pigments bropunctamine and monascorubramine, whereas their reduced forms are the yellow pigments monascin and ankaflavin.[17] Monascorubrin and rubropunctatin have a unique structure responsible for their high-affinity for compounds with primary amino groups (so-called aminophiles). Reactions with amino acids yield the water-soluble red colorants, monascorubramine and rubropunctamine. In reality, various biocolorants derivatives with improved functional properties in the color range of orange-red can be produced by *M. purpureus* fermentation using different substrate and solvent extracts.[18] Ten

*Corresponding author e-mail: chordclri@lycos.com

Manuscript received July 16, 2017, accepted for publication January 25, 2018.

different kinds of red biocolorants have been isolated and identified from monascusspecies.[19-26] The effect of biocolorant from *M. purpureus* in the dyeing of full chrome goat crust leather has been evaluated in the present study.

Experimental

Reagents and Chemicals

Basic chromium sulfate (BCS) and other post tanning auxiliaries used for post tanning are of technical grade and obtained from BASF India Ltd. The reagents used for the production of biocolorants were of analytical grade.

Microorganism

A culture *M. purpureus* MTCC1880, available in the microbial type collection and gene bank, Institute of Microbial Technology, Chandigarh, India, was used for the present study. MSG medium was used for spore development and vegetative seed culture in the production of red biocolorants. The MSG[17]media contained (g dm^{-3}deionised water): MSG-Maltose, 50.0; anhydrous Sodium Glutamate, 12.6; K_2HPO_4, 2.4; KH_2PO_4, 2.4; $MgSO_4 \cdot 7H_2O$, 8.0; KCl, 0.5; $FeSO_4 \cdot 7H_2O$, 0.01; $ZnSO_4 \cdot 7H_2O$, 0.01; $MnSO_4 \cdot 7H_2O$, 0.003. The pH of the MSG media was adjusted to 5.5 using 0.1M NaOH and sterilized at 121˚C for 15min. MPI-glucose, 40.0; K_2HPO_4, 3.0; yeast extract, 10.0. Before sterilization the pH values of media MPI was adjusted to 5.6 using 1 M HCl. The culture was preserved at 4˚C and sub-cultured once in every three weeks.

Table I
Control experimental post tanning process using biocolorant.

PROCESS	%	CHEMICALS	DURATION	pH ADJUSTMENTS
MATERIAL: Wet blue from goat, shaved to 1.0 -1.2 mm				
WASHING	200% 0.2%	Water, 35˚C Acetic acid, 85%	 20 min	 pH 3.2
RECHROMING/ NEUTRALISATION	150% 4% 2% 2% 0.4%	Water, Chrome syntan Neutralizing syntan Sodium formate Sodium bicarbonate	 60 min 20 min 60 min	 pH 4.2 pH 6.0
RETANNING	100% 2% 4% 4%	Water, 35˚C Acrylic syntan Phenol, Naphthalene syntan Melamine Syntan	30 min 60 min	
DYEING - Biocolorant (Experimental trial groups) Trial 1 – Treatment at varying concentration (2, 4, 6, 8, 10 and 12%) at pH 5 , 60˚C & 90 min Trial 2 – Treatment at varying pH conditions (3, 4, 4.5, 5 and 6) at 8% conc. offer, 60˚C & 90 min Trial 3– Treatment at varying temperature (30, 40, 50 and 60°C) at 8% conc. offer, pH 5 & 90 min Trial 4 – Treatment at varying running time (15, 30, 45, 60 and 75 min) at 8% conc. offer, pH 5 & 60˚C				
DYEING - Control **FATLIQUORING**	2% 10% 8% 1.0% 10%	Acid black dye Water, 35°C Synthetic fat liquor Formic acid, 85% Water, 35°C	 60 min 60 min 3x10min + 30 min	 pH 3.5
Leathers piled over night; next day set, hooked to dry, staked, trimmed and buffed				

Substrate and Solid-State Fermentation

Spores and mycelium fragments from 5-day-old agar MSG slant were suspended in 5 mL of sterile deionized water using a sterile potter and then used to inoculate a 250 mL Erlenmeyer flask containing 50 mL of medium MPI. Once the culture had been incubated on a rotary shaker at 150 rev. min^{-1} and 30˚C for 24 h, it was homogenized and then centrifuged at 3780 × *g* for 10min. The residue was dispersed in 50 mL of deionized sterile water and used to inoculate (10 mL per flask) 500 mL Erlenmeyer flasks containing 100 mL of production medium (MSG). Each culture was incubated on a rotary shaker at 180 rev. min^{-1} and 30˚C for 7–8 days.

Extraction of Biocolorant

From the fermented solid substrate, a known amount has been taken for pigment extraction using 90% isopropyl alcohol (5mL of solvent per gram of wet fermented material). The extractable mixture has been placed in a rotary shaker at 200 rev min^{-1} at 30˚C for 60 min. The supernatant collected has been filtered through Whatman GF/C disc (47mm). The absorption spectrum of the supernatant has been recorded in the range of 350-700 nm using a Hitachi U-2000 spectrometer (Hitachi Ltd, Tokyo, Japan). The solvent extracted pigment has been evaporated in a rotary vacuum flash evaporator at 60 ± 2˚C. The pigment has been pulverized to a fine powder of average size 6-10 µm. 4% (based on collagen weight) of the pigment powder has been dissolved in 10% water at 60 ± 2˚C, cooled to 40 ± 2˚C and used for the dyeing experiments.

Experimental Trials

Conventional chrome tanned leathers from a same lot of similar weight range and grade were selected for the study. The leathers were sammed and shaved to 1.0 mm thickness and cut into 15 X 15 cm size samples. Five samples were taken for each trial – quantity of chemicals calculated on shaved weight. The samples were processed into upper leathers as per the process described in Table I. The effect of dyeing has been studied, employing the biocolorant in the process as mentioned in Table I. Various experimental trials (trial 1 to trial 4, mentioned below) have been carried out at different conditions of dyeing as mentioned in Table I.

Effect of Concentration (Trial 1)

Five sets of cut samples of wet blue goat leathers were rechromed, neutralized and retanned as per the process given in Table I. After retanning, bio colorant was given at different concentrations viz., 2, 4, 6, 8, 10 and 12% at pH 5 at 60˚C and the duration of treatment was 90 min.

Effect of pH (Trial 2)

Five sets of cut samples of wet blue goat leathers were subjected to post tanning using the process mentioned in Table I. The bio colorant treatments were carried out at different pH conditions viz., 3, 4, 4.5, 5 and 6. The treatment was carried out atat 8% dye for 90 min at 60˚C.

Effect of Temperature (Trial 3)

Three sets of cut samples of wet blue goat leathers were subjected to post tanning using the process mentioned in Table I. The biocolorant treatments were carried out at three different temperatures viz., 30, 40, 50 and 60˚C at pH 5 for 90 min.

Effect of Time (Trial 4)

Five sets of cut samples of wet blue goat leathers were subjected to post tanning using the process mentioned in Table I. The bio colorant treatments were carried out at varied running times viz., 15, 30, 45, 60, and 75 and min, keeping the pH and temperature constant at pH 5 and at 60˚C respectively.

Control Process

One set of cut samples of wet blue leathers was subjected to post tanning as mentioned in Table I without any biocolorant treatment. The process liquors from all the experimental and control trials were analyzed for the exhaustion of dye. The leathers were washed, set, hooked to dry and stored at room temperature before color measurement and fastness properties.

Comparison of Experimental and Control Trials

Matched pair comparison of control and experimental trial at optimized bio colorant treatment was carried out using ten goat wet blue leathers. Ten left halves were used for control process and right halves were processed using optimized bio colorant process. The leathers were compared for color, fastness, strength and organoleptic properties.

Analysis of Dye Exhaustion in the Process Liquor

Exhausted dye liquor was collected and analyzed for the unspent dye using a spectrophotometric method by measuring the absorbance value at the λ_{max} of the dye used, after suitably diluting the spent dye liquor using Hitachi *UV–visible spectrophotometer.* Then the amount of dye present in the spent liquor was calculated from the calibration graph drawn for the known concentration of the dye.

$$\% \text{ dye exhaustion} = [(C_g - C_t)/C_g] \times 100$$

Where C_g is the concentration of dye given and C_t is the concentration of dye in the spent liquor.

Determination of Color Difference

The control and experimental leathers made in this study were subjected to the reflectance measurements using a Milton Roy Color mate HDS instrument. Color measurement (L, a, b, h and C) were recorded and the total color difference (ΔE) and hue difference (ΔH) were calculated using the following equations:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

$$\Delta H = \sqrt{\Delta E^2 - \Delta L^2 - \Delta C^2} \quad (2)$$

Where ΔE = overall color difference; ΔL = Lightness difference; Δa and Δb = difference in a and b values, where 'a' represents the red and green axis and 'b' represents the yellow and blue axis; ΔH, hue difference, ΔC, chromaticity difference.

ΔL < 0 sample is darker, ΔL > 0 sample is lighter,

Δa < 0 sample is greener, Δa > 0 sample is redder,

Δb < 0 sample is bluer, Δb > 0 sample is yellower,

Δc < 0 sample is brighter/more saturated, ΔL > 0 sample is duller/less saturated,

Visual Color Assessment

The leather samples made from matched pair control and optimized experimental processes (full skin leathers) were subjected to visual assessment for uniformity of color, depth of shade, color shift from control and general appearance by standard tactile evaluation technique. Four experienced tanners rated the leathers on a scale of 0 -10 points for each functional property with 0 as the lowest and 10 as the best. The average of ratings was calculated for each property and taken for comparison.

Determination of Fastness to Light

The leather samples made from matched pair control and optimized experimental processes were tested for light fastness after conditioning according to IS 6191 - 1971 (LF:10).[27] The samples were exposed to xenon arc light under prescribed conditions for 20 h along with the dyed blue wool standards. The black panel temperature was maintained at 63 ±˚C and the relative humidity was 30 ± 5%.

Determination of Fastness to Wet and Dry Rub

Samples of appropriate size (5 x 14 cm) were cut from the samples and were tested according to IS 6191-1971 (LF: 10).[28] This method uses a SATRA Crock meter.

Physical Testing Analysis

The matched pair leather samples made from control and optimized experimental processes of full skin leathers were taken for physical testing measurements and the samples were cut from the official sampling position (IUP 2 method).[28] The leather samples were conditioned at 20±4˚C and 60±4% R.H. for 48 h. The tensile strength, elongation at break, tear strength and grain crack strength were measured as per IUP,[29] IUP 8 [30] and IUP 9 [31] methods.

Assessment of Bulk Properties

The leather samples were also subjected to visual assessment for bulk properties such as softness, fullness, grain smoothness, grain flatness, grain tightness and general appearance was assessed by four experienced tanners. Assessment was done on a scale of 0 – 10 points for each functional property and higher value indicates better property. The average of ratings was calculated for each property.

Results and Discussion

Biocolorant Production

In this study, an attempt has been made to color the tanned leather using biocolorant extract from *M. purpureus*. The approach is based on the concept that the functional groups available in the biocolorant interact to the available functional sites in the fibrous collagen network thereby imparting permanent color to the leather matrix. Hydrogen bonding and electrostatic interactions are involved in the interaction of red biocolorant and Leather. Table II provides the results of the 10th day fermentation trials of *M. purpureus* producing red color. The extract obtained from the media presented an absorption maximum at 525 nm. The cultural pH of the extract has been 6 and the production of red biocolorant has been predominant as a consequence of the presence of carbohydrate and sodium glutamate as sole nitrogen source.

Natural colorant applied for Dye Exhaustion: Influence of Process Parameters

The influence of natural biocolorant under various conditions has been studied in order to reach increased exhaustion and distribution of dyes in to the leathers. The wet blue leathers have been retanned followed by trials varying the different parameters of biocolorant such as pH, temperature and time.

Effect of Concentration

The exhaustion of dye at different concentrations of biocolorant treated is shown in Figure 1. From the figure, it is seen that the uptake of dye by the leather increases with increasing the offer of red biocolorant. The exhaustion of dye at an offer of 10% has been found to be maximum. Further is the offer of dye has not shownnot shown considerable in the exhaustion. Hence, from this study, it is found that offer of 10% dye is optimum for dying process, of the biocolorant.

Effect of pH

The results obtained with respect to the effect of pH on the exhaustion of dye in the process liquor are shown in Figure 2. From the table it can be seen that the exhaustion of dye increases gradually with increase in pH up to 5 and further increase in pH resulted in decrease of the exhaustion of dye. Maximum exhaustion of dye is observed when the biocolorant application is done at pH 5. Hence, offer of biocolorant at pH 5 has been taken as optimized pH. However, the biocolorant from the *Monascus sp.* used in this study has pH 6 as the pH of maximum activity.

Effect of Temperature

The results obtained for the exhaustion of dye with the presence of biocolorant at different temperatures are given in Figure 3. It is seen that there is an increase in the exhaustion of dye with the increase in temperature of biocolorant treated (up to 50). The increase in exhaustion of dye could have been improved with the distribution of dye aiding access to more reactive sites for interaction, as the biocolorant used is active at higher temperatures around 60˚C. Diffusion of dye and also other auxiliaries could have favored improved fixation and enhanced exhaustion resulting in higher and maximum fixation.

Effect of Time

The fixation of dye to the leather in terms of percentage exhaustion values of dye in process bath at different time intervals is shown in Figure 4. It is evident from the figure that the uptake of dyes increases gradually with time. It requires maximum of 1 h to bring about significant exhaustion in dye bath. At higher time intervals, the fixation of dye is proportionally higher and did not increase. Increased time intervals of biocolorant may not to be ideal for processing, as the leathers may exhibit looseness. Hence 90 min of biocolorant treated has been taken as optimum duration.

Effect of Surface Color at Varied Condition of Biocolorant Treatment

Variation in color of the leather at different treatment condition of red biocolorant viz., concentration, pH, temperature is presented in Table III. The color difference values of the leathers, when compared with control treated at varying concentrations of biocolorant viz., 2% to 12% are seen in the Table III. From the ΔL and ΔC values shown in the table, it is observed that there is an increase in the darkness and the intensity of the leathers with increase in the offer of biocolorant up to 12%. The leathers tend to become lighter at concentration values above 8%. Similar trend is observed with intensity of the color on treatment with different concentrations of biocolorant. The total color difference ΔE is found to be maximum at 10% offer of biocolorant. The hue difference is marginal in the biocolorant treated leathers compared to the control.

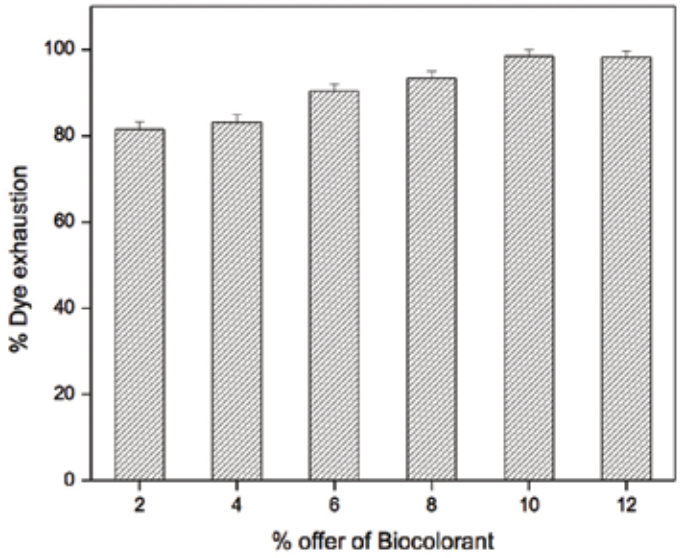

Figure 1. Effect of concentration on the uptake of biocolorant in the leather, run for 30 min at 30°C and pH of 4.5.

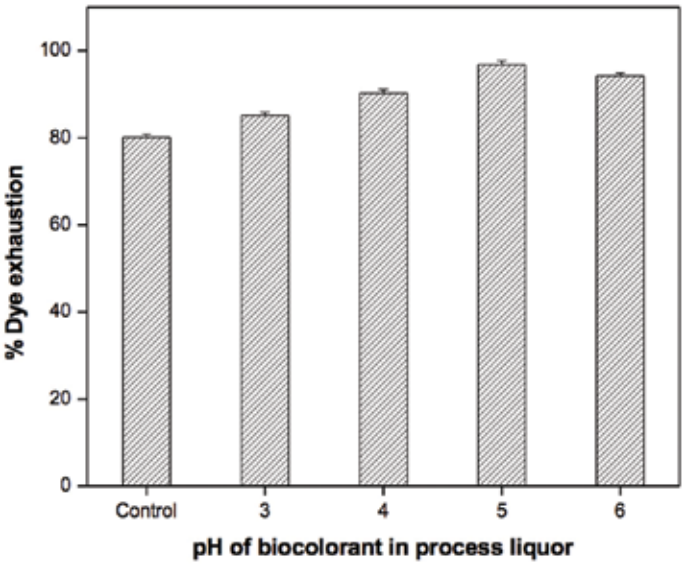

Figure 2. Effect of pH on the uptake of biocolorant in the leather.

Table II

Results of activity of *M. Purpures*.

Culture medium	Inoculum Concentration	Extract pH	Biocolorant Yield	λmax	Extract color
Rice with Sodium glutamate	$1.6x10^3$	6.0	1g/50ml extract	525 nm	Red

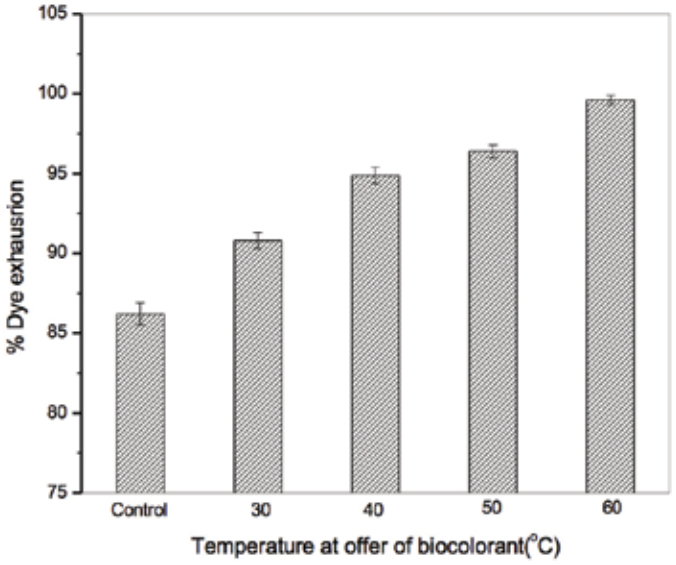

Figure 3. Effect of temperature (˚C) on the uptake of biocolorant.

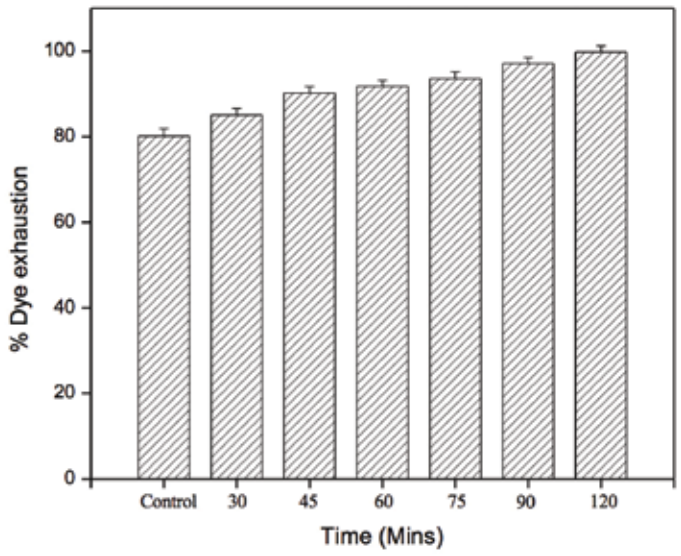

Figure 4. Effect of time on the uptake of biocolorant in the leather.

The study on the color differences at different pH condition indicated that there is an enormous increase in the darkness and color intensity of the leathers at pH 3. This may be due to the higher surface fixation when dye is offered after biocolorant treated at pH 3 and at this lower pH, aggregation of dyes will be favored that leads to increase in darkness and color intensity. Also, the penetration of dye will be limited at low pH conditions (high affinity of dye to the surface) resulting in the deposition on the surface of the grain. Also, biocolorant treated at lower pH conditions, the grain fiber structure in the leather may not be opened up (low colorant activity at pH 3), which might have resulted in the deposition of the aggregated dye intermediates causing surface darkness in dyed crust. A similar trend is observed with the surface color intensity, which is the highest at

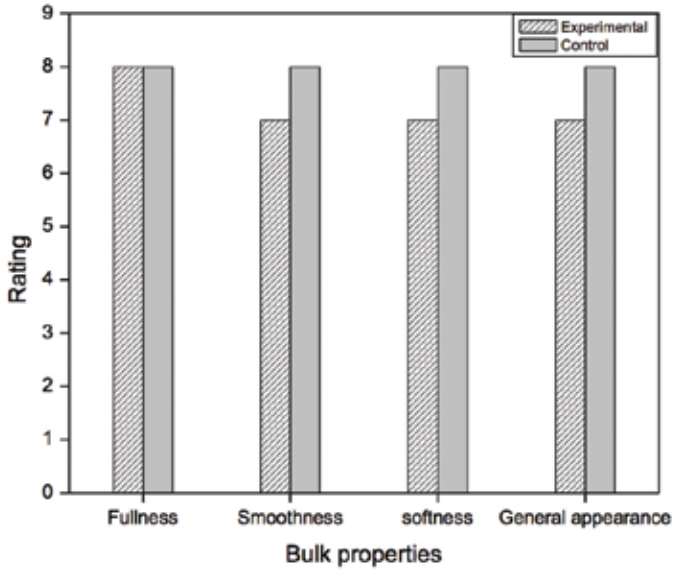

Figure 5. Graphical representation of organoleptic properties of control and biocolorant treated leather (Optimized conditions). *Ratings presented are mean of the rating values from three experienced tanners and the standard deviation for all properties is found to be <±0.5

pH 6. The hue difference is marginal in the biocolorant treated leathers at different pH conditions.

The color difference values of leathers treated with 10% biocolorant at pH 6 for 90 minutes at varied temperature conditions are also given in Table III. The darkness (ΔL) is found to be maximum at 60˚C. Beyond this temperature there is a decrease in ΔL values and the leathers have been found to be lighter. The intensity is also found to increase when the temperature of biocolorant treatment is increased. Maximum intensity is observed at 60˚C, which subsequently decreases with further increase in temperature. The hue difference is marginal in the biocolorant treated leathers, which had indicated that the color of the leathers is not deviated from its original color.

Optimized Condition of Biocolorant Treatment

Based on the effect of color and exhaustion of dyes at varied conditions, 10% offer of biocolorant for duration of 90 min at pH 5 and 60˚C has been taken as optimized conditions. The color difference values of leathers treated with biocolorant at optimized conditions are also given in Table IV. Experimental leathers processed at the optimized conditions have been used for comparison with the control undyed leathers for various parameters.

Visual Assessment of Leathers

Visual assessment for shift in color, uniformity of color, depth of shade and general appearance for experimental leathers (optimized biocolorant conditions) is carried out by standard tactile evaluation technique (Figure 5). The depth of shade has been uniform for biocolorant treated leathers, which is in agreement with the reflectance measurement values. The intensity

Table III
Color difference values of control and bcolorant treated leathers (various conditions).

Parameters		Δ_L	Δ_C	Δ_H	Δ_a	Δ_b	Δ_E
Concentration#	2%	L=0.137	W=-0.744	D=-0.002	LR=-0.460	LY=-0.585	1.051
	4%	d=-0.930	W=-0.380	D=-0.158	LR=-0.133	LY=-0.390	1.046
	6%	d=-1.226	S=0.030	D=-0.041	MR=0.049	MY=0.072	1.243
	8%	d=-2.269	S=1.184	I=2.047	MR=0.912	MY=2.474	1.512
	10%	d=-2.790	S=1.364	D=-2.119	MR=1.117	MY=2.263	2.212
	12%	d=-2.990	W=-0.250	D=-2.201	LR=-1.277	MY=2.362	2.522
Temperature^	30˚C	d=-2.269	S=1.184	I=2.047	MR=0.912	MY=2.474	1.512
	40˚C	d=-2.546	S=0.853	I=0.296	MR=0.528	MY=2.380	2.323
	50˚C	d=-2.998	S=0.993	I=0.506	MR=0.498	MY=2.580	2.716
	60˚C	d=-0.453	S=0.272	D=-0.015	MG=0.268	MB=-0.043	0.552
Dye fixative treated leathers		l=2.341	S=0.261	D=-0.035	MR=0.134	MY=0.226	2.333

#→pH 5, 60˚C, 90 min
^→10%, pH 5, 90 min

Table IV
Color difference values of control and experimental leathers.

Parameters	L	a	b	c	H	
Control	73.324±3.12	2.071±0.18	6.710±0.42	7.022±0.39	72.844±3.44	
Experiment	31.385±1.42	39.945±2.46	37.583±2.81	54.846±1.92	43.255±2.82	
Parameters	Δ_L	Δ_a	Δ_b	Δ_c	Δ_h	Δ_E
	Darker	More red	More yellow	Stronger	Decrease	
Experiment	41.940±2.68	37.874±1.72	30.873±2.16	47.824±2.32	10.023±0.86	64.394±2.24

of the biocolorant treated leather is moderate. There is no appreciable change or shift in color within the leather sample. The uniformity of color and shade has been better for the biocolorant treated samples. The overall general appearance of the biocolorant (optimized conditions) leathers was found to be of good quality.

Light and Rub Fastness Characteristics of Leathers

The fastness of control and experimental leather to rubbing and light is given in Table V. From the table, it is evident that the fastness to wet and dry rubbing of the biocolorant treated leather is slightly lower. The biocolorant treated leathers exhibited moderate light fastness (rating 3 on grey scale), equivalent to the Blue wool standards 4-5. The effect of fastness of leather on ageing for six months has also been studied. The fastness properties measured after ageing has been found to be marginally varying from values before ageing as given in Table V.

Table V

Fatness to wet rub, dry rub and light fastness of control and biopigment treated leathers.

Sample	Before ageing			After ageing		
	Wet rubbing	Dry rubbing	Light fastness*	Wet rubbing	Dry rubbing	Light fastness*
Biocolorant treated	3±0.25	4±0.25	3±0.25	4±0.25	4±0.25	3±0.25
Control	3±0.25	4±0.25	4±0.25	4±0.25	4±0.25	4±0.25

***Value in parenthesis indicates the corresponding blue wool standard**

Table VI

Strength properties of control and biocolorant treated leathers.

Sample	Tensile Strength	Elongation at break	Tear Strength	Grain Crack Resistance Load Distension	
	N/cm^2	(%)	N/cm	(Kg)	(mm)
Control	272±12	66±3	69±4	43±2	10.8±0.7
Biocolorant treated	278±11	76±5	77±3	46±3	12.2±1.1

Evaluation of Strength Characteristics

It is essential to analyze the strength characteristics upon treatment with biocolorant as one may expect open up of fiber structure influencing the strength of the leather. The various strength characteristics of the experimental crust leathers treated at optimized conditions of biocolorant along with control leathers are given in Table VI. It is observed that the strength characteristics of the experimental leathers are not affected due to biocolorant treatment. The values of various strength properties of experimental leather are found to be comparable to that of the control leathers.

Assessment of Bulk Properties

The ratings of the bulk properties such as softness, fullness, feel, grain smoothness and general appearance of the leathers treated with biocolorant (optimized conditions) and control is presented in Figure 5. It is seen that all the organoleptic properties are comparable or better than the control leathers. Especially the grain smoothness and softness of the biocolorant treated leathers have been found to be better than the control leathers. As the fibres are opened up in grain and corium, the experimental leathers are soft with smooth grain characteristics.

Conclusion

The concept of using biocolorant in the dyeing process helps in opening up of fibrous in the leather network. This leads to enhancement in the diffusion of dyes in to leather exposing large surface area. This in turn increases the dye-leather interaction. The offer of 10% biocolorant, at pH 5 for 90 min and 60˚C is found to be optimum with respect to the uptake of dye and intensity of the color. Uniform dyeing, intense and bright shade is resulted by the use of natural colorant treated leather at optimized conditions. The light and rub fastness of the leathers treated with biocolorant is comparable with those obtained by control leathers. The strength and bulk properties like softness, fullness, grain smoothness, feel and general appearance have been improved by the application of natural biocolorant in the process bath.

References

1. Brown, D., Anliker, R., and Richardson, M.L.; Risk Assessment of Chemical in the Environment: Royal Society of Chemistry, London, UK, 1988.
2. Pekhtasheva, E., Neverov, A., Kubica, S., Zaikov, G.; Biodegradation and Biodeterioration of Some Natural Polymers. *Chem. Chem. Tech.* **6**, 263-280, 2012.
3. Bechtold, T., Turcanu, A., Ganglberger, E., and Geissler, S.; Natural dyes in modern textile dye house-how to combine experiences of two centuries to meet the demands of the future. *J. Clean. Prod.* **11,** 499, 2003.
4. Marotti M.; *Le Piante Coloranti:* Edagricole, Bologna, 1997.
5. Angelini, L.G., Pistelli, L., Belloni, P., Bertoli, A. and Panconesi, S.; *Rubiatinctorum* a source of natural dyes: Agronomic evaluation, quantitative analysis of Alizarin and industrial assays. *Ind. Crops. Prod.* **6**, 303-311, 1997.
6. Moresi, M., De Santis, D., Cerrato, A.; Estrazione di pigmenti da *Reseda* e *Robbia*, in *Colture Alternative per le Aree Collinari delLazio.* ARSIAL-Area Studie Progetti, *Tipografia Flaminio:Rome*. **51**-63, 2001.
7. Kokubun, T., Edmonds, J., John, P.; Indoxyl derivatives in woad in relation to medieval indigo production: *Phytochem.* **49,** 79-87, 1998.
8. Cerrato, A., De Santis, D. and Moresi, M.; Production of luteolin extracts from *Reseda luteola* and assessment of their dyeing properties. *J. Sci. Food Agric.* **82**, 1189-1199, 2002.
9. De Santis, D., Ball,P.; *Colore*, 1st ed. RCS Libri. SpA: Milan, 207-239, 2002.
10. De Santis, D., Moresi, M., Gallbghjao, A., Petruccioli, M.; Assessment of the dyeing properties of pigments from *Monascus purpureus. J. Chem. Tech. Biotech.* **80**(9), 1072-1079, 2005.
11. Baranov´a, M., Mal'a, P., Burdov´a, O., Hadbavn´y, M. and Sabolov´a G.; Effect of natural pigment of *Monascus purpureus* on the organoleptic characters of processed cheeses. *Bull. Vet. Inst. Pulawy.* **48,** 59-62, 2004.
12. Hajjaj, H., Kla´eb´e, A., Loret, M.O., Tz´edakis, T., Goma, G., Blanc, P.J.; Production and identification of *N*-glucosylrubropunctamine and *N*-glucosylmonascorubramine from *Monascus ruber* and occurrence of electron donor–acceptor complexes in these red pigments. *Appl. Environ. Microbiol.* **63,** 2671-2678, 1997.
13. Yongsmith, B., Tabloka, W., Yongmanitchai, W. and Bavavoda, R.; Culture conditions for yellow pigment formation by *Monascus*sp. KB 10 grown on cassava medium. *World J. Microbiol. Biotechnol.* **9,** 85-90, 1993.
14. Sato, K., Iwakami, S., Goda, Y., Okuyama, E., Yoshihira, K., Ichi, T.,Odake, Y., Noguchi, H. and Sankawa, U.; Novel natural colorants from *Monascusanka*U-1. *Heterocycles.* **34**, 2057-2060, 1992.
15. Berger, R.G.; Colors/flavors derived by fermentation, in *Encyclopedia of Food Microbiology*, ed. by Robinson, R.K., Batt, C.A. and Patel, P.D.; Academic Press: New York, 729-735, 1999.
16. Mart´ýnkov´a, L. and Pat´akov´a, P.; *Monascus*, in *Encyclopedia of Food Microbiology*, Academic Press: New York, 1481-1486, 1999.
17. Pastrana, L., Blanc, P.J., Santerre, A.L., Loret, M.O. and Goma, G.; Production of red pigments by *Monascusruber*in synthetic media with a strictly controlled nitrogen source. *Process. Biochem.* **30**, 333-341, 1995.
18. Blanc, P. J., Loret, M.O. and Santerre, A.L.; Pigments of *Monascus. J. Food. Sci.***59,** 862- 865, 1994.
19. Hajjaj, H., Klae´be´, A., Goma, G., Philippe, J. , Barbier, E., Francois, J.; Medium chain fatty acids affect citrinin production in the filamentous fungus *Monascus ruber. Appl. Environ. Microbiol.* **66**(3), 1120-1125, 2000.
20. Ju¡zlova´, P., Martý´nkova´, L.. Kren.; Secondary metabolites of fungus Monascus: A review. *J. Ind. Microbiol* .**16**, 163-170, 1996.
21. Kyoko, S., Yukihiro, G., Shiho. S. S.; Identification of major pigments containing D-amino acid units in commercial *Monascus* pigments. *Chem. Pharm. Bull.* **45** (1), 227-229.
22. Lin, T. F., Yakushijin, K., Bu¨chi, G. H.; Formation of water-soluble *Monascus*red pigments by biological and semi-synthetic processes. *J. Ind. Microbiol.* **9**, 173-179, 1992.
23. Teng, S.S., Feldheim.; Analysis of anka pigments by liquid chromatography with diode array detection and tandem mass spectrometry. *Chromatographia.* **4(9),** 529-536, 1998.
24. Turner, W.B.; Fungal metabolites. London: *Academic- Press,* 245-253, 1971.
25. Yongsmith, B., Chaisrisook, C., Chimanage, P., and Krairaak,S.; Production of yellow pigment by a *Monascus* mutant growing on cassava: In The symposia on *Monascus* culture and applications meetings/symposia. Toulouse: France, 1998.
26. S.1691, LF: 4, Determination of fastness to artificial light -Xenon lamp of colored leather: Indian *Standards,* 1971.
27. Determination of color fastness to rubbing (Croaking test); *Indian Standards,* IS 6191 (LF: 10), 1971.
28. IUP 2: Sampling; *JSLTC* **84**, 303-309, 2000.
29. IUP 6: Measurement of tensile strength and percentage elongation; *JSLTC* **84**, 317, 2000.
30. IUP 8: Measurement of tear load – Double edge tear; *JSLTC* **84**, 327-332, 2000.
31. IUP 9: Measurement of distension and strength of grain by the ball burst test, Official methods of analysis, *JSLTC* **80**, 1996.