Microbial Pigments – Novel Benign Colorants for Leather Finishing

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Abstract

The Worldwide demand for natural colorants is rapidly increasing due to greater consumer awareness. Microbial sources of pigment production are novel to leather and many other industrial sectors. *Monascus ruber* MTCC 1880, potential pigment producing fungi were studied to produce three different colored pigments. Traditional mode of cultivation by solid state fermentation using carbohydrate as the substrate was used to produce red pigments at different environmental conditions. Environmental conditions controlling aeration, pH and agitation in solid state culture system were followed to produce different colors. The medium for cultivation was benign and designed form carbon source with rice (30 g/L), nitrogen source from mono sodium glutamate (2 g/L) and the inoculums size was maintained at 10 ml ($5x10^5$spores/ml) at pH:5.5 and temperature 30°C with an incubation time of 6 days. The produced microbial pigments were used for leather finishing. The finishing characteristics in terms of color measurements, compatibility with other finishing chemicals, gloss, light and color fastness, dry and wet rub fastness, organoleptic properties like surface smoothness, surface characteristics and overall performance of the microbial pigments for leather is highlighted in this paper.

Keywords: Microbial colors, Solid state fermentation, Natural Pigment, Fungi, leather finishing.

1. Introduction

The chemicals used to produce dyes today are often highly toxic, carcinogenic, or even explosive. The dye industrial unit across the world is dumping millions of tons of dye effluent into water bodies. Because they are synthetic, the natural environment cannot recognize them or degrade their toxicity. Even a very low concentration of these dyes in industrial effluents is enough to do great damage to the environment. If polluted fluids are discharged directly into the aquatic environment, the toxin present will be consumes or absorbed by aquatic organisms and will eventually find its way through the food chain into human beings. To overcome these limitations, an alternative is the natural origin from microbial source. This has been traditionally used for manufacturing food colorants, fermented foods and beverages in Southern and Far Eastern Asia for coloring.

The worldwide demand for food and textile colorants is rapidly increasing and they are important determinants for acceptability from the public. For imparting pleasing and attractive colors to food and textile, many natural colors have been in use since ancient times (Francis, 1987). These colors were obtained from several vegetables and animals. But after industrial revolution, color conscience of the people increased rapidly. Necessity in this regards led to invention and production of synthetic colors, which took the place of natural colors. Synthetic colors are found technically more suitable than natural colors and become popular because the former are known for their fastness, available in a wide range of colors, low cost even at high concentration in low volumes (Pattnaik, 1997). Fungal pigment are produced both by submerged and solid state fermentation. Both methods are followed based on their own merits and demerits. The East follows the traditional solid state fermentation on rice, wheat, sorghum, maize, cassava etc. while the West follows the submerged fermentation because of their deep commitment to fermentors and sophisticated control devices. Though many elaborate studies have been made on submerged fermentation, the yields of pigments have been much lower than in solid state fermentation (Hajjaj *et al*., 1999 and Vijayalakshmi and Tamilselvi, 2009). Characteristics of pigments make ineffective the use of the simple isocratic development mode in effective. *Monascus* pigment fermentations have been performed mainly in solid cultures (Johns and Han1975), however production yields have been too low to allow industrial scale production (Kim *et al*., 2002). Several factors such as gaseous environments agitation and aeration (Hajjaj *et al*., 2000), carbon and nitrogen (Pastrana *et al*., 1995) can influence the pigment production of *Monascus.*

The conventional method of pigment production is by solid state fermentation using rice as substrate. A submerged culture system could overcome the drawback of the low yield, its large area requirement and the difficulties in controlling the aeration, pH and agitation in solid state culture system. In this study, to improve the production of *Monascus* red pigments, many factors such as carbon source, nitrogen source, inoculum size, temperature, pH and incubation time that influence the pigment production have been evaluated in solid state fermentation. The extracted red pigments have been tested for leather dyeing as an alternative to a commercially used synthetic pigment.

2. MATERIALS AND METHODS:

Microorganism, Maintenance and Ascospore suspension preparation

Monascus ruber MTCC 1880 obtained from Microbial type Culture Collection, Chandigarh, India were used for all the experiments. The fungal species were maintained on Potato Dextrose Agar (PDA) slants at 30°C and stored at 4°C and sub cultured monthly. The fungus was routinely grown on PDA plates at 30°C. Ascospores were prepared by flooding the plates with 20ml sterile phosphate buffer solution (pH 7) and gently scraping of the medium with a sterile spatula.

Cultivation of Monascus - Submerged fermentation

Submerged fermentation was used for the production of red pigments from *Monascus ruber*. *C*arbon sources like ethanol, glucose, fructose, soluble starch and maltose were used with the rice powder. 30g/l of these carbon sources were used for submerged cultivation of *Monascus ruber* in 250ml flasks containing 100ml medium inoculated with 5ml of spore suspension at 30°C and 150rpm and the red pigment productions were compared after 4 days. 2g/l of the nitrogen sources with 30g/l of the carbon source were used for cultivation in 250ml flasks containing 100ml medium inoculated with 5ml of spore suspension at 30°C and 150rpm and the red pigment productions were compared after 4 days.

Effect of inoculum size

The effect of inoculum concentration on pigment production was studied by adding different volumes of inoculum of 1, 5, 10, 15 and 20 ml to 250 ml flasks containing 100ml medium with 30g/l of carbon source and 2g/l of nitrogen source and cultivated for 4 days at 30°C at 150rpm and the red pigment productions were compared.

Effect of pH and temperature

To study the effect of initial pH on red pigment production, the medium was adjusted to pH values from 4.5 to 7.0 with HCl and NaOH and then used for cultivation. To study the effect of incubation temperature, the culture flasks were incubated at various temperatures from 25°C to 45°C.

Effect of incubation time

To study the effect of incubation period on red pigment production, culture flasks were incubated for varying period ranging from 1 to 12 days. After fermentation, the medium was filtered and the filtrate was said to contain extra cellular pigments. For extracting intracellular pigments, the filtered mycelium was subjected to cell destruction using mortar and pestle and 50ml of 90% ethanol was added to extract the pigment. It was agitated in rotary shaker at 200rpm for 24 hrs and the filtrate was said to contain intracellular pigments.

Pigment estimation

The pigment production was indirectly evaluated by means of absorbance measurements. The analysis of pigment produced was carried out by measuring the optical density of pigment extracts at 500 nm for red pigments respectively using spectrophotometer. Pigment yield was expressed as maximum absorbance (*A*max) at corresponding wavelength.

Pigment preparation

The red pigment was extracted with ethanol and concentrated by evaporating the solvent till the pigment was obtained as powder. This was the extracted red pigment and it was used to study its efficacy in leather finishing.

Leather finishing trails

Leather finishing season was prepared with the composition as given in Table 1.

Table 1. Composition of the finishing Season

Component	**Quantity (g)**
Microbial Pigment	100
Mesdium soft resin binder	200
Protein binder	50
Wax Emulsion	30
Ammonia	10
Water	610

The prepared season was finished using spray technique white chrome tanned Goat crust. A total of 7 cross coats were applied followed by a cellulose butyrate acetate lacquer finishing. Conventional red colored chemical based pigment and the extracted *Monascus* red colored natural pigment were used to processed the finished crust leathers for comparison.

Colour measurements

Undyed crust leather was taken as the control. The colour measurements (L, a, b, c, H) of the finished leather samples were taken using reflectance spectrophotometer. The colour difference (dl, da, db, dc, dE, dH) measurements between the control and the samples were taken and the surface colours of the finished leather samples were assessed.

Colour fastness to wet and dry rubs

The finished leather samples were tested according to circular rubbing test method using a crock meter. For dry rub test, circular dry standard wool felt was used to rub against leather surface for 128 cycles and for wet rub test, circular standard wool felt wet with water was used to rub against leather surface for 64 cycles. The change in colour of the leather samples was assessed.

3. Results and Discussions

Effects of various factors on red pigment production

The study was based on the effect of various carbon and nitrogen sources, pH, incubation temperature, incubation time and the inoculum size on red pigment production. The yields of the red pigments were calculated in each case and the culture media was designed accordingly to improve the red pigment production as in Table 2.

Table 2. Submerged culture conditions for red pigment production

Carbon source	Rice powder (30g/L)
Nitrogen source	Mono sodium glutamate (2g/L)
Inoculum size	10 ml
pH	5.5
Temperature	30°C
Incubation time	6 days

The culture medium and the conditions were maintained as given in Table 2 and the extracted red pigments were used for further studies in finishing leather and compared with the synthetic pigment.

Leather dyeing

The surface colours imparted by the three dyeing emulsions were assessed using reflectance spectrophotometer and the colour measurements were taken. The overall color difference between control and leather finished with *Monascus* red pigments was found to be 16.283. This shows that the leather finished with the Monascus red pigment was darker, redder, more yellow and brighter than the control.

Table 3. Colour values of Control and *Monascus* based finished leather

Colour Values					
	L	**A**	**b**	**C**	**h**
Control	39.691	16.528	30.182	34.411	61.295
Extracted red pigment	28.812	27.916	34.313	44.235	50.869

Table 4. Colour difference values of control and *Monascus* based finished leather

	Colour Difference Values					
	Dl	Da	db	dc	dh	dE
Extracted red pigment	Darker -10.879	More Red 11.389	More Yellow 4.131	Stronger 9.824	Decrease -7.089	16.283

Colour fastness test to dry and wet rubs

Colour fastness to dry and wet rubs was studied and grades were given to the samples according to the resistance of the pigments to the rubs.

Table 5. Grades given to the leather samples based on the pigment resistance to dry and wet rubs

Tests	Synthetic pigment	Extracted red pigment
Dry and wet rub	3/5	4/5

From the grades given in Table 5, it was observed that the extracted red pigment can be a possible alternative to synthetic pigment.

4. Conclusion

Submerged fermentation technique can be followed for the cultivation of *Monascus ruber* and production of red pigment. There is a promising chance of replacing the synthetic pigments by natural pigments in the near future using this process in leather technology. The produced microbial pigments in red color were used for leather finishing. The pigments were compatible with other ingredients of finishing process. The color measurements clearly indicate that they produce color as well as uniform finish characteristics. The organoleptic properties with respect to feel, visual color, uniformity of color, smoothness of finish, gloss and overall performance of *Monascus ruber* based microbial red color pigments for leather was good. The color fastness, dry and wet rub fastness is also found to be satisfactory and recommended for finishing of leather. However, further study on improving and optimizing the submerged culture medium, production of other colored pigments and their efficiency for use in finishing process support the possibility of using natural pigments based microorganisms for leather.

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6. References

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