Optimisation of process parameters for coloration and antibacterial finishing of wool fabric using natural fungal extract

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The present study aims at evaluating and optimizing the dyeing potential of fungal pigments for wool fabric specimen. The fungal pigments are extracted from the species of Thermomyces, purified and characterized using UV-Vis and FTIR spectra and then used for dyeing. An experiment has been designed using Box-Behnken with three levels and three variables using pH, temperature and time as independent variables and K/S, wash, rub & light fastness and bacterial reduction (%) as dependent variables and the process conditions are optimized. Regression equations have been obtained to analyse color intensity, fastness properties and bacterial reduction and the optimum process parameters are identified. The results show that the optimum concentration of the pigment is 2% on weight of the fabric and the optimum conditions for dyeing are 60°C, 30 min at 3 pH.

Keywords: Box-Behnken, Bacterial reduction, Fungal pigment, Regression equation, Wool

1 Introduction

The worldwide demand for colorants of natural origin, especially yellow or red pigments, is rapidly increasing in the food, cosmetic and textile sectors. Several research projects have so far been carried out to evaluate the techno-economic feasibility of today’s alternative dye crops. Among the species examined, common madder (Rubbia tinctorum L) and woad (Isatis tinctoria L) are proved to be quite interesting sources of red (alizarin) and yellow (luteolin) dyes respectively, either for their agronomic characteristics or for their dyeing properties. In fact, all three dyes were extensively exploited until the commercial success of their synthetic analogues. The main disadvantages of these natural dyes are the order of magnitude of their extraction yield factors (a few grams of pigment per kg of dried raw material). This makes their current market price about US$ 1/g, thus limiting their application to high value-added natural-colored garments only. To overcome this limitation, it was suggested to exploit the potentiality of other biological sources such as fungi (both moulds and yeasts), bacteria, algae and plant cultures, since appropriate selection, mutation or genetic engineering techniques are likely to improve significantly the pigment production yields with respect to wild organism. Among the several pigment-producing micro-organism described in the literature, the fungus Thermomyces has been thoroughly studied. It has been traditionally used for manufacturing food colorants and fermented foods and beverages in southern and far eastern Asia, the latter being also used in medical therapy to promote blood circulation and proper cholesterol levels, prevent gastric and intestinal disorders, stimulate digestion, etc. The several pigments produced by Thermomyces are oligoketides and have been subdivided into three groups; rubropunctain and monascorubrin are orange pigments, presenting different side chains on the ozolactone ring. Their two azoto analogues are the red pigments rubropunctamine and monascorubramine, where- as their reduced forms are the yellow pigments monascin and ankaflavin. A 3-factor, 3-level Box-Behnken design was used to derive a second order polynomial equation and construct contour plots to predict responses. In this research, attempt has been made to study the dyeing potential and microbe resistant characteristics of pigment extracted from the species Thermomyces.

2 Materials and Methods

2.1 Materials

Bleached 100% wool fabric, having the specifications plain weave, 40 Ne yarn count with warp 56 ends/cm, weft 27 picks/cm and 120 gsm, was used.

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2.2 Methods

2.2.1 Extraction and Estimation of Pigment (Air-drying Process)

Extracellular pigment producing fungi *thermomyces* species was isolated from soil. The fungal cultures were inoculated on to potato dextrose broth and incubated at 35°C for 5-7 days. Supernatant was filtered through the filter paper. Broth having the pigment was taken in a clean glass petriplates and placed under hot air in a dust free chamber at 40°C. The plate was covered with a thin muslin cloth to avoid contamination due to dust. After 8 hours drying, the volume of the broth was reduced to one third. The condensed broth was lyophilized and the powered pigment was stored at 4°C [Figs 1(a) and (b)].

2.2.2 Selection of Mordant

Various synthetic mordants like stannous chloride, alum, ferrous sulphate and natural mordants like myrobolan, neem oil were identified for the above process. Considering the ecofriendliness and cost effectiveness, the natural mordant myrobolan was chosen for the dyeing process. It was initially pre mordanted with the fabric specimen before treating with the natural fungal extract pigment for the dyeing process. The fabric samples were treated with myrobolan solution (2-5g owm) at 30° C for 20 min. After mordanting, the samples were well squeezed and immersed into the dye bath (MLR 1:20, temp. 30° C, time 20 min and pH 3). After dyeing the samples were dried at 60° C for 20 min. To optimize the process parameters, Box-Behnken experimental design was used (Table 1).

2.3 Statistical Analysis

The traditional approach for developing a formulation is to change one variable at a time. By this method, it is difficult to develop an optimized formulation, as the method reveals nothing about the interactions among the variables. Hence, a Box- Behnken statistical design with 3- factors, 3- levels, and 15 runs was selected for the optimization study. The experimental design consists of a set of points lying at the midpoint of each edge and the replicated center point of the multidimensional cube. The independent and dependent variables are listed in Table 1. The polynomial equation generated by this experimental design (using Statistica Release 6, Statsoft Inc) is as follows:

\[ Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3 + b_{11} X_1^2 + b_{22} X_2^2 + b_{33} X_3^2 \]

where \( Y \) is the dependent variable; \( b_0 \), the intercept; \( b_1 - b_{33} \), the regression coefficients; and \( X_1, X_2 \) and \( X_3 \), the independent variables that are selected from the preliminary experiments.

2.4 Testing

The untreated and treated samples were tested for various measurements by standard test procedures. Color fastness properties of the samples were assessed using AATCC standards fastness to washing (AATCC Test Method 61-2009), fastness to rubbing/crocking (AATCC Test Method 8-2007) and fastness to light (AATCC Test Method 16-2004). The dyed samples were analysed for the spectral values \( K/S \) determined using a Minolta 508 spectrophotometer with Macbeth Match View software (X-Rite, USA) in D65 daylight.

2.4.1 Determination of Antibacterial Activity (AATCC 100-1993)

Bacterial strains were grown in nutrient broth at 37° C for 18-24 h. Using sterile cork borer a well was formed. Culture broth (0.01 mL) was spread on nutrient agar plate by spread plate method and impregnated with 100µl of methanolic extract and crude extract. The plates were incubated at 37° C for 24 h at the end of the incubation period. The susceptibility of the test organism was determined by measuring the zone of inhibition around the well.

| Table 1 — Details of various levels of process parameters |
|---------------------------------|---|---|---|
| Process parameter | Different levels |
| Temp. (\( X_1 \)), °C | -1 | 0 | +1 |
| Time. (\( X_2 \)), min | 20 | 30 | 40 |
| \( p \text{H} \) (\( X_3 \)) | 2 | 3 | 4 |
2.4.2 Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was performed to test the antimicrobial activity of the methanolic extract of *P. purpuroscens*, *thermomycetes* sp. and *chatomium* sp. using tube dilution method. The MIC is defined as the lowest concentration of antibiotics or extracts that does not show any growth of tested pathogens at a minimum concentration. This test was performed at four concentration of the plant extract (10, 1, 0.1 and 0.01mg/mL). Twenty-four hours old culture of each organism was used for the study. 4/10 dilution of each organism was prepared by serial dilution technique. A four number of sterilized Eppendorf tubes were taken and to this 900µl of 4/10 diluted test organism were added. To the first tube, 0.1 mL of prepared culture extract was added and serially diluted to the last tube. The four tubes corresponding to four concentrations of the culture extract were obtained (10, 1, 0.1, 0.01mg/L). Likewise, a set of Eppendorf tubes was prepared for each organism, for each test samples. Simultaneously controls were also kept for the experiment. For the second set of Eppendorf tubes (4 numbers), 0.1 mL of the negative control (100 % ethanol ) was added to first tube and serially diluted to the last tube. For the third set of Eppendorf tubes (4 numbers), 0.1mL of the positive control, ketaconazole for fungi and chloramphenicol for bacteria (10 mg/mL) was added to the first tube and serially dilute to the last tube. For the fourth set of Eppendorf tubes (2 numbers), nothing was added so that the tubes contained only the microbial cells. Similar to the agar well diffusion method, the Petri plates were divided into 4 equal quadrants. After incubation of the Eppendorf tubes for an hour, 50ul from each of the tubes were spotted on the petri plates. The plates were then covered and incubated for 24 h. The growth of the organism for each dilution was observed and thus the minimum inhibitory concentration of the fungal extract was calculated as shown in Table 2.

3 Results and Discussion

Table 3 shows the fastness properties and bacterial reduction (%) of silk fabric treated with natural fungal extract at different temp, time and pH. The regression equations ($R$ & $R^2$ values) for the various properties are given in Table 4.

A checkpoint analysis was performed to confirm the role of the derived polynomial equation and contour plots in predicting the responses. Values of independent variables were taken at 3 points, one from each contour plot, and the theoretical values of the dependent variables were calculated by substituting the values in the polynomial equation.

After developing the polynomial equations for the responses PDE and MVD with the independent variables, the formulation was optimized for the response PDE. Optimization was performed to find out the level of independent variables ($X_1$, $X_2$, and $X_3$) that would yield a maximum value of antibacterial efficacy with constraints on temp, time and pH.

<table>
<thead>
<tr>
<th>Table 2 —Minimum inhibitory concentration for <em>thermomycetes</em> species against pathogens</th>
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<td>Pathogens</td>
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<td><strong>Gram positive bacteria</strong></td>
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<td><em>Enterococcus</em></td>
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<td><em>Bacillus subtilis</em></td>
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<td><em>B. cereus</em></td>
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<td><strong>Gram negative bacteria</strong></td>
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<td><em>Escherichia coli</em></td>
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<td><em>Vibrio cholerae</em></td>
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<td><em>Salmonella typhi</em></td>
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<td><strong>Fungi</strong></td>
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<td><em>C. albicans</em></td>
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<td><em>C. neoformans</em></td>
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1— Thermomyces sp; 2— *P. purpuroscens*; and 3— Chaetomium sp.
NI—No inhibition; I—Inhibition.
3.1 Effect of Process Parameters on K/S Value

Contour plot shown in Fig. 2 represents the color strength (K/S) value of the pigment vs temp., time and pH. Here, the K/S value decreases with increase in temperature and pH but increases with increase in time, reaching a maximum value of 1.5 at 30°C and 40 min (Fig. 2). A positive value of the coefficient for $X_2$ (surfactant loading) indicates a favorable effect on K/S value. Optimized K/S value is observed at 30°C, pH 3.0 and time 40 min. The reason might be due to the influence of the diffusion characteristics and depth of penetration of the natural fungal pigment onto the fabric specimen.

3.2 Effect of Process Parameters on Wash Fastness

Contour plot shown in Fig. 3 represents the wash fastness ratings of the fabric specimen vs temp., time and pH. The plot corresponding to 30°C is linear, but above this value, plots are found to be nonlinear in relationship to $X_2$ and $X_3$. A high value of wash fastness (5.0) can be obtained with $X_2$ level range from –1 to 0.81 and $X_3$ level range from –1 to 0. Here, wash fastness rating decreases with increase in temperature and pH but increases with increase in time, reaching a maximum rating of 5.0 at 30°C and pH 3.0. Optimized wash fastness rating is observed at 30°C, pH 3.0 and time 40 min. The results infer that the substantivity characteristics of the natural fungal pigment influence the fabric properties.

3.3 Rub Fastness

Contour plot shown in Fig. 4 represents the rubbing fastness ratings of the fabric specimen vs temp., time and pH. Here, the rubbing fastness rating decreases with increase in temperature & pH but increases with increase in time, reaching a maximum rating of 5.0 at 30°C and pH 3.0. This signifies that there is no direct linear relationship among the selected independent variables. Optimized rubbing fastness rating is observed at 30°C, pH 3.0 and time 40 min. The reason might be due to the reactivity and fixation levels of the natural fungal pigment on to the fabric specimen.

3.4 Light Fastness

Contour plot shown in Fig. 5 represents the light fastness ratings of the fabric specimen vs temp., time and pH. Here, the light fastness rating decreases with...
Fig. 2 — Influence of (a) time and temp., (b) temp. and pH, and (c) time and pH on K/S value

Fig. 3 — Influence of (a) time and pH, (b) temp. and pH, and (c) time and temp. on wash fastness
Fig. 4 — Influence of (a) time and temp., (b) time and pH, and (c) temp. and pH on rub fastness

Fig. 5 — Influence of (a) time and temp., (b) time and pH, and (c) temp. and pH on light fastness
increase in temperature and pH but increases with increase in time, reaching a maximum rating of 5.0 at 30°C and pH 3.0. All the contour plots for a high value of light fastness ratings are found to be nonlinear. Optimized light fastness rating is observed at 30°C, pH 3.0 and time 40 min. The results infer that the surface adsorption characteristic of the natural fungal pigment influences the fabric properties.

3.5 Influence of Process Parameters on Antibacterial Activity

Contour plot shown in Fig. 6 represents antibacterial reduction % of the fabric specimen vs temp, time and pH. Here, the antibacterial reduction % decreases with increase in temperature and pH but increases with increase in time. A high value of antibacterial efficacy can be obtained up to a certain level of all 3 independent variables, but above this an increase in the level of independent variables leads to a decrease in the antibacterial efficacy. Optimized values are observed at 60°C, pH 3.0 and time 40 min. The results infer that natural mordant and pigment influences the pathogenic bacterial reduction. The same result is also reported by Velmurugan and Balachandar in five different fungal pigments dyed with textile and leather samples.

3.6 Characterization of Pigment from Thermomyces

The optical density of the pigment extract was determined in a wide range of spectra using a UV-Visible spectrometer. The spectrum shows the maximum absorbance of the specimen (250-300 nm), which confirms the presence of protein and carbohydrate groups in the fungal pigment. In FTIR absorption spectra, the absorption in the region 3000-3500 cm⁻¹ confirms the presence of N-H (str) group. The absorption in the region 1500-1750 cm⁻¹ confirms the presence of C=O (str) group. Hence, from the observation, the affinity of sample towards protein fibres is confirmed.

4 Conclusion

100% wool fabric has been dyed using the natural fungal extract, Thermomyces and the effect of coloring behavior and fastness results are analyzed. The process parameters like pH, temp. and time duration have been varied and the results are optimized. The results are examined for coloring the protein fabric specimens at 2% on weight of the fabric and the optimum conditions for coloration and antibacterial finishing are found to be 30°C, 60 min.
and 3 pH. The process proves to be more eco-friendly in nature and it can be used for specific product development in hospital and medical applications.

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References
