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Color Evaluation of Wool and Silk Fabrics using Microbial Sludge from Actinomycete Bacteria

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Abstract- Three naphthoquinone compounds have been studied which were produced by liquid cultures of Actinomycete. These napthoquinones compounds are 5,8-Dihydroxy-2,7-dimethoxy-l,4-naphthoquinone;2,7-Dimethoxy-5-hydroxy-l,4-naphthoquinone;2,5,7-Trimethoxy-l,4naphthoquinone. Results obtained from dyeing of wool and silk fabrics with these naphthoquinone compounds (used as natural dyes) were studied. Dyeing was carried out by using two different methods i.e. with and without mordant. To study the reflectance values of both the substrates the spectrophotometric evaluation was done. The results of colour strength for wool as well as silk, with and without mordanting were compared. Thin layer chromatography, NMR and washing fastness testing were carried out. The dye uptake and fastness properties were found to be good for both wool and silk.

***Index Terms*—Actinomycete bacteria, Wool, Silk, Fastness.**

I. INTRODUCTION

In recent times a revitalization attention in the use of natural dyes in textile coloration has been rising. This is a consequence of the severe ecological values forced by various countries in reaction to the deadly and allergic reactions associated with synthetic dyes [1]. Natural dyes are friendly to the milieu than synthetic dyes and can reveal better biodegradability along with higher compatibility with the environment [2- 4]. Moreover, natural dyes are better as they are biodegradable, less toxic, less polluting, non-carcinogenic, no chemicals such as leveling agent, pH regulator etc. are required, it can be recycled after use and full range of colors using various mordants can be produced [5]. With the introduction of synthetic dyes in the middle of 19th century, the decline in the use of natural dyes started. The synthetic dyes have replaced the natural dyes. During the manufacturing process of synthetic dyes many carcinogenic chemicals are used. The toxic and unfriendly by products formed have to be discharged in the rivers, ponds or into the atmosphere. Keeping in mind the environmental awareness and hence production of eco friendly textile, the natural dyes are coming back to the driver's seat. For this reason, natural dyes are again getting prime importance now a day [6].

The invention and appraisal of microbial colors as textile colorants is presently being explored [7]. Fungi are more biological appealing resource of pigments, as some fungal genus are rich in stable colorants such as anthraquinone [8-10]. Anthraquinone derivatives were previously isolated from the fungus *Dermocybe sanguinea* [11]. A number of naphthoquinones.derivatives, Figure 2, has been identified from *Actinomycete* [12]. These metabolites are of concern because numerous of them acquire considerable antibiotic activity, primarily against Gram-positive bacteria and Pseudomonas aeruginosa..

Sludge or broth produced from *Actinomycete* contains some colored substances which can act as colorant for textile substrate. *Actinomycetes* are best known for their ability to produce antibiotics and are gram positive bacteria which comprise a group of branching unicellular microorganisms (Fig. 1).

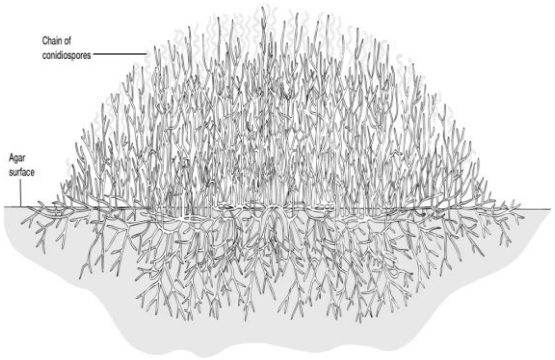

Fig. 1. An Actinomycete colony growing on agar. [13]

Gram staining [14] is an empirical method of differentiating bacterial species into two large groups: Gram-positive and Gram-negative. Both are based on the chemical and physical properties of their cell walls. The Gram stain is almost always the first step in the identification of a bacterial organism. Gram-positive bacteria are those that stain dark blue or violet by Gram staining, whereas Gram negative bacteria are those that do not stain by gram staining. The morphology of these bacteria is that it has filamentous growth like fungi on substrate, grow on and in it. In addition to this, tissue like mass is generally grown in culture.

In this study, production and evaluation of microbial pigments as textile colorants are investigated. The dyeing of cotton, wool and silk fabrics using a natural dye obtained from the sludge of Actinomycete culture has been studied using both conventional as well as ultrasonic dyeing methods. The effect of mordants on the dyeing outcome in terms of shade output as well as fastness properties of the dyeing is rationalized. The results of fastness properties of the dyed fabrics were fair to good. CIELAB values of the dyeing have also been recorded.

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II. MATERIALS AND METHODS

A. Dye material

The sludge extracted from the culture of Actinomycete is used as natural dyes. Actinomycete is grown at 28°C on starch casein agar medium. Then broths are inoculated with the growth and incubate at 28°C on shaker. A culture is filtered after 2 weeks. After pigment production it is extracted using acetonitrile as a solvent and dried on rotary evaporator.

B. Source of Actinomycete bacteria

Bacteria was taken from Department of Microbiology, Guru Nanak Dev University, Amritsar (Punjab).The sludge extracted from the culture of Actinomycete was used as a dye.

C. Fabric and mordants

Scoured and bleached wool twill (2/2), silk plain and cotton plain fabrics were purchased locally. The mordants used for study were alum, copper sulphate and ferrous sulphate.

D. Procedure for the dyeing of cotton

For cotton two methods of dyeing were used: (1) In the presence of alkali; (2) without alkali in dye bath. After dyeing, the dyed samples were rinsed with cold water followed by soaping and final rinsing.

1) *Recipe for dyeing of cotton without alkali*
   - Dye shade – 4%
   - NaCl - 5 gpL
   - Na2CO3 - Nil
   - Temperature - Boil
   - Time - 45 min
   - MLR - 1:30
2) *Recipe for dyeing of cotton with alkali*
   - Dye shade – 4%
   - NaCl - 5 gpL
   - Na_2CO_3 – 3 gpL
   - Temperature - Boil
   - Time - 45 min
   - MLR – 1:40

The dyeing process was carried on laboratory water bath machine.

E. Dyeing of wool and silk substrates

The sample was taken and weighed. The process was moved further by using following recipe.

- Dye shade – 4%
- pH- 4 (Adjust by acetic acid)
- Temperature - Boil
- Time - 45 min
- MLR - 1:30

F. Mordanting

Pre-mordanting treatment was conducted using metal salts such as alum, copper sulphate and ferrous sulphate. Fabric was treated with a solution (5.0% owf) of an appropriate mordant at material to liquor ratio of 1:30, for 30 min. Mordanting in case of alum and copper sulphate was conducted at 80^0C, whereas in case of ferrous sulphate the process was run at room temperature. After mordanting the samples were rinsed in cold water to remove the excess of mordant and used in the respective dye baths as described below.

G. Dyeing of mordanted samples

Dyeing (4% owf) of the pre-mordanted samples was performed for 1 h at 100 oC in an open bath beaker dyeing machine at 30:1 liquor to material ratio. The dyed samples were rinsed in cold water and dried in open air.

H. Soaping

Soaping of substrate was performed to remove superficial dye so as to improve washing fastness.
Recipe of soaping is given below:-

- Standard soap - 2gpL
- Temperature - 60°C
- Time - 15 min
- MLR - 1:30

I. Fastness testing and colorimetric evaluation [15]

Color fastness to washing was evaluated using standard ISO C06 C2S test. ECE reference detergent (4gL-1) and sodium perborate (1gL-1) at pH 10.5 and steel balls (25) were employed in the test. A 10 x 4 cm dyed fabric strip stitched through the short end to SDC's multifibre test fabric was washed on a Washtec (RBE) at 60 o C for 30 min, the samples was rinsed with cold water air dried and analyzed for the color change and staining. Likewise color fastness to rubbing was conducted using IS-766-88 test using a crock meter. The light fastness was tested on a xenon arc lamp following the test method ISO 105/B02 using blue wool reference samples.

The colorimetric properties [16] of the dyeings (D65 illumination, 10o observer) were determined using a Spectraflash 600 colorimeter (Datacolor International). Relative colour strengths (K/S values) were determined using the Kubelka Munk equation.

$$K/S = \frac{(1-R)^2}{R} - \frac{(1-R)^2}{2R_0}$$

Where R is the decimal fraction of the reflectance of dyed fabric, R_0 is the decimal fraction of the reflectance of undyed fabric, K is the absorption coefficient, and S is the scattering coefficient.

III. RESULTS AND DISCUSSION

The experiments showed that cultures of *Actinomycete* developed heavy floating mycelia rugs, and began elaborating dark reddish orange pigments which leaked into the medium after one month from incubation.

A. Dye extraction

Extraction of culture filtrate of *Actinomycete* and spectrophotometric measurements show that there are three compounds. Sludge of *Actinomycete* contains mainly these three compounds, which are derivatives of naphthoquinones.

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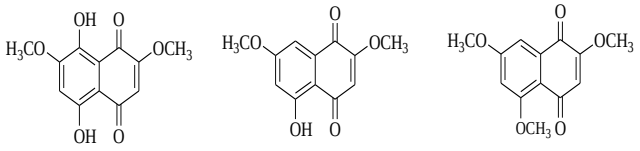
These compounds [17] are

1. 5,8-Dihydroxy-2,7-dimethoxy-l,4-naphthoquinone; (I)
2. 2,7-Dimethoxy-5-hydroxy-l,4-naphthoquinone; (II)
3. 2,5,7-Trimethoxy-l,4naphthoquinone.(III)



I II III

Fig. 2. Chemical structures of compounds

First compound is in its impure form gives dark red color. Chloroform elution of the main dark red compound after evaporation of the chloroform, and titration of the residue with hexane give reddish black crystals which after recrystallization from acetic acid-water mixture and then from chloroform-hexane, gives pure compound of blue color with alcoholic ferric chloride. The compound can reversibly decolorize using sodium hydrosulfite. This compound is insoluble in water, concentrated hydrochloric acid and 5% sodium bicarbonate, but gets dissolved in dilute aqueous solution of sodium hydroxide and produces purple colored solution.

Second compound is of orange color which is also in impure form. Chloroform elution of the compound gives orange crystals which after recrystallization from dioxane-water gives pure compound having brown color with alcoholic ferric chloride. Compound can reversibly decolorize with sodium hydrosulfite. It is insoluble in water, concentrated hydrochloric acid, and 5% sodium bicarbonate but gets dissolved in dilute aqueous sodium hydroxide and produces red-purple colored solution.

Third compound is also of orange color same as of second compound. Elution with chloroform followed by 1% methanol in chloroform brought down this orange color to dark orange.

The compounds mentioned above are soluble in acid and show the affinity for protein fibres.

B. Fastness Results

The K/S values and colors obtained in the dyeing are shown in Table 1 are shown, a slight variation in the color could be seen when dyeing was conducted using different mordants and without mordants.

Comparison of fastness results shows that

S1 > S2: It shows that washing fastness obtained on wool was better than silk.

S4 > S3: It shows that washing fastness properties obtained in wool by using copper sulphate are better than using alum.

S5 > S6, shows that washing fastness results of silk premordanted with copper sulphte are better than premordanted using alum .

The fastness properties are quite satisfactory for practical dyeing with many cases, particularly with the mordanted samples. It can be seen that the dyeings using extracted pigments were yellow and brown in hue.

TABLE 1. COMPARISON OF SHADES ALONG WITH K/S VALUES AND WASHING FASTNESS

S. No	Sample Code	Samples used	Dyed Samples	K/S Values	Staining on Cotton	Staining on Wool	Change in Color
1	S1	Wool		9.3795	4-5	3-4	4
2	S2	Silk		7.6921	4-5	3-4	3-4
3	S3	Wool (alum)		8.5418	4-5	4-5	3-4
4	S4	Wool (copper sulphate)		17.0013	4-5	4	4
5	S5	Silk (alum)		8.5418	4-5	3-4	3-4
6	S6	Silk (copper sulphate)		18.5409	4-5	4-5	4-5

IV. CONCLUSIONS

Broth obtained from Culture of *Actinomycete* bacteria shows good dyeability on wool and silk. Derivatives of naphthoquinones compounds which were produced by stationary cultures of *Actinomycete* bacteria could be used for dyeing wool and silk with good fastness properties and high dye uptake on mordanted substrates. In both cases of dyeing i.e. with and without premordanting, depth obtained on wool was better than silk. Also, in case of both wool and silk, by using CuSO4 as mordant, exhaustion behavior as well as depth obtained was better than the case of alum as mordant. In case of wool, by using alum and CuSO4 as mordant, the fastness properties has been found best ranges between 4-5.

This study shows that natural naphthoquinones dyes can provide bright hues and good colour fastness properties. They can serve as a remarkable resource of raw material in the future. Chemical alteration of natural complexes could be an interesting field of study as it could significantly ease production of dye molecule.

Dye extracted from different microbial cultures may also be used on different textile substrates as well as can be considered as future enhancement. Naphthoquinones based dyes may be used commercially on nylon fibres.

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