# Incorporation of Biocolours in Textiles: An Eco Friendly Approach

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## Abstract

Repeated and prolonged use of synthetic colours has thrown up many issues related to environment and human health. Hence, there is an increasing demand for "biocolour" from natural sources which can replace artificial synthetic colours. Natural colourants are generally obtained from plant, animal or micro organisms. Microbial pigments have some advantages over plant and animal species and so it is of much importance for pigment production. *Leifsonia aquatica, Pseudomonas aeruginosa, Serratia marcescens* were isolated for yellow, green and red colour pigment respectively, from natural environmental source and highest colour production was achieved at 25 $^{o}$C, 150 rpm in 72 h incubation time. Glycerol content was added for enhancement for good visibility of colour. Isolated pigment producing species were confirmed by fully automated Vitek 2 and Phoenix 100 system. Extracted pigments were analysed by TLC and various confirmation tests were carried out. Antimicrobial properties of extracted pigments were also evaluated. Extracted pigment can be applied on selected fabric at optimized conditions, using combination of mordants and may be checked/evaluated for washing, rubbing, light and temperature fastness. This indicates the ability of the pigment to be used as colourant for fabric and a good alternative source to replace synthetic dyes in textiles.

**Key words:** Biocolour, Vitek 2, TLC, mordant.

## INTRODUCTION

Bacteria are considered as a good source of pigments. As they are originate from living organisms, they are also referred to as “Biocolours”. Plant and animal origin pigments are also available in nature but due to some drawbacks like availability throughout the year, its solubility in water, solubility in organic-inorganic solvents, sensitivity against heat and light, bacterial pigments are mostly preferred. Generally, aerobic microorganism produce pigments. Biocolours are identified as their secondary metabolic products. They have good potential for various industrial applications.

The terms “dyes” and “pigments” both are different according to its solubility. Generally dyes are soluble in water and pigments are soluble in solvents. Also pigments score higher on the longevity parameter when compared to dyes. Dyes are generally chemically synthesized and available in large number whereas pigments are derived majorly from natural sources and available in limited numbers. Pigments need a binding agent for gluing on fibre.

Textile industries utilize enormous amounts of synthetic dyes and produce the textile effluent, which is difficult to treat and dispose. The harmful effect of synthetic dyes used in textile have forced to concern about the alternative natural source which has less adverse effect on environment and give a product that is eco friendly (Juailova *et al.,* 1997). Natural dye can provide the much needed alternative to the complex word of chemical dye. Biocolourants when incorporated into textiles, can be directly applied on fabrics or with specific mordants which are generally used for the stability of natural pigment. Such pigments also show antibacterial activity and have capacity to absorb ultraviolet radiation and these characters increase the value of dyed fabric.

## Materials and Methods

- **Isolation of Pigment Producing Bacteria:**

Microorganisms which are capable to produce bio colours (pigments), were isolated from the soil sample collected from the campus of Veer Narmad South Gujarat University, Surat and grown on Nutrient agar plate (HiMedia, Mumbai).

From Nutrient agar plate, isolated 3 chromogenic bacterial colonies were recorded for the production of considerable amount of yellow, green and red pigmentation on the agar as well as in the broth.

Selected strains were identified based on the morphological and biochemical characteristics, as described in Bergey's Manual of Systematic Bacteriology (Buchanan and Gibbons, 1974) as well as with the help of VITEK 2 system and ID-GNB card.

- **Standardization of Procedure for Extraction of Pigment:**

Maximal yield of pigment for extraction of culture, broth was standardized, using different solvents *viz*; methanol, ethyl acetate, petroleum ether, chloroform, diethyl ether and distilled water.

50 mL of freshly prepared Nutrient broth (HiMedia) was taken in 250 mL Erlenmeyer flasks were inoculated directly from the colonies of pigment producing microorganisms grown on Nutrient agar plate and incubated for 72 h as stationary cultures on a rotary shaker at 150 rpm, at 25 ± 2°C temperature. 1 % glycerol was added for the enhancement of pigment production.

Extraction of the pigment was done by the modified method given by P. Gunasekaran, 2005. After incubation, the grown cultures were centrifuged (REMI-laboratory Centrifuge, India) at 2200 rpm for 10 minutes. The coloured pellets were re-suspended in 95% methanol until pellets becomes colourless. The coloured supernatants were then analyzed by scanning in a U.V.-Visible spectrophotometer (Shimadzu, Japan) for detecting their absorbance. The scanning range selected was 400-600 nm.

Extracted pigments were filtered by Whatman filter paper no.1 (0.1 mm) and filtrates were concentrated by evaporation of the solvent. Evaporated extracted pigments were re-dissolved in ethyl acetate and methanol.

- **Analysis of Colourants by Chromatography:**

Extracted Pigments were separated by Thin Layer Chromatography. The solvent system used for the TLC was chloroform: methanol: acetic acid: water (90: 8 :1 :0.8). Rf value of separated pigments was compared with standard values of pigments.

- **Antibacterial Activity of Partially Purified Pigment:**

Bacterial cultures were used for the antibacterial activity namely *Staphylococcus aureus, Salmonella paratyphi B, Escherichia coli* and *Bacillus subtilis.* The extracted pigments having concentration were 0.052 g/mL ,0.185 g/mL 0.131 g/mL, was used respectively for yellow, green and red pigments to determine its antibacterial activity by disc diffusion technique given by Patel R. J., 2011.

- **Presumption Test for Extracted Pigment:**

**For Yellow Biocolourant:**

The colony of yellow bacteria was scrapped & flooded with 20 % (w/v) KOH. Colour of the colony was changed from yellow-orange to red-brown (basic condition test). Resulting colour of the colony was compared with original one which was not in contact with KOH. Acidic solution was used to revert the initial colour (acidic condition) (Balows *et al.,* 1992).

**For Green Biocolourant:**

Add 2-3 mL chloroform in extracted pigment. Bluish colour was developed. After addition of 0.2N HCl, it gives pinkish red colour which is confirmation test for Pyocyanin pigment. (Sudhakar T. *et al*.,2013, Wa'ad Mahmood Ra'oof *et al.,* 2010).

**For Red Biocolourant:**

Extracted pigment was tested against acidic and alkaline conditions. Red or pink colour in acidic condition and yellow or tan colour in alkaline condition confirmed a positive presumptive test for prodigiosin (T. Sathis kumar and H. Aparna, 2014, Gerber and Lechevalier, 1976).

- **Application of Pigments in Textiles:**

Bacterial pigments in methanol were used as the stock solution for yellow, green and red pigment respectively. Attempts were made to dye cotton, viscose and polyester fibres with the extracted microbial biocolourants. The dyeing of cotton/polyester and viscosa fibres were carried out at $100^0$C for 60 min. One set of experiment may be done with the addition of thiourea, alum, copper sulphate, ferrous sulphate and lime as a mordant for increasing binding capacity of pigments to fibres (Shirata *et al.*, 2000).

- **Wash Performance/ Fastness Properties of the Textile Materials:**

**Washing fastness:**

The preheated soap solution (Tide, at 60ºC) and water was taken in the ratio of 1: 50 (0.5 g/25 mL) in beaker.Dyed fibres was added in the solution for 30 minutes. Then the specimen was removed and rinsed in cold water.

**Rubbing fastness:**

The rub fastness of the dyed fibres was carried out by rubbing the fibres manually and checking for fading of colour.

**Light fastness:**

The dyed fibres were exposed to sunlight for 24 h. The colour fastness to light was evaluated by comparison of colour change of the exposed portion to the unexposed original material.

The same procedure can be repeated for the dyed textile material treated with any mordant.

The rating for rubbing, light and washing fastness was determined to respect to staining on cotton, polyester showing rating between 1 to 5.

## Result and Discussion

- **Selection and Extraction of Biocolourants**

The isolated microorganisms were obtained from soil sample showed considerable amount of yellow, green and red pigment production both on the agar medium as well as in the liquid medium.

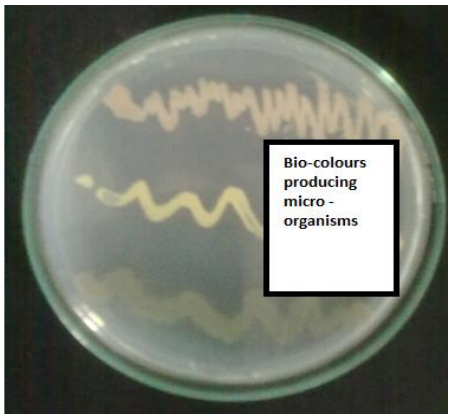


**Figure 1: Bio-colour producing microorganisms**


**Figure 2: Extracted Pigments in solvent-Methanol**

The pigment produced by isolates were water insoluble except green pigment producing isolate and methanol was found to be an ideal solvent for the maximum extraction of the pigment among the different solvents studied.

Isolated organisms were further confirmed by VITEK 2 (bio-Me´rieux) system in conjunction with the ID-GNB card. VITEK 2 testing required from 6 to 13 h. The sensitivity, specificity and positive and negative predictive values for the VITEK 2 test system were calculated against the result of the molecular comparison method.

Identified and evaluated result of VITEK 2 gave 99 % probability that isolated red colour producing organism was *Serratia marcescens* having bionumber 6025711455054220, yellow colour producing organism was *Leifsonia aquatic* having accession number 1502100750 and green colour producing organism was *Pseudomonas aeruginosa* having bionumber 0043041003500242.


**Figure 3: Pigment producing isolates in liquid media**

VITEK 2 system is a promising new tool for identifying gram-negative and gram-positive bacteria regarding both speed and accuracy.

- **Presumption Test for Extracted Pigment:**

The colony of yellow coloured pigment, when flooded with 20 % (w/v) KOH, there was a change in its colour from yellow to red-brown colour which is assured test for presence of flexirubin type of pigment.

Addition of 2-3 ml chloroform in extracted pigment, bluish colour was developed and after addition of 0.2N HCl, it gave pinkish red colour which is confirmation test for Pyocyanin pigment.

On addition of concentrated HCl to the supernatant containing pigment, the colour changed to deep pink or red colour and addition of ammonia solution, the colour changed to tan or yellow colour, which assured the positive presumptive test for prodigiosin.

- **TLC Result:**

After running TLC plate using solvent system (chloroform: methanol: acetic acid: water / 90: 8 :1 :0.8) (**Figure 4**). Rf value was compared with standard Rf value of flexirubin, pyocyanin and prodigiosin a shown in **Table 1**.

| Pigment | Rf value | Standard Rf Values |
|---|---|---|
| Pinkish red | 0.5 | 0.65 |
| Yellow | 0.8/0.84 | 0.85/0.71 |
| Green | 0.93/0.92 | 0.72 |

**Table 1: Result of TLC plate**

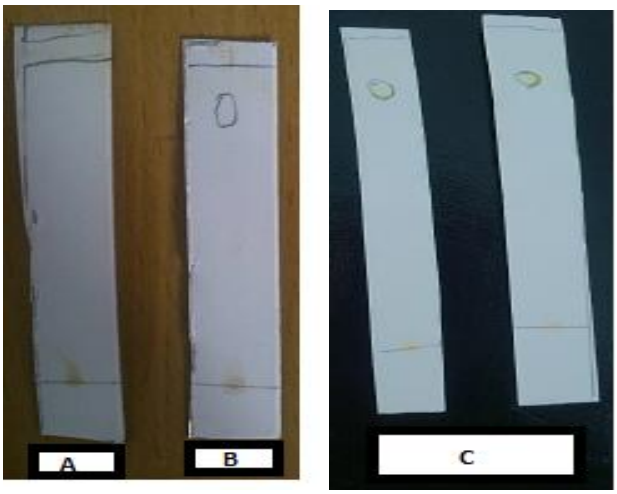


**Figure 4: Separation of pigment by TLC plate (A: green pigment, B: red pigment, C: yellow pigment)**

- **Antibacterial Activity:**

The antibacterial activity of Prodigiosin and Pyocyanin pigments were found significant towards the tested organisms as shown in **Table 2**. Red pigment has shown the antibacterial activity against *Staphylococcus aureus* and *Salmonella paratyphi B*. It has shown 9 mm & 15 mm diameter zone of inhibition respectively. Green pigment has given 10 mm & 13 mm diameter zone of inhibition against *Escherichia coli* and *Staphylococcus aureus* respectively.

| **Name of the test organisms** | **Ethyl acetate** | **0.131 g/20 mL broth/1 mL in ethyl acetate** | **0.185 g/20 mL broth/1 mL in ethyl acetate** |
|---|---|---|---|
| | **Control** | **Pinkish red pigment ( zone size)** | **Green pigment ( zone size)** |
| ***Escherichia coli*** | - | 10 mm | - |
| ***Staphylococcus aureus*** | - | 13 mm | 9 mm |
| ***Bacillus subtilis*** | - | - | - |
| ***Salmonella paratyphi B*** | - | - | 15 mm |

**Table 2: Susceptibility of test organisms towards pinkish red and green pigments**

- **Dyeing in textiles**

The fibres dyed with microbial biocolourants have shown uniformity and levelness. The dyeing of cotton/polyester was carried out at $100^0$C for 60 minutes. The results of which are shown in **figure 5**.


**Figure 5: Dyed fibres, 1- control, 2- yellow biocolour, 3- green biocolour, 4-red biocolour**

- **Fastness properties of dyed fibres:**

The dyed fibres of cotton/ polyester were tested for assessment of fastness properties such as, washing fastness, light fastness and rubbing fastness. The results of which are compiled in **Table 3**.

| **Fastness property** | **Rating** | | |
|---|---|---|---|
| | **Yellow** | **Green** | **Red** |
| **Fastness to washing** | | | |
| **Cotton** | **4** | **4** | **4** |
| **Polyester** | **3** | **3** | **3** |
| **Viscose** | **4** | **4** | **4** |
| **Fastness to rubbing** | | | |
| **Cotton** | **4** | **4** | **4** |
| **Polyester** | **2** | **2** | **2** |
| **Viscose** | **4** | **4** | **4** |
| **Fastness to light** | | | |
| **Cotton** | **3** | **3** | **3** |
| **Polyester** | **2** | **2** | **2** |
| **Viscose** | **2** | **1** | **2** |

**Table 3: Fastness properties of dyed fibres**

**( Rating: 5-excellent, 4-very good, 3-good, 2-poor, 1- very poor)**

## Conclusion

The yellow, green and red-pigment producing bacteria from natural source showed significantly good application as a natural dye/biocolourant for colouring of cotton, viscose and polyester. The pigment also showed antibacterial property enhancing its business worth. Pigment yield can be maximised with optimisation of media and extraction parameters. Pigment can be further analysed and characterised to elucidate its structure successively, which shall facilitate in describing the most effective application for that pigment in foods, cosmetics, paper, or any other such industries. Application of the natural pigment promotes consumers health protection and allows manufacturing of fully eco friendly pigment without any synthetic mix.

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