International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.4, pp 219-225, 2017

Production of Natural Dye by Solid State Fermentation

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Abstract : Dye is an intensely colored complex organic substance used for colouring other materials. They can be synthesized artificially or obtained from natural products. Natural colours are also called biocolors since they are extracted from biological materials like fruits, vegetables, seeds, roots, insects and microorganisms. In this study, microbial fermentation using macro algae as substrate was utilized to extract dye. Different macro algae such as *Chaetomorpha antennia, Gracilaria corticota and Ulva fasciata* were collected from Kovalam, Mahabalipuram, Tamil Nadu and were used as a substrate for fermentation. *Aspergillus niger* was used as an inoculum for fermentation. SSF was carried out for 10 days and the dye was extracted. Quality of the colour was analysed by adding fixative and non fixative at different time intervals. Results revealed that the colour intensity was more in the addition of fixative. Different pigments such as chlorophyll a (20.625 mg/l) was found more in *U.fasciata* , chlorophyll b (12.486 mg/l) was to be more in *G.corticata* and high amount of total carotene was found in (14.701mg/l) in *C.antennina* and least was found in *U.fasciata* (3.39 mg/l). Among all seaweed substrates, *C.antennina* was found to be the good one to impart colour.
Keywords : Dye, *Chaetomorpha antennia, Gracilaria corticota, Ulva fasciata, A.niger,* SSF.

Introduction

Natural dyes are an important alternative to harmful synthetic pigments[1]. Recent research and development on dye production and application is observed due to rising popularity of more natural lifestyle based on natural substances. Natural organisms have full of delicate colors, fascinating and attracting humans towards it. A large number of plant and animal and insect have been identified for extraction of color and were used in textile dyeing and other uses[2-4]. Nature has gifted us with more than 500 dye-yielding plants[5].

Natural dyes are also extracted from turmeric and beetroot are used as a substitute to red and yellow colour in textile industries because they are eco-friendly, readily available, biodegradable and will not harm environment as well as humans[6]. Plenty of industrial wastes are generated from food and beverages. These bio wastes can be used for extracting dyes.

Microbial production of carotenoids are the great interest due to the stability of the pigments produced and their cultivation technology[7-11]. The microbial production of dyes from various vegetables or chemical synthesis, have problems of seasonal and geographic variability in the production and marketing[12]. The advantages of pigment production from microorganisms comprise easy and fast growth in the cheaper culture medium, independence from weather conditions and colors of different shades.

Several studies reported that the microorganisms of the genus Monascus produce red pigments, which are used in food and textiles industries[13-15]. Monascus species are grown on rice grains, and used for coloring some foods in several asian countries[16].Solid state fermentation (SSF) has emerged as an effective alternative for liquid, culture-based fermentation technology. The substrates used in SSF supply the basic nutrients to the microorganisms to grow[17]. Recent studies revealed that SSF provides a more sufficient habitat for fungi, resulting in production of pigment in cheaper cost. Wastes generated from agro industries such as wheat bran, rice bran, sesame oil, coconut oil cake, palm kernel cake, cassava powder, groundnut oil cake, spent jackfruit seed powder and brewing grain have been screened to select the best substrate for producing pigment[17]. Many investigations have been performed to reduce the costs and optimize the pigments production. Seaweeds are one of the good source of pigment production. In Asian countries, seaweeds are regularly consumed as marine vegetables[18]. But in India most of seaweeds were not used effectively and were treated as waste. Hence, in this study, production of microbial pigments using macro algae as solid substrate and analysis of the pigment's parameters were carried out.

Materials and Methods

Collection and Preservation of Seaweeds

Different seaweeds such as *Chaetomorpha antennia, Gracilaria corticota and Ulva fasciata* were collected from Kovalam, Mahabalipuram and were identified by Dr.Balusamy, Professor and Head, Department of Plant Biology, Madras Christian College, Chennai. Then it was thoroughly washed with water to remove dirt particles and dried under sunlight. After complete drying it was ground well using mixer and stored.

Production and Extraction of Dye

Estimated amount of each seaweed samples were weighed and taken in a conical flask and 5 discs of pure culture of *A.niger* was inoculated in to the conical flask. Then 1% sucrose solution was added to the solid substrate and was kept for 7 to 10 days for fermentation.

After fermentation was completed, 90% methanol was added to extract the dye.

Pigment Analysis

The extracted dye was subjected to UV spectroscopy for pigment analysis.

Dyeing of Fabric

i. **With fixative**

A vinegar fixative was prepared in the ratio of 1:4 by adding 1 part of vinegar in 4 parts of water. Two different fabric such as nylon and cotton was soaked in the dye at different time intervals such as 15mins, 30 mins and 1 hour at 60° C and the color of fabric was analysed.

ii. Without fixative

The fabric was soaked in the dye for same time intervals at 70 to 80°C without adding any fixative and the color of fabric was analysed.

Fastness Test

After the fabric was soaked in the dye, it was washed and kept under tap water. After that the color of the fabric was analysed.

Rubbing Test

The fabric was washed and rubbed to observe the dye absorption.

Concentration of Chlorophyll A

$C_a = 16.72\ A_{665} - 3.62\ A_{649}$

Concentration of Chlorophyll B

$C_b = 25.06\ A_{649} - 6.5\ A_{665}$

Concentration of Total Carotenes

$(1000\ A_{470} - 2.860\ C_a - 129.2\ C_b)/\ 245$

Results and Discussion

In this work, various seaweeds were utilized as a solid substrate using *A.niger* for the production of dye. Different fabrics such nylon and cotton were soaked in dye at different time interval to analyze the color consistency of the dye (Fig.1).

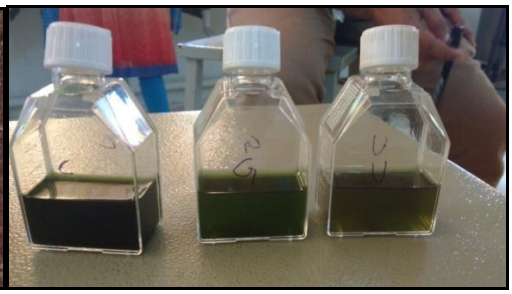

Fig.1 Fresh Fabric (Cotton and Nylon) **Fig.2 Dyes**

Raja Rajeswari et al.,[19]suggested that SSF was found to be the good practice in the microbial pigment production.

Results showed that, adding of fixative with extracted dye showed stable colour on the fabric even after underwent fastness test and rubbing test (Fig.3 – 14)

Fig.3 Fastness Test after dyeing without fixative (*U.fasciata* as substrate)

Fig.4 Fastness Test after dyeing without fixative (*C.antennina* as substrate)

Fig.5 Fastness Test after dyeing without fixative (*G.corticata* as substrate)

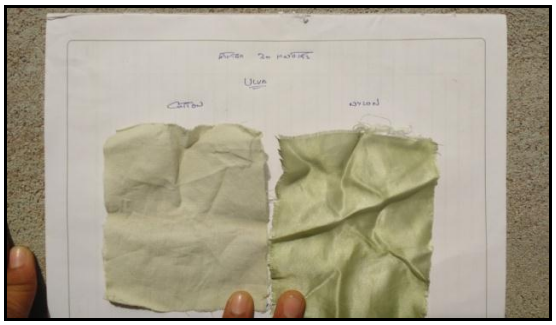

Fig.6 Rubbing test after dyeing without fixative (*U.fasciata* as substrate)

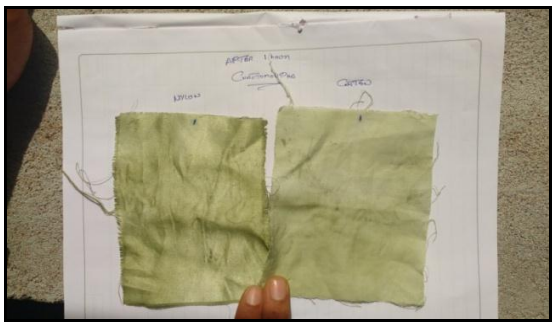

Fig.7 Rubbing test after dyeing without fixative (*C.antennina* as substrate)

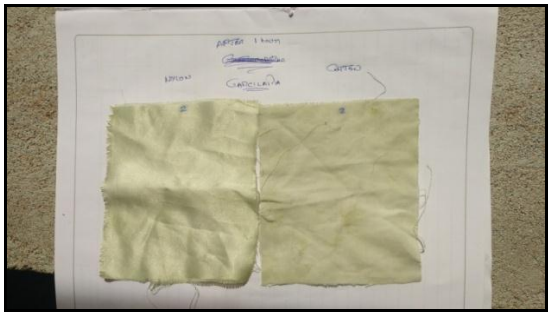

Fig.8 Rubbing test after dyeing without fixative (*G.corticata* as substrate)

Fig.9 Fastness Test after dyeing with fixative (*C.antennina* as substrate)

Fig.10 Fastness Test after dyeing with fixative (*G.corticata* as substrate)

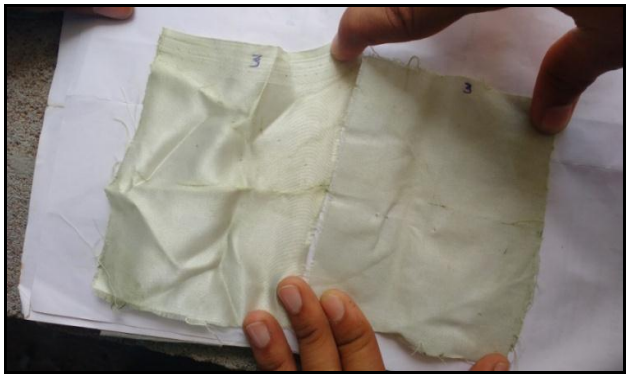

Fig.11 Fastness Test after dyeing with fixative (*U.fasciata* as substrate)

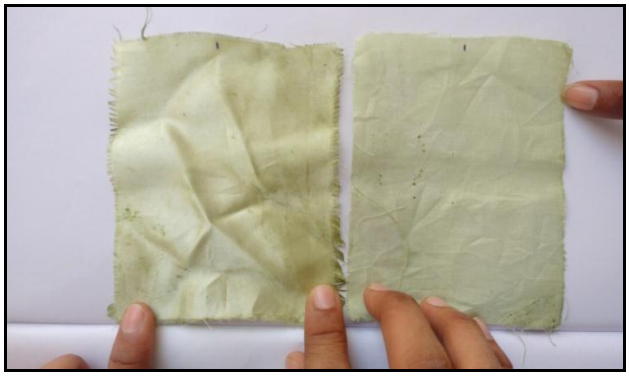

Fig.12 Rubbing Test after dyeing with fixative (*C.antennina* as substrate)

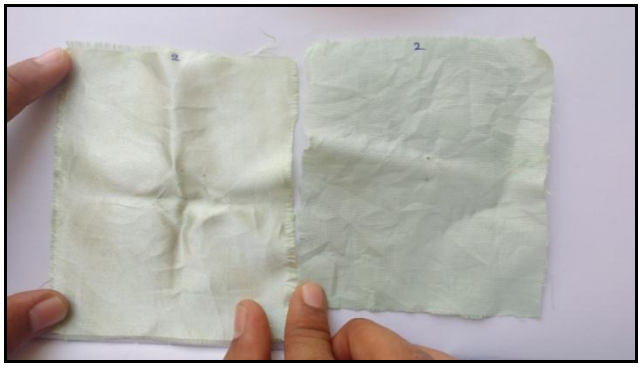

Fig.13 Rubbing Test after dyeing with fixative (*G.corticata* as substrate)

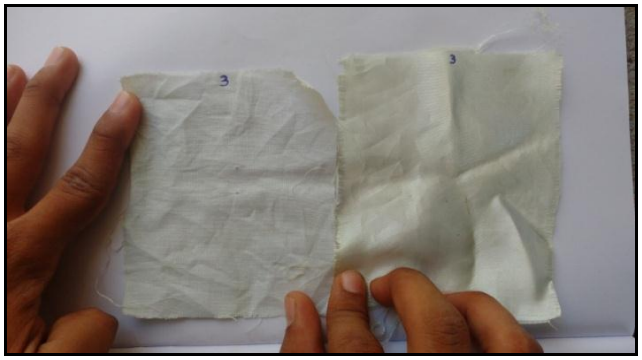

Fig.14 Rubbing Test after dyeing with fixative (*U.fasciata* as substrate)

Various pigments such as Chlorophyl a, Chlorophyl b and total carotenes was found to be present in the dye. (Table.1)

Table.1 Analysis of pigments in dye

Seaweed Substrate	**Ch a (mg/L)**	**Ch b (mg/L)**	**Total carotenes (mg/L)**
Chaetomorpha antennia	2.4786	1.4679	14.701
Gracilaria corticata	18.807	12.486	8.627
Ulva fasciata	20.635	8.345	3.39

Concentration of Chlorophyl a was found to be maxiumum(20.635 mg/l)in *U.fasciata* substrate followed by *G.corticata* (18.807 mg/l) and very less was found in *C.antennina.* In case of presence of Chlorphyl b, *G.corticata* was found to be the best substrate in which maximum amount of (12.486 mg/l) Chlorphyl b was found followed by *U.fasciata* (8.345 mg/l) and least amount was found in *C.antennina* (1.4679 mg/l). Total carotene content was found to be more (14.701 mg/l) in *C.antennina* followed by *G.corticata* (8.627 mg/l) and least (3.39 mg/l) was found in *U.fasciata*. (Table.1).

Conclusion

Production of natural dye using microbial fermentation is a developing technology in this modern era. This work showed that the using of seaweed waste as a substrate for microbial fermentation was found to be very much effective to produce dye. In future, a systematic screening of other microbes for pigment production is highly recommended. Optimization of pH, time, and amount of substrate may be carried out in future to increase the yield and improve the quality of dye.

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