



Production of Microbial Pigment from Bacteria and its application in Textiles and Cosmetics

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Abstract

All life on Earth, from microorganisms to plants and people, depends on pigments. Recent awareness of the importance of pigments in various industries has led to a renewed interest in natural sources of bacterial pigments. These pigments, which are produced by microbes, have better biodegradability, compatibility with the environment, and wide applications in food, pharmaceutical, cosmetic, and textile industries. The study aims to isolate bacterial pigments from soil samples and make dye from them, finding applications in various fields. Soil samples were collected from diverse environmental niches and pigment-producing organisms were enriched in nutrient broth. The spread plate approach was used to isolate pigments in substantially pure and concentrated forms. Bacterial pigments are secondary metabolites produced when the right conditions are present for a particular type of bacteria to flourish. The industrial microbial fermentation method offers benefits such as lower production costs, simpler extraction, increased yields, no shortage of raw materials, and no seasonality.

Introduction

Pigments are chemical compounds that absorb light from the visible spectrum, creating colours through a chromophore structure. They are used in various industries, including food, dyes, cosmetics, and pharmaceuticals.[6] H. Perkin founded the first organic synthetic paint factory in 1856, producing purple. Since then, synthetic organic dyes have been developed, creating a cheaper and wider range of pigments.[4][15]

The economic impact of the paint industry is evident in the large number of compounds synthesized, with up to 700 dyes currently available. Pigments produced from natural sources, such as microbial pigments, are of global interest due to their safe use as natural food coloring instead of synthetic colors. [5-8]

Microbial production of carotenoids, vegetable pigments, or chemical synthesis presents challenges due to seasonal and geographical variations in production and marketing.[7] Advantages of microbial pigment

production include simple and rapid growth in a favorable nutrient medium, independence from weather conditions, and colors in different shades. Microbial dyes are already used in the fishing industry, improving the pink color of farmed salmon.[11] Carotenoids like β -carotene and xanthophylls like astaxanthin are essential for maintaining good vision and maintaining the metabolism of the macula and retina of the eye.[2] They also act as nutraceutical, averting carcinogenesis through anti-oxidative and anti-free radical mechanisms. [1]

Pigment production is a crucial aspect of the development of any pigment production process, as it is influenced by various factors such as incubation temperature, pH, carbon source, nitrogen source, and minerals.[13] The growth conditions of microorganisms, particularly physical and nutritional parameters, are of utmost importance in the process. The "one factor at a time" method is often used, but it is time-consuming, labor-intensive, and expensive.[12]

Recent optimization efforts have relied on statistical experimental design and response surface analysis, as well as artificial intelligence techniques like genetic algorithms. Statistical design is a potent tool that accounts for the main and interactive influences of fermentation parameters on process performance, producing valuable data with little testing, which cuts down on the time and expense of process development.

There is a growing interest in natural colouring alternatives due to concerns about the potential negative effects of synthetic colourants on consumers and the environment. Microbial colorants, or pigments, have several benefits, such as the use of household and agro-industrial wastes as low-cost substrates for pigment production. Techniques and procedures have been developed to use less expensive substrates and wastes as substitute substrates for the microbial pigment manufacturing process.[11]

This study focuses on isolating pigment-producing microorganisms, simple production of commercial pigments in higher amounts by purchased strains, and optimizing the primary factors that impact pigment synthesis as required.

MATERIAL AND METHODS

2.1 Sample collection:

Soil samples were collected from Bhayandar East Khadi, Mira Bhayandar, Maharashtra. Samples were collected from the soil surface with a sterile spatula, and about 10g of soil were obtained from a depth of 2–5cm. Samples were transported immediately to the laboratory and processed for further analysis.

2.2 Screening method:

Soil samples were serially diluted up to 10^{-6} . 10^{-5} to 10^{-6} dilutions were spread on sterile modified mineral salt medium plates by the spread plate technique and incubated at 37°C for 24 to 48 hours. The modified

mineral salt medium plates had been found to be growing after 48 hours of incubation. The pigment-producing microorganisms were used for additional studies. The selected pigmented colonies were selected and streaked on modified mineral salt slants and modified mineral salt medium plates to obtain a pure culture of the colony. Stress conditions for pigment were created using a variety of salts, including magnesium sulphate dihydrate, sodium chloride, calcium chloride, and potassium dihydrogen phosphate. Nutrient agar and agar were used as growth media to offer nutrients necessary for bacterial growth and pigment formation. Pure cultures were subcultures on sterile modified mineral salt slants and kept as stock cultures for future studies.

2.3 Characterization of isolated pigmented bacteria:

Gram staining was used to analyze bacterial isolates, observing their morphological characteristics, including form, colour, texture, opacity, elevation, and margin, were observed. The isolated colony was viewed under a microscope, and bacterial size was assessed using micrometers.

2.4 Production of pigment:

For the production of the pigment, the isolated bacterial cultures were suspended in four petri plates containing modified mineral salt medium. They were incubated at 37 °C for 7 days for observation of maximum pigment production.

2.5 Extraction of pigment:

Different organic solvents include ethanol, DMF (Dimethylformamide), chloroform, and acetone were screened for maximum extraction of pigment. The inoculation of 1 gram of pigment was dissolved in each of the tubes containing the extrant. The dissociation of the colourant gives a positive result.

2.6 Effect of pH:

To determine the optimum pH for the extracted pigment, potassium phosphate buffer was used, which contains potassium dihydrogen phosphate and dipotassium phosphate. By inoculation of the pigment in different pH solutions ranging from 5 to 12. pH was determined.

2.7 Effect of temperature:

Temperature optimization of the pigment was achieved when it was exposed to different temperatures. The pigments were subjected to temperatures ranging from 30 to 100°C. To determine the best temperature.

2.8 Spectrophotometric analysis:

Spectrophotometric monitoring of pigment expression is possible because most pigments absorb light at a certain wavelength. The extract was analyzed in the range of 328_{nm} to 390_{nm} by an UV-visible spectrophotometer to find out the highest absorption spectra.

2.9 Antibacterial test:

The antibacterial assay was performed by the Kirby-Bauer disc diffusion method. The sample microorganisms were collected, and the pigment was tested for its antibacterial activity. Two wells were drilled in the NA plates, and a 24-hour-old microbial culture was used to seed the nutritional agar plate. One of the wells has been filled with a suitable quantity of pigment, and the other has had ethanol poured into it and incubated at 37 °C for 24 hours. The end result was found by using the measuring zone of inhibition in diameter.

2.10 Antifungal test:

The antifungal test was done using the Kirby-Bauer disc diffusion technique. The sample microorganisms were collected, and the pigment's antifungal activity was determined. The sabouraud dextrose agar plate was previously seeded with a 24-hour-old microbial culture, and two wells were drilled in the SDA plates. One of the wells was filled with an appropriate amount of pigment, while the other was filled with ethanol and incubated for 24 hours at 37°C. The final result was obtained by measuring the zone of inhibition in diameter.

2.11 Catalase Test:

One drop of 3% hydrogen peroxide was added for this test, and the effervescence was recorded.[8]

2.12 Applications of pigmented bacteria

2.12.a Cosmetic industry: Because of the widespread use of diverse coloring in cosmetics and their global market, efforts have been made to research the use of biopigments in cosmetics, particularly skincare products. Skincare is crucial for both skin beauty and health, including barrier function. As people age, their skin thins, losing suppleness and moisture capacity. Exposure to chemicals and UV radiation can deplete the skin's antioxidant capacity and accelerate ageing.

Cosmetic goods often contain synthetic chemicals that might have negative effects on the body. For instance, pigments can damage cells and UV filters can induce tumours. Metals are commonly used as pigments in cosmetics, including eye shadow, lipstick, blush, and eyeliner. Certain metals, such cadmium and chromium, can injure the human body and disrupt metabolism. Cosmetic toxicity was not considered early on, resulting in undesirable outcomes including as deformities, blindness, and death. While there are some limitations to their use, they are not totally effective.[10]

✓ Application of bacterial dye in cosmetics

Ingredients for making nourishment cream from dye :

1. Gulab jal,
2. Aloe vera gel,

3. Bees wax,
4. Liquid paraffin,
5. Sodium benzoate,
6. Borax, citric acid,
7. Natural bacterial pigment - as much as required.

✓ **Procedure is as follows:**

In a heat-resistant container, mix beeswax and liquid paraffin. Heat carefully until the beeswax melts completely. Incorporate Gulab jal, aloe vera gel, citric acid, and sodium benzoate into the melted mixture. Stir thoroughly to mix. In a separate container, dissolve borax in a little quantity of water. Slowly add the borax solution to the wax oil mixture and stir continuously. Allow the mixture to cool somewhat without solidifying fully. Add natural pigment to the colour mix well to achieve equitable distribution. Pour the cream into a sterilized container and let it solidify entirely. Label the jar with the date of preparation and store in a cool, sterilized area away from sunlight.

2.12.b Textile industry: Synthetic dyes are widely utilized in textile industries to meet the needs of both dyers and consumers. Before the 19th century, natural dyes were only utilized in the textile industry. Perkin's development of mauveine in 1856 led to the popularity of synthetic dyes and the extinction of natural colours. Chemists in the textile business use innovative concepts to generate synthetic dyes that meet diverse demands. Synthetic dyes are widely utilized around the world. The dye industry has a significant impact on the environment, with effluent contaminating drinkable water and negatively impacting human health.[14]

Nowadays, individuals prefer natural products and adopt a green or bio-friendly lifestyle. Many researchers focused on producing natural dyes from plants, animals, and microbes. Microbes, including bacteria, actinomycetes, fungi, and algae, create pigments. Pigments yield the least amount of colours compared to other bacteria. Therefore, research is focused on increasing pigment synthesis through fermentation. Microbial pigment are a better alternative to synthetic dyes due to their biodegradability, ecological balance, and increased market value.

✓ **Procedure is as follows:**

- We purchased silk cloth from a nearby vendor.
- We used 40 ml of dye plus 0.3 gramme of NaCl in one beaker and dye in another since the salt acts as a fixator here and allows the dye to adhere to clothing.
- After soaking the cloth in both dyes, we heated it in a water bath to high temperatures to see if the colour would fade or remain.
- The tested cloth had stained well with the dye.

RESULT AND CONCLUSIONS

3.1 Sample Procurement: Soil samples from Bhayandar East Khadi, were collected and processed for further analysis.



Figures 3.1 Soil sample

3.2 Isolation of pigment-producing bacteria: Soil samples were diluted and spread on modified mineral salt medium plates for 24 to 48 hours.

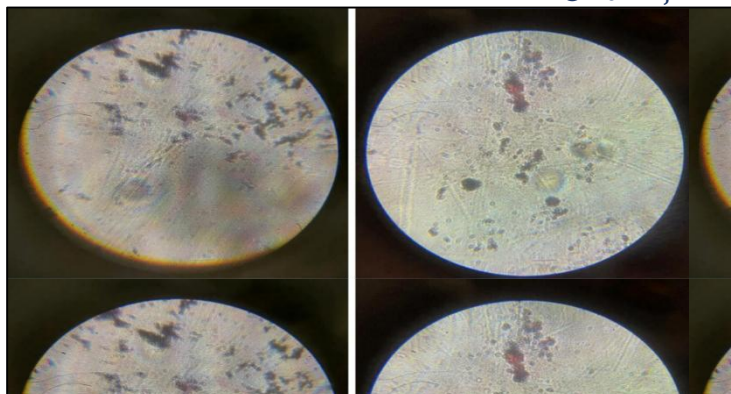
The plates were found to be growing after 48 hours of incubation.

When exposed to stress and provided with salt, they create pigments in the hues orange, and pinkish red as a means of surviving.



Figures 3.2 Pigment production on modified mineral salt medium

3.3 Characterization of isolated pigmented bacteria: Gram staining was used to investigate the form and colour, of each isolate's morphological colony. The bacterium was identified as gram-negative cocci.



Figures 3.3 Gram staining of Microbial pigment

3.4 Extraction of pigment: It dissolved in acetone and ethanol solution

The pigment was decolorized by Dimethylformamide (DMF)



Figures3.4 Pigment extraction with an organic solvent

3.5 Optimization-effect of pH and temperature: Potassium phosphate buffer was used to establish the ideal pH for the extracted pigment. The pigment's growth and pigment synthesis peak was maintained at pH 7.

The pigment's temperature was optimized by exposure to various temperatures.

Because this bacterial pigment retains its colour even at 80 °C, it is perfect for use in the dyeing process. The ideal dyeing temperature is 80 °C, and the process takes 60 minutes.

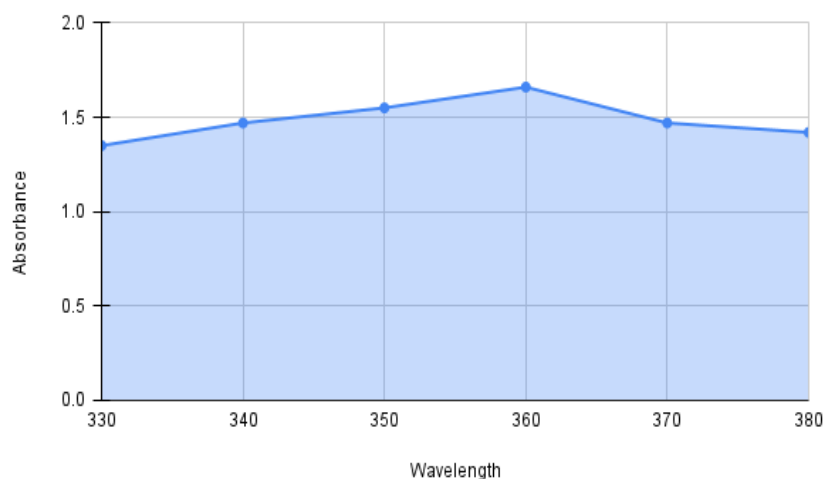
3.7 Spectrophotometric analysis: The wavelength at which the microbial pigment shows maximum absorbance is called the absorption maximum, which depends on the concentration of the sample. By plotting

the graph of absorbance at different wavelengths, it was found that the microbial pigment showed the maximum absorbance at 360 nm, which was 1.66.

Wavelength	Absorbance
330	1.35
340	1.47
350	1.55
360	1.66
370	1.47
380	1.42

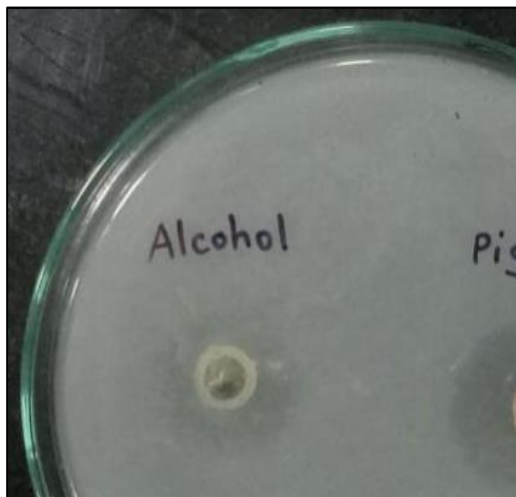
Table 3.1 Reading of absorbance at different wavelengths.

Standard graph of Wavelength v/s Absorbance



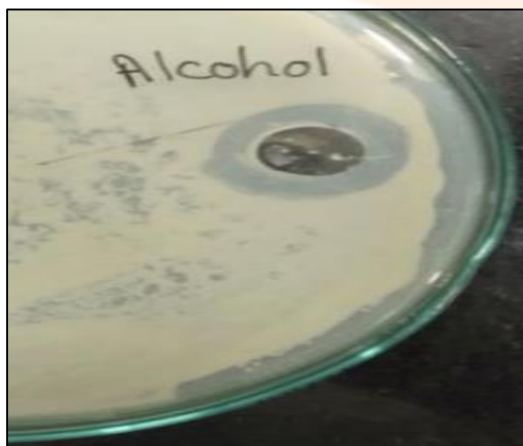
Figures 3.5 Standard graph of wavelength v/s absorbance

3.8 Antibacterial test: The Kirby-Bauer disc diffusion method was used to conduct an antibacterial test. A microbial culture was created using sample microorganisms and cultured for 24 hours. Two created wells filled with ethanol and pigment were placed on nutritional agar plates. The bactericidal action of pigments was effective.



Figures 3.6 Microbial pigments' antibacterial properties

3.9 Antifungal test: The Kirby-Bauer disc diffusion method was used to conduct the antifungal test. A 24-hour-old microbial culture and a Sabouraud dextrose agar plate were used for the antifungal test. One well was filled with ethanol, pigment was added, and it was then incubated at 37°C. There was no antifungal action in the pigments.



Figures 3.7 Microbial pigments' antifungal properties

3.10 Catalase Test: Bubbles formed on colored bacteria after hydrogen peroxide addition. Catalase test results indicate bacteria's catalase enzyme generation. Bacteria tested positive via catalase test.



Figures 3.8 Catalase test of microbial pigment

3.11 Applications of pigmented bacteria

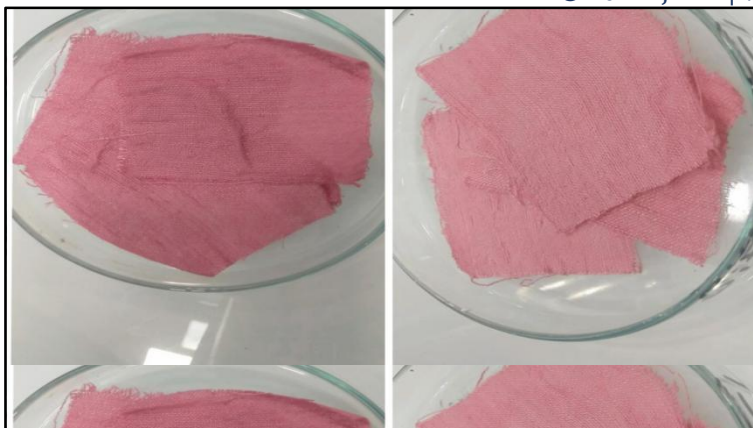
3.11.a Cosmetic industry: Beeswax and liquid paraffin mixture melted, incorporated Gulab jal, aloe vera gel, citric acid, and sodium benzoate. Borax solution dissolved in water added to the wax-oil mixture, cooled, and natural pigment added.

Cream made from bacterial dye solidified, giving a pinkish appearance and no irritation after application.



Figures 3.9 Cream made from pigmented bacteria

3.11.b Textile industry: Dye to absorb to clothes was tested using 40 milliliters of dye and 0.3 grams of NaCl. The fabric that was left to soak in dye and sodium chloride became a light pink colour, whereas the fabric that was just soaked in dye took on the proper colour.



Figures 3.10 Microbial pigment used to tint silk fabric

CONCLUSION

Throughout the observation of this particular procedure, it was evident that the biological activity exhibited by bacterial pigments is influenced by various stress conditions, temperatures, and pH levels. These factors play a crucial role in inducing the production of pigments as secondary metabolites by the bacteria. The resulting biological compound derived from this process possesses a diverse array of applications that offer innovative solutions to contemporary challenges that pose threats to both mankind and the natural environment. In order to fully harness the potential benefits of these bacteria, further extensive studies are indispensable to facilitate their integration into industrial processes. The utilization of these bacteria spans across a multitude of industries including therapeutic, cosmetic, dye production, and the food sector, underscoring their significance in diverse fields.

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