Original Article
ISSN (Online): 2582-7472

ShodhKosh: Journal of Visual and Performing Arts
April 2024 5(4), 500–506

# OPTIMIZING PIGMENT PRODUCTION FROM PENICILLIUM SP. FOR SUSTAINABLE SILK DYEING

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## ABSTRACT

This study explores the use of microbial pigments from Penicillium sp. as a sustainable alternative to synthetic dyes in textile dyeing, focusing on optimizing pigment production and assessing color fastness on silk. Static Potato Dextrose Broth (PDB) at 28°C produced the highest concentration of red pigment after 27 days of incubation, with an optical density (O.D) of 1.010 at λmax = 530 nm. In contrast, 15°C resulted in slower pigment production (O.D = 0.860), and 37°C showed negligible growth. Adjusting the PDB pH to 5 increased pigment yield, aligning with previous studies showing Penicillium sp. thrive in acidic conditions. The final broth pH decreased to 4.0 due to organic acid production by the fungus.

Silk samples dyed with these pigments were evaluated for color strength and fastness. The colorimetric values indicated moderate color intensity (K/S = 4.25) with a bright, pastel-like appearance (L* = 81.23), and a warm reddish-yellow hue (a* = 9.23, b* = 6.48). Fastness tests showed excellent performance for dry rub (rating 5), good results for wet rub (rating 4), and favorable wash fastness (rating 4 for staining and color change). However, light fastness was lower, suggesting potential fading under prolonged exposure.

Overall, the study identifies Penicillium sp. as a promising source of natural dyes for silk, with optimized fermentation conditions (PDB, 28°C, pH 5, 27 days) leading to high pigment yield and strong fastness performance, offering a sustainable alternative to synthetic dyes in textiles.

**Keywords:** Silk, Dyeing, Penicillium, Fastness

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**DOI**
10.29121/shodhkosh.v5.i4.2024.2093

**Funding:** This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.



## 1. INTRODUCTION

Fungi are well-known for producing a wide variety of secondary metabolites that play a crucial role in their adaptation and survival across diverse ecological environments (Fox & Howlett, 2008). These metabolites have attracted considerable attention from researchers due to their potential for biotechnological applications in fields such as drug development, cosmetics, and food production (Shwab & Keller, 2008). In particular, fungal pigments have garnered interest for their biological activities and potential as natural dyes (Celestino et al., 2014). Filamentous fungi synthesize various pigments as secondary metabolites, including carotenoids, melanins, flavins, phenazines, and quinones (Dufossé et al., 2014; Mapari et al., 2010). These natural pigments offer a promising alternative to synthetic dyes, which are often linked to harmful effects on health and the environment, as well as mutagenic and carcinogenic risks (Lopes et al., 2013).

Furthermore, many fungal pigments exhibit beneficial biological properties, such as antibacterial, antifungal, and herbicidal activities, making them valuable for a range of biotechnological applications (Geweely, 2011; Premalatha et al., 2012; Teixeira et al., 2012).

Synthetic dyes, on the other hand, pose significant environmental and ecological risks. Their production is largely dependent on non-renewable energy sources, such as fossil fuels. Processing 1 (Mg) of textile material requires vast amounts of water (230–270 $m^3$), and up to 90% of the chemicals used in the finishing process end up in the waste dye baths, creating serious disposal challenges from an environmental perspective (De Santis et al., 2005).

With growing public awareness about environmental conservation and the demand for safer, biologically-friendly alternatives, there has been a renewed interest in natural dyes. These dyes are eco-friendly, biodegradable, and environmentally sustainable (Nagia & El-Mohamedy, 2007). Microorganisms, particularly fungi, have emerged as ideal candidates for natural colorant production due to their ability to produce a broad spectrum of colors, high yields (Liu et al., 2013), and stable pigments (Räisänen et al., 2002). Unlike plant-based natural dyes, microbial pigments are not affected by seasonal fluctuations in raw material supply, minimizing batch-to-batch variability (Mapari et al., 2010). Moreover, advancements in biotechnology, such as strain improvement and fermentation techniques, have further improved the efficiency of microbial pigment production (Kim et al., 1999; Parekh et al., 2000).

## 2. METHODOLOGY

**2.1. MATERIAL:** A Scoured 100% Mulberry Silk Fabric with a Thread Count Of 234 was Purchased from Nehru Place, Delhi, India**.**

## 2.2. MICROBIAL COLORANT

Isolation of Penicillium sp.: A soil sample weighing 0.1 g was collected from Lodhi Garden, New Delhi, India, and suspended in 10 ml of sterile normal saline. From this suspension, 0.1 ml was spread onto several potato dextrose agar (PDA) plates (Himedia, India), which were then incubated at 28±2°C for 3-5 days. Following incubation, fungal colonies producing red pigmentation were observed on the PDA plates. These colonies were subsequently purified and identified as Penicillium species by the Department of Plant Pathology, IARI, Pusa, New Delhi, India. To ensure long-term preservation, silica gel stock cultures were prepared. (Grivell & Jackson, 1969; Windels et al., 1988)

## 2.3. OPTIMIZATION OF INCUBATION TYPE

Penicillium sp. was cultured under different Incubation conditions viz. Shaking and Static to optimize the parameters of growth favoring maximum color production.

## 2.4. OPTIMIZATION OF TEMPERATURE

The purified culture of Penicillium sp was inoculated into the optimized medium at different temperature viz. 15° C, 28° C and 37° C and kept under optimized pH, incubation type, and time. For measuring color production, optical density (O.D) at the maximum wavelength (λmax) was recorded using a spectrophotometer (Visible spectro 105, Systronic, India).

## 2.5. OPTIMIZATION OF PH

The purified culture of Penicillium sp was inoculated into the optimized medium at different pH viz. 3.0, 4.0, 5.0, 7.0, and 9.0 and kept under optimized incubation type, temperature, and time.

## 2.6. EXTRACELLULAR RED PIGMENTS

The colored culture filtrate, obtained after cultivation in PDB (potato dextrose broth) medium, was filtered through 47 mm pre-weighed Whatman GF/C microfiber filter paper. The production of red pigment was assessed by measuring the absorbance of the filtrate at 530 nm using a spectrophotometer. Optical density (O.D) at the maximum wavelength (λmax) was recorded using a spectrophotometer (Visible spectro 105, Systronic, India). To ensure accurate and

consistent O.D readings, optimal dilution was determined prior to measurement. This was done by preparing various dilutions of the filtered culture filtrate in distilled water, including 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, and 1:10 ratios. After several tests, the 1:4 dilution proved to be the most stable, showing minimal fluctuations in O.D, and was therefore selected for all subsequent measurements. The fermentation process was optimized based on the highest O.D readings and reliable pigment production after four repeated culturing trials under the same conditions. All optimization procedures were performed using 50 ml of medium in 100 ml conical flasks (Borosil, India).

## 2.7. DYEING OF SILK

Dyeing was carried out in a static water shaker bath (NSW, India) at 80° C for 40 min with 50 ml of the colored liquor

## 2.8. ASSESSING THE COLOR FASTNESS AND STRENGTH OF DYED SILK SAMPLES

The color strength (K/S) and the colorimetric values (L*, a*, and b*) of all dyed samples were measured using a computer-aided color matching system (Macbeth, Color Eye 3100, USA). The color fastness to washing of the dyed silk samples was evaluated according to IS: 764:1984 (IS-3 method) using a digiWASH-INX™ machine (ISO 9001 certified group, Paramount Instruments Pvt. Ltd., India). The color fastness to rubbing was assessed following the IS: 766-1984 method using a crockMETER-1 (ISO 9001:2000 group, Paramount Instruments Pvt. Ltd., India). Additionally, the color fastness to light was determined in accordance with the ISO 105 B02 standard.

## 3. RESULTS AND DISCUSSION

At the end of the incubation period, only the static PDB broths maintained at 15°C and 28°C exhibited the production of extracellular pigments, appearing in various shades and tints of red. In contrast, the broths subjected to agitation formed large, dense mycelial masses due to the shaking during incubation. When these mycelial clumps were filtered, they produced a minimal amount of liquid, which was devoid of color. (Purwanto et al., 2019). As a result, shaking incubation was excluded from further experiments. Based on the O.D measurements taken at λmax = 530 nm as shown in Table 1, it was found that the static PDB at 28°C produced the highest concentration of red pigment on the 27th day, compared to other temperature conditions. The highest O.D values and consistent fermentation results indicated that the optimal conditions for maximum color production were static incubation in Potato Dextrose Broth (PDB) at 28°C for 27 days.

## 3.1. OPTIMIZATION OF PH

The data clearly shows that pH 5 is the most favorable for pigment production by Penicillium sp. This pH supports rapid growth and maximum pigment yield, reaching an O.D of 1.021 by day 27. Lower and higher pH values (pH 3, 7, and 9) result in poor pigment production, while pH 4 shows moderate but suboptimal results. Thus, pH 5 is the optimal condition for maximizing colorant production in this study.

**Table 1 O.D of PDB (28°C) at Different Ph Over a Period Of 27th Day**

| O.D at λmax=530 nm at different days interval | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **pH** | **3rd** | **6th** | **9th** | **12th** | **15th** | **18th** | **21st** | **24th** | **27th** | **30th** |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.012 |
| 4 | 0 | 0 | 0 | 0.021 | 0.062 | 0.113 | 0.216 | 0.465 | 0.608 | 0.607 |
| 5 | 0 | 0.021 | 0.036 | 0.049 | 0.209 | 0.424 | 0.731 | 0.891 | 1.021 | 1.021 |
| 7 | 0 | 0 | 0.01 | 0.002 | 0.01 | 0.019 | 0.031 | 0.076 | 0.091 | 0.095 |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.024 | 0.091 |

With these optimized conditions maintained, a further increase in O.D was observed when the pH of the PDB was adjusted to 5, as shown in Table 1. This finding is in line with the work of Mendez et al., who reported that a species of Penicillium sp exhibited the highest color production at lower pH levels compared to higher ones (Mendez et al., 2011).

Many Penicillium species are known to thrive in acidic environments, typically showing enhanced growth and metabolic activity between pH 5.0 and 6.0 (Abubakar et al., 2013). Interestingly, it was also observed that the pH of the broth became more acidic after incubation, reaching pH 4.0. This acidification may result from the production of organic acids by phosphorus-solubilizing fungi, such as Penicillium sp, which dissolve insoluble phosphorus in the culture medium through the release of organic acids (Scervino et al., 2011; Yasser et al., 2014). Furthermore, pH 5.0 was deemed reliable, as the O.D values showed minimal variation.

## 3.2. OPTIMIZATION OF TEMPERATURE

The Table 2 presents optical density (O.D) values measured at λmax = 530 nm for Penicillium sp. grown in potato dextrose broth (PDB) at different temperatures (15°C, 28°C, and 37°C) over a 30-day period. These O.D values indicate the pigment production in the culture filtrate, with higher O.D values corresponding to greater pigment concentration.

**Table 2 O.D of PDB showing Color in Different Temperature**

| O.D at λmax=530 nm at different days interval | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 3rd | 6th | 9th | 12th | 15th | 18th | 21st | 24th | 27th | 30th |
| PDB 15°C | 0 | 0.018 | 0.026 | 0.061 | 0.101 | 0.113 | 0.316 | 0.565 | 0.86 | 0.808 |
| PDB 28°C | 0.014 | 0.021 | 0.036 | 0.049 | 0.209 | 0.424 | 0.731 | 0.891 | 1.01 | 1.01 |
| PDB 37°C | 0 | 0 | 0 | 0.01 | 0.002 | 0.01 | 0.019 | 0.031 | 0.076 | 0.071 |

**PDB at 15°C:** The O.D values at 15°C increase gradually, showing slow pigment production in the early stages. The O.D reaches 0.061 by day 12, indicating a slow rate of growth and color production. After day 12, there is a more pronounced increase in O.D values, peaking at 0.860 on day 27. However, there is a slight decline to 0.808 by day 30, indicating that pigment production slowed down or plateaued toward the end of the incubation period. While pigment production at 15°C is significant, it occurs at a slower rate compared to higher temperatures. The final O.D value of 0.808 suggests moderate pigment yield.

**PDB at 28°C:** Pigment production at 28°C begins slowly, but by day 12, the O.D has reached 0.049, showing a slightly faster growth rate than at 15°C during the early stages. A sharp increase in O.D is observed after day 12, with the O.D rising rapidly from 0.209 on day 15 to 1.010 by day 27, which remains stable through day 30. This indicates robust growth and high pigment production, with no decline in O.D even at the end of the incubation period. 28°C is clearly the most favorable temperature for Penicillium sp. growth and pigment production, with the highest O.D (1.010) and the fastest growth rate observed throughout the experiment. The stability of the O.D after day 27 further suggests that the culture is well-suited to this temperature for producing the desired pigment.

**PDB at 37°C:** At 37°C, growth is minimal, with O.D values remaining at 0 until day 9 and only reaching 0.010 by day 12, indicating very poor pigment production or no color production. The O.D values at 37°C remain extremely low, peaking at only 0.076 by day 27 and slightly declining to 0.071 by day 30. This indicates that Penicillium sp. struggles to grow and produce pigment at 37°C.

The data shows that 37°C is not conducive to the growth or pigment production of Penicillium sp., with very low O.D values throughout the incubation period. Based on the data, 28°C is the optimal temperature for the growth of Penicillium sp. and for maximizing pigment production. The O.D values at 28°C show the highest and most stable increase, indicating robust growth and consistent pigment yield. In contrast, 15°C shows slower growth and moderate pigment production, while 37°C is too high for effective growth, resulting in minimal pigment formation.

The temperature of 28°C is the most favorable for growing Penicillium sp. to achieve maximum pigment production, as reflected in the highest O.D value (1.010). This temperature supports both rapid and sustained growth, making it the best condition for fermentation and pigment extraction.

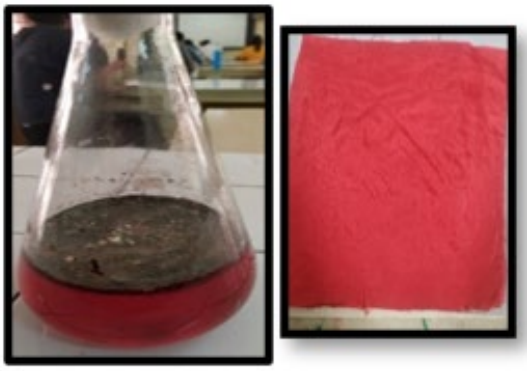

Figure 1 Penicillium sp. on PDB and Silk Dyed Sample

## 3.3. ASSESSING THE COLOR FASTNESS AND STRENGTH OF DYED SILK SAMPLES

The given data represents the colorimetric values and color strength (K/S) for the dyed silk samples. The K/S value indicates the color strength of the dyed silk fabric. A K/S value of 4.25 suggests a moderate intensity of color on the fabric. The value of 4.25 suggests that the dye penetrated the silk fibers well, producing a reasonably strong and noticeable coloration.

The L* value represents the lightness of the color on a scale from 0 (black) to 100 (white). A value of 81.23 indicates that the dyed silk is quite light in appearance. This high lightness value suggests that the dye imparts a relatively bright and pastel-like effect rather than a dark, rich color. The fabric maintains a light tone, likely making it visually soft and less intense, which could be desirable for applications that require subtle and elegant shades.

The a* value indicates the position of the color on the green-red axis, where positive values represent a shift toward red and negative values indicate a shift toward green. The a* value of 9.23 means the dyed fabric leans slightly toward a red hue. This red component gives the fabric a warmer tone, which can enhance the perceived richness of the dye, despite the relatively high lightness (L*) value.

The b* value represents the color position on the blue-yellow axis, with positive values indicating a shift toward yellow and negative values toward blue. A b* value of 6.48 shows that the fabric has a slight yellowish undertone, which complements the red (a*) to produce a warm, vibrant hue.

**Table 3**

| Table 3 Color Strength and Color Values of Silk Dyed with the Colorants of Penicillium Sp | | | | |
|---|---|---|---|---|
| **Color Measurement** | **K/S** | **L*** | **a*** | **b*** |
| **Silk Sample** | 4.25 | 81.23 | 9.23 | 6.48 |

The Table 4 presents the color fastness ratings of silk samples dyed with Penicillium sp., assessed using the SDCE Grey Scale (1 to 5, with higher numbers indicating better performance). The fastness tests include rub fastness (dry and wet), wash fastness, and light fastness. The silk samples dyed with Penicillium sp. showed varying results across different fastness tests. The rub fastness, both in dry and wet conditions, displayed strong performance, particularly in dry rubbing where the highest rating of 5 was achieved for staining. The wet rub fastness also showed good results with a rating of 4 for both staining and color change, indicating that the dyed silk demonstrates a solid resistance to color transfer and fading when subjected to friction, even under wet conditions.

The wash fastness results are similarly favorable, with ratings of 4 for both staining and color change. This suggests that the dye is fairly resistant to washing, with only minimal staining on adjacent fabrics and a slight loss of color in the dyed silk. The silk dyed with Penicillium sp. can be expected to hold up well under routine washing, making it suitable for textile applications where durability is a concern.

**Table 4**

| Table 4 Color Fastness of Silk dyed with the Colorants of Penicillium sp | | | |
|---|---|---|---|
| **Color Fastness of Silk sample dyed with *Penicillium sp.*** | | | **Ratings on Greyscale** |
| Rub Fastness | Dry | Staining on white cloth | 5 |

| | | | |
|---|---|---|---|
| | | Change in color | 4 |
| Rub Fastness | Wet | Staining on white cloth | 4 |
| | | Change in color | 4 |
| Wash fastness | | Staining on adjacent fabric (cotton) | 4 |
| | | Change in color on specimen | 4 |
| Light Fastness | | Change in color | 2 |
| The Ratings Were Recorded Using the SDCE Grey Scale, Which Provides A Scale From 1 To 5, To Evaluate Both Color Change (A02) And Color Staining (A03) | | | |

## 4. SUMMARY AND CONCLUSION

The colorants produced by Penicillium sp. demonstrated a strong affinity for protein-based fibers, such as mulberry silk. The dyed silk samples exhibited good to excellent fastness properties against both rubbing and washing, indicating the durability of the color. Given the growing need for sustainable dyeing solutions in the textile industry, this microbial dye from Penicillium sp. offers a promising eco-friendly alternative for dyeing silk fabrics and can be effectively utilized for color production in textiles.

## CONFLICT OF INTERESTS

None.

## ACKNOWLEDGMENTS

None.

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