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Investigation of microbial pigment production and its assessment of wool fiber dyeing

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Abstract The production of natural red pigment using the filamentous fungus *Pencillium purpurgenium* under different growth forms of inocula (mycelia and spores) was investigated. Meanwhile, different physiological condition involving magnesium sulphate concentration, inoculums size and different age as well as some additives were tested. The results showed that the specific production of pigment was obtained by using mycelia as inocula, the yield increased by 30.08% as compared to spore form. The study extended to dye wool fibers with the extracted pigment using innovation technique to save energy and time. In order to obtain color of aimed specific red hue, the influence of certain dyeing process condition namely dyestuff concentration, pH, temperature, and duration of the dyeing process has been reported.

Keywords: Production, Pigment, *Pencillium*, Wool, Dyeing

Introduction

Many natural pigments are derived from different sources involving plants (Ferruzzi and Schwartz, 2005), animals specially insects as well as microorganisms (Tulli *et al*., 2014). However, a first requirement for the application of microbial pigments is that they must be non-toxigenic and nonpathogenic to human. The use of eco-friendly natural dyes non allergic, nontoxic in textiles has become with significant importance results in increased environmental precautions and safety. Natural dyes have very uncommon, soothing and soft shades as compared to synthetic one (Ali and Abd- Elsalam, 2020). Synthetic colors are widely available due to economical price in wider range of colors but these dyes produce skin alergies, less stable, toxic and also produce highly harmful wastes cause environmental pollution (Morales *et al.*, 2015). The pre requisites for industrial application of a given dye are involving,

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color stability under extreme temperature, wide pH range. The filamentous fungi have been found more versatility on pigment production specially *Penicillium* and *Monascus* sp., than other fungal species (Jakovijevic *et al.*, 2014). Increasing pigment productivity by selected microbial strain involved optimization of the main growth requirement as carbon, nitrogen sources, pH, temperature and growth time. The textile industries are one of the most popular industrial sectors in the world. Synthetic dyes are almost exclusively used in commercial textile dyeing (Clark *et al.*, 1988, Moore and Ausley, 2004). In the meantime, it classified as one of the most polluting industry that discharges effluents contaminated with many pollutants (Nagia and El-Mohmedy, 2006, Zhang *et al.*, 2007 and Herrero *et al.*, 2008). The colorants are not biodegradable were the most hazardous (Van der torn *et al.*, 2019). Therefore, natural dyes now have more attracting and attention from both academia and industry due to its eco-friendly attributes (Nagia and El- Mohamedy, 2006).

Rubropuncatmin (red pigment)

Figure 1. Structure of microbial pigments under investigation

Nowadays, modifications and dyeing of some fibers have been conducted under the effect of microwave irradiation. Microwave irradiation is one of the powerful technique of non-contact heating, and has been used for reacting, dye extraction, and dyeing of different type of fabrics and fiber. The classical processing of fabric consumed a large amount of energy. Many researchers, Nagia and El-Mohmedy (2006) and Zhang *et al.* (2007) studied some new technique and methods for saving both time and energy. Microwave heating, has been proved to be more rapid, uniform, efficient, and it can be used as alternative to conventional heating technique. Microwave irradiation can penetrate easily inside fiber particles, consequently reducing heat transfer

problems. It has been supposed that the microwave irradiation modification could effect on dye ability of textile fiber.

The main purpose of study was optimization of red pigment production by the filamentous fungus *Pencillium purpurgenium* and quantify how the inocula type (mycelia or spores) effects on pigment productivity as well as the application of the obtained pigment on wool fiber dyeing.

Materials and methods

The culture of *Penicillium purpurogenum* was purchased from Assiut University Mycology Centre (AUMC), the spore suspension (2×10^7 spores/ml) was used to inoculate Czapeck yeast agar (CYA) growth media that contained the following ingredients (g/l): glucose 30, yeast extract 2, peptone 10, NaNO3 3, KCl 0.5, MgSO4 0.5 and Agar 25(Pitt, 1985), incubated for 7 days at 28℃ and stored at 4℃ . Mill scoured 100% wool fibers used for this study was supplied from Misr Co. (El Mehalla El-Kobra Egypt for spinning and weaving). The fibers was washed in a bath containing 2g/l non-ionic detergent (Nonidet) at 40℃ to remove any impurities and then thoroughly washed with water and then dried by air at room temperature. The microwave equipment used in this experiment was the Samsung M 245 with an output of 1,550 watts operating at 2450 MHz.Wool fibers10/2, and silk fibers supplied by El Mahalla Company Egypt. Neem oil extract was purchased from ELGmhoria Company, Egypt. UV spectrophotometer (V630 spectrophotometer serial no. C 316661140 was used for the pigment estimation.

Growth medium and production media

The fungus was grown in a rich Czapeck yeast agar medium, Dox'S production medium (50 ml) in 250 ml Erlenmeyer flasks was inoculated with the fungus cell suspension (2×10^7 spores/ml).The culture was shake at 200 rpm and 28℃ for 7 days. Modified Dox'S medium was prepared with the following nutrients (g/l)Glucose (20), NaNO3 (3.0), KCl (0.5), MgSO4.7H2O (0.5), KH_2PO_4 (1.0), FeSO4.7H_2O (0.1).

Pigments extraction and analysis

At the end of fermentation period mycelium growth was filtered through filter paper (What man No.1), the biomass was dried at 50℃ for 48 h and weighed. Red pigment was extracted by solvent. The extract was concentrated

till dryness and dissolved in 10 ml distilled water, absorbance was read at 500 nm by a UV-visible spectrophotometer .

Inoculum age and size

Different inoculum ages of 24, 48, 72, 96 h were investigated under the optimal other fermentation conditions, and the production of pigment was quantitatively estimated.

Significance of different glucose concentration on pigment production

The effects of different glucose concentrations of 1, 15, 20, 25, 30 g/L on pigment production using the (mycelia and spores) inoculum was investigated.

Suitability of magnesium sulphate

Different magnesium sulphate concentrations of 0.1, 0.5, 0.75, 1 and 1.25g/L on pigment production were tested using the experimental microorganism.

Glucose estimation

The concentration of glucose after the fermentation process was determined according to the method described by Eman *et al.* (2018) and Van der Toorn *et al.* (2019).

Dyeing procedure

Dyeing of wool fibers was carried out using microwave heating. Dye under investigation was applied at different pH of 3-7 for periods of 1-5 minutes. After dyeing, wool fibers were rinsed with water and then dried at room temperature. K/S values of dyed wool fibers were measured.

Measurements of color strength (K/S value)

An Ultra Scan PRO spectrophotometer was used to measure the reflectance of the samples and hence, the K/S was measured spectrophotometric ally at wave lengths (λ_{max} 515nm). K/S where K and S are the absorption and scattering coefficients.

Leveling properties

The leveling of the dyed wool fabrics were assessed by measuring the color differences with each sample at five separate points and the average color difference (ΔE) between these points was determined P^P by using Ultra Scan PRO. Respectively CIELAB coordinates (L* a* b*) measurements of un-dyed and dyed wool fibers were determined using an Ultra Scan PRO spectrophotometer (Hunter Lab) with a D65 illuminant and 108 standard observers.

Measurements of fastness properties

According to ISO standard methods. The specific tests were ISO 105X12 (1987), ISO 106-C06 (1989), ISO 105-E04 (1989), and ISO 105-B02 (1989), corresponding to color fastness to rubbing, washing, perspiration and light, respectively. The color changes of the samples were assessed against an accurate Gray scale.

Results

Investigation of pigment production using different spore concentrations

The different spore concentrations were applied and pigment output was relatively affected by spore concentrations. The results revealed that at low spore concentration of 1x 10^7spore/ml reduced level of pigment was obtained (18mg/ml). The best output (59 mg/ml) was obtained at 2 x10^7 spore /ml. On the other hand, at the high spore concentration of 5x10^7 spore /ml showing a remarkable reduction of pigment productivity was noticed (30mg/ml) as shown in Table 1.

Table 1. Effect of different spore concentrations on the production of pigment

Spores conc. (ml)	Final pH	Biomass (mg/ml)	Glucose conc.(mg/ml)	Pigment conc.(mg/ml)	Specific production %
1x10^7	5.62	59	431	18	30.59
2x10^7	5.57	178	376	59	33.14
3x10^7	5.88	293	278	43	14.67
4x10^7	6.00	288	265	33	11.45
5x10^7	6.00	379	210	30	7.91

Effect of different inoculum age

The different inoculum ages of 24, 48, 72, 96 h were tested for their effects on pigment product. The results showed that the best specific production (46.91%) was obtained by 72h. At the longer inoculum time ages of 96 h showing low level of specific production to pigment were obtained (29.1%) as seen in Table 2.

Table 2. Effect of different inoculum age on the production of pigment

Inoculums age (h)	Final pH	Biomass (mg/ml)	Glucose conc.(mg/ml)	Pigment conc.(mg/ml	Specific production %
24	5.62	121	533	34	28.09
48	5.57	143	355	41	28.67
72	5.88	162	276	76	46.91
96	6.00	185	210	54	29.18

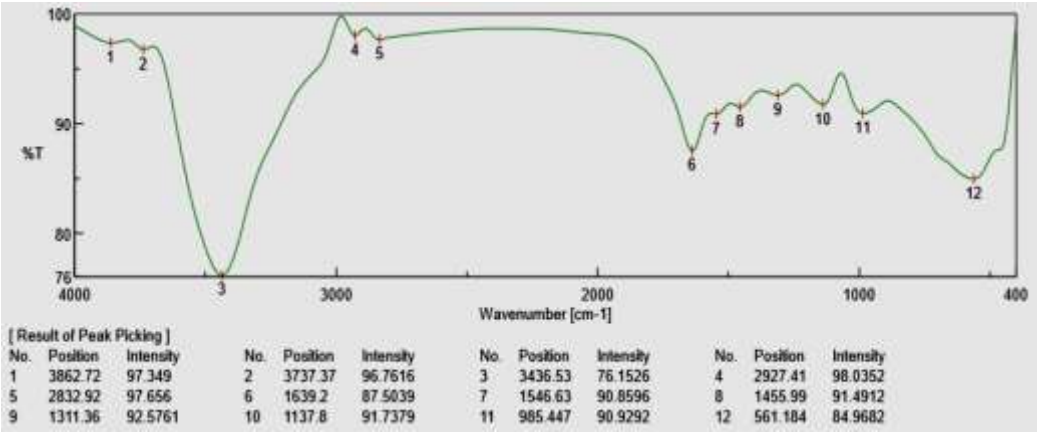

Figure 2. Infrared spectroscopy of the pigment

Effect of different inoculum size on the production of pigment

The productivity of pigment was remarkably affected by the inoculum size. Result indicated that the best production of 36.86% was obtained by using inoculum size of 3 ml / 100ml medium (Table 3). The sizes above 3 ml showed more biomass production, but low production out puts reached to 21.48% at 5 ml inoculum size.

T**able 3.** Effect of different inoculum size on the production of pigment

Inoculum size (ml/100ml)	Final pH	Biomass (mg/ml)	Glucose conc.(mg/ml)	Pigment conc.(mg/ml)	Specific production %
1	5.57	143	355	41	28.67
2	5.57	233	265	73	31.33
3	5.88	255	231	94	36.86
4	6.00	351	179	79	22.50
5	5.99	349	188	75	21.48

Effectiveness of high glucose concentration on the production of pigment

The investigation on different glucose concentrations of 10, 15, 20, 25and 30 g/L were tested. The results showed that by increasing glucose concentration enhancement of the productivity (57.61%) was recorded at 25g/L (Table 4). On the other hand, the higher concentrations produced low yield of 37.45 % at 30 g/L.

Table 4. Effect of different glucose concentrations on the production of pigment

Glucose conc. (g/l)	Final pH	Biomass (mg/ml)	Pigment concentrations(mg/ml)	Specific production
10	5.89	140	46	32.85
15	5.87	172	59	34.30
20	6.00	177	89	50.28
25	6.11	210	121	57.61
30	5.93	267	100	37.45

Effect of magnesium sulphate on pigment production

Effect of different magnesium sulphate concentrations on the production of pigment were investigated. The results revealed that the best specific pigment production 64.02% was obtained at magnesium sulphate concentration of 1g/L (Table 5). However, at the lower concentrations of 0.1, and 0.5 g/l showing low pigment production was noticed at33.08 and 46.91%) respectively, which reflected the importance of the magnesium ions on the biosynthesis pathway of the pigment. On the other hand, at the higher concentrations above (1 g/L) reduced output was noticed 58.33%.

Table 5. Effect of different magnesium sulphate on pigment production

Magnesium sulphate conc. (g/l)	Final pH	Biomass (mg/ml)	pigment concentration (mg/ml)	Specific production
0.1	5.66	133	44	33.08
0.5	5.57	162	76	46.91
0.75	6.00	177	89	50.28
1	5.66	189	121	64.02
1.25	5.66	168	98	58.33

Effect of dye concentrations

Microwave dyeing takes into account the dielectric and the thermal properties of matter. The dielectric property refers to the intrinsic electrical properties that is affected the dyeing by dipolar rotation of the dye and influences the microwave field upon the dipoles. The aqueous solution of dye was two components which are polar in the high frequency microwave field. It influenced the vibration energy in the water molecules and the dye molecules**.**

The color strength of dyed wool fibers was affected by concentrations. The dye under investigation was high affinity for the fibers. Result showed that the color strength of wool fibers dyed with the dye under investigation gave the highest value of color strength (K/S) at conc. 8g/L (Figure 3).

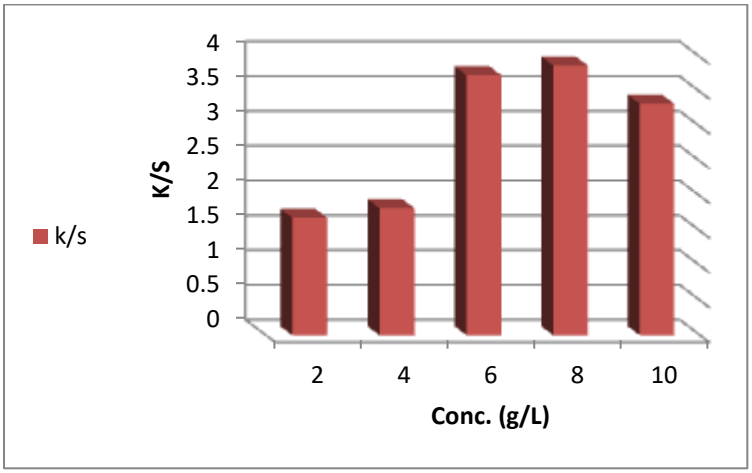

Figure 3. Effect of dye concentrations on the color strength for wool fibers dyed by microwave

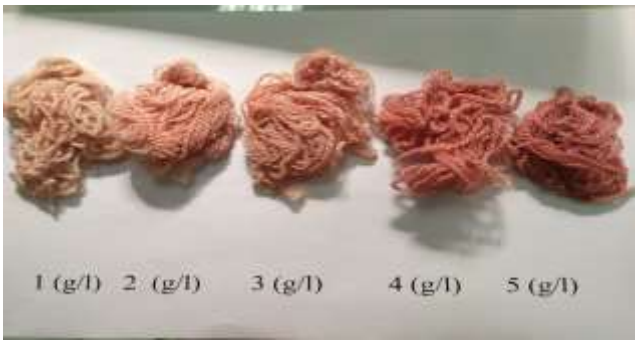

Figure 4. Wool fiber samples dyed by the investigated natural dye at different concentrations

Study of the colorimetric CIE

The colorimetric CIE L*a *b*C*h data were evaluated for dyed wool fibers were shown in Table 6**.** The color strength changed a remarkably as different concentrations of dye were used while the Lab values showed that samples dyed with 8g/L were darker in shades.

Table 6. Effect of natural dye by microwave method on the K/S and color difference values

Conc. the investigated natural dye(g/L)	K/S	L*	a*	b*	C*	h	ΔE
2	1.69	58.54	22.6	15.23	26.5	35,.83	83.5
4	1.83	57.98	21.14	14.55	25.66	34.53	88.1
6	3.74	51.51	25.5	12.8	28.66	25.7	85.5
8	3.88	45.74	21.17	13.82	25.15	31.12	85.5
10	3.33	50.31	25.64	13.02	25.15	31.12	84.6

Effect of the dye bath pH

The color strength of wool fibers dyed with investigated natural dye was affected by dye bath pH. The dye has high affinity for the fibers in acidic medium. In acidic medium, the cationized amino groups can be adsorbed an anionic dye molecules by the electrostatic attraction. Result showed that the color strength of wool fibers dyed with the dye under investigation gave the highest value of color strength (K/S) at pH 5 (Figure 5 and 6).

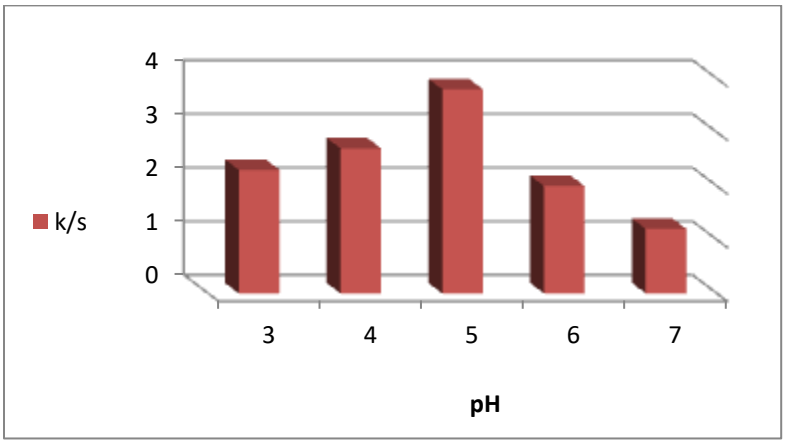

Figure 5. Effect of the dye bath pH on the color strength for wool fibers dyed fibers using microwave

Figure 6. Wool fiber samples dyed by the investigated natural dye at different pH

Effect of time on the color strength

The color strength of dyed wool fibers is dyed by microwave that affected by duration of time. The dye under investigation was high affinity for the fibers. It showed that the color strength of wool fibers dyed with the dye under investigation gave the highest value of color strength (K/S) after 5 min (Figure 7 and 8).

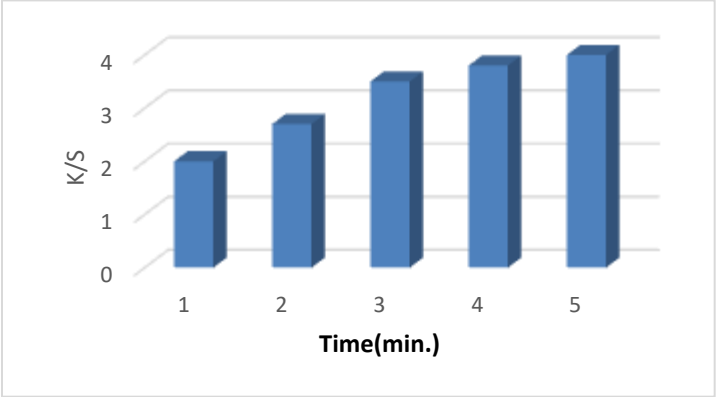

Figure 7. Effect of time of dyeing on the color strength for fibers using microwave

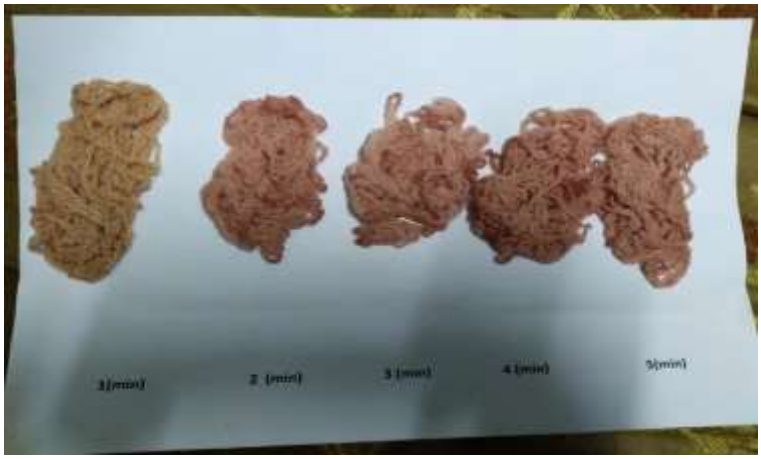

Figure 8. Wool fiber samples dyed by the investigated natural dye at different Time intervals

Fastness properties

The durability of colour on wool fibre samples after dyeing by using microwave irradiation as a heating source were evaluated in term of fastness towards rubbing, washing, perspiration and to light fastness using the grey scale. Result showed that the wool fibre more resistant against washing and perspiration using microwave irradiation which is displayed higher colour one by microwave. It may be attributed to increase dye penetration and its interaction with fibres (Table 7).

Table 7. The fastness properties of the investigated dye on wool fibers at different concentrations

`Conc.g/L	Rubbing Fastness		Washing fastness		Perspiration Fastness				Light fastness
	Wet	Dry	Alt.	S. c	Alkaline		Acidic		
					Alt	S.c	Alt	S. c	
0	4	4-5	4	4	4	4-5	4	4	5
2	4	4	4	4	4	4	4	4-5	6
4	4-5	4	4-5	4-5	5	5	4-5	4-5	5
6	4	5	4-5	5	4-5	5	4-5	5	5
8	4	5	4-5	5	4-5	5	4-5	5	7
10	5	5	4-5	5	4-5	5	4	5	7

Alt = change in color, SC = staining on cotton, SW = staining on wool

Discussion

The productivity of pigment was strongly affected by the type of inoculum. The fact that spore would require a longer lag phases for germination to compare the mycelia as inoculum. It would suggest the better productivities that might be obtained with the higher growth achieved by using mycelium (Morales *et al*., 2015). Thus, if the microorganism presents a raped adaptation to the culture, it may use the available substrate for its growth by synthesis of other metabolites and higher growth could actually results in less pigment production. Papagianni and Moo-Young (2002) showed that inoculum type of free mycelia and spores, and the levels were the determinant factors for the development of certain fungal morphology for secondary metabolites production. The high spore inocula concentration led to more mycelial growth at longer time compared to firstly using mycelia as inoculum. It confirmed the impact of the inoculum on the final production of pigment and reduced the time to reach it by 40% (Santos –Ebinuma *et al.*, 2013).

The obtained results indicated that glucose consumption effects on pigment production and biomass. Alexandra *et al.* (1999) stated that the maximum growth was recorded using glucose containing medium (10g/L). It was highly significant at $p=2.6x10^{-14}$ when the fungus grew rapidly using glucose and a slow growing phase which favoring secondary metabolites.

Moreover, Griffin (2000) indicated that a slowly metabolized compounds like starch and sucrose stimulates the production of secondary metabolites when added to the culture medium.

The fermentation *M. purpureus* with maltose and glucose as carbon sources gave very dark liver pigment, whereas sucrose produced a light and uneven red pigment. For pigment production from *Phaffia rhodozyma* where cellobiose supported more pigmentation than others. D-mannitol also supported pigmentation whereas glucose promoted both growth and pigmentation (Joshi *et al.,* 2003). The sugar-type also influences the shade of pigment.

Acknowledgments

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