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IMMOBILIZATION OF *PENICILLIUM PURPUROGENUM* AND APPLICATION OF THE PRODUCED PIGMENT IN PAINT INDUSTRY

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Abstract

Penicillium purpurogenum was known to produce red pigment in the fermentation medium. The aim of this article was to study the effect of different temperatures, pH values and recycling of the immobilized cells on pigment output. Additionally, the yield of pigment in a medium supplied with nutrients milled to Nano scale was also investigated. Furthermore, physical and mechanical properties of the dyed paint were evaluated.

The nutrient powder mixture was blended for 15 h and then, milled at different times up to 10 h in planetary ball. After milling, the milled powders were investigated by transmission electron microscopy (TEM). Maximum production of pigment (226) mg/ml was got using milled nutrients powder (114 nm) in the fermentation medium. The optimum temperature and pH values for pigment production with immobilized cells were (30°C and 7) respectively and after the first cycle. Moreover, in paint application, an increase in viscosity and a decrease in density were observed by addition of dry pigments to the white paint. An increase in hardness of the coated paint films was detected by increasing the pigment content as well. Most painted-films exhibited good chemical resistivity to diluted alkali, acetic acid, and water and all samples passed the heat resistivity test.

Abbreviations :

WP White emulsion paint, DP dyed-WP, S.C solid content, W_i final weight, W_0 initial weight

Key Words: *Penicillium purpurogenum*, pigments, immobilization, Nano scale, paint application.

Introduction

Fungi were reported as biological producers of organic compounds and pigments like anthraquinone, anthraquinone carboxylic acids and pre-anthraquinones. These dyes are eco-friendly and nontoxic. They were used as alternatives to the harmful synthetic dyes and natural vegetable colorants (Iffat *et al.,* 2015). Pigments are very important in industry, they are used as additive in food industry, pharmaceuticals, and textiles. Microbial pigments are widely used due to their high stability and solubility in many solvents (Gunasekaran and Poorniammal, 2008).

Some pigments from fungi are found nowadays in the market; *Monascus* pigments and Arpink red from *Penicillium oxalicum*. Fungal hydroxyanthraquinoid pigments are widespread in nature in many filamentous fungi that belong to *Aspergillus* sp. and *Penicillium* sp. (Dufosse *et al.,* 2014). Filamentous fungi produce secondary metabolites that include carotenoids, melanins, flavins, phenazines and quinones. Most fungal pigments are biologically active, they have antibacterial, antifungal and herbicidal potentials, and these properties make them important organic compounds for many biotechnological applications (Geweely 2011, Premalatha *et al.,* 2012, Teixeira *et al.,* 2012).

This study was conducted to investigate the effect of the Nano scale milled nutrients on the production of the pigments. Additionally, immobilization of the fungal cells and the influence of temperature, pH and recycling

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on the productivity were evaluated. Application of the produced powder of pigment in paints was an important target in this article.

Material and Methods

Organism

Penicillium purpurogenum 2603 was brought from Assiut University Mycology Centre (AUMC). The spores were cultured on a rich Czapeck Yeast Agar (CYA) which containing the following nutrients (g/l): glucose 30, yeast extract 2, peptone 10, $NaNO_3$ 3, KCl 0.5, $MgSO_4$ 0.5 and agar 25 (Ahmed *et al.*, 2018). The Cultures were then incubated at 28°C for 8 days. After incubation a spore suspension (3×1010) of the fungus was prepared and transferred to inoculate Czapeck Yeast fermentation medium.

Effect of Nano scale particles of medium's nutrients on pigment production

The powder mixture of the fermentation medium's nutrients (g): (Mannitol 30, Peptone 4, KCl 1, and $MgSO_4$ 0.5 and KH_2PO_4 0.5) was mechanically blended for 15 h with ball-to-powder ratio (BPR) equals to 1:2 and the diameters of balls were 10 mm. Then, these mixtures were milled for 2, 5, 10 h in a planetary ball mill (type MTI SFM (QM-3SP2)) with rotation speed equals to 350 rpm and ball-to-powder (BPR) weight ratio was 10:1. The milling was performed using Al_2O_3 vial and balls. It is worth to mention that the milling was done in a cycle of 2h and paused for 2h.

Morphology and particle size of the milled powders were examined by transmission electron microscopy (TEM), (type JEOL JEM-1230).

The powder of the nutrients was milled to different Nano scale particles (31.5, 70.4, 114.5) nm and added to the fermentation medium to be utilized by the fungus. By ending of incubation period, the concentration of the pigment in the production medium was calculated.

Immobilization of *Penicillium* *purpurogenum* in fermentation medium

P. purpurogenum spores were cultured in a fermentation medium containing the following nutrients (g/l): Mannitol 30, Peptone 4, KCl 1, KH_2PO_4 0.5 and $MgSO_4$ 0.5. The medium was shaken and incubated at 28°C for 4 days. Fungal cells (5g) were centrifuged and immobilized in 50 ml of 3 % sodium alginate. The immobilized cells were subjected to the following experiments:

Effect of temperature

Immobilized cells were cultured in the fermentation medium and incubated in shaking incubator at 200 rpm for 8 days at different temperatures from (20 - 45) °C. The concentration of the produced pigments was calculated according to the equation reported by Beer's Lambert Law: A= abc. Where A is the absorbance of the solution, a is the molar absorptivity, b is the light path length, c is the concentration, mold m^{-3}.

Effect of different pH values

The pH of the media was adjusted to cover the range from 3 to 8. The immobilized cells (15) beads were seeded in 50 ml broth media, incubated at 28°C in a shaker at 200 rpm for 8 days, the absorbance of the dissolved pigment was measured at 500 nm.

Recycling of the immobilized cells

The production medium was prepared and adjusted at the optimum pH and temperature. The immobilized cells in sodium alginate (15) beads were inoculated for 8 days in 50 ml production media and the concentration of the pigment was calculated. The beads were centrifuged and reused to inoculate new fermentation media for three cycles.

Application of *P. purpurogenum* pigment in paint industry

The fungus was cultured in 1 L of the production medium for 8 days at 30°C. The cells were separated by centrifugation and the medium was dried. The powder of the pigment was weighed.

Coloring of white emulsion paint with the dye

White emulsion paint (WP) was kindly supplied by Scib® Paints Company and was colored by adding the powder pigment directly to the WP in different solid ratios such as 1%, 2%, 3%, 4%, 5%, 6%, 7%, and 8% to produce dyed-WP (DP), respectively. The produced DPs were symbolized by 1DP, 2DP, 3DP, 4DP, 5DP, 6DP, 7DP, and 8DP, respectively.

Characterization techniques

The solid content (S.C.) was determined according to ASTM D4209-07, 2013 by weighing an adequate amount of the sample in a petri dish and heating it in an aerated oven at 105°C till constant weight was obtained. S.C.% was calculated as the average of three experimental determinations.

$$S.C. \quad \frac{Wi}{W0} \quad 100$$

Where W*i* is the final weight of the sample after drying and W0 is the initial weight of the sample.

The viscosity of WP and the prepared DP was measured by Sheen Krebs digital viscometer according to ASTM D2196-10, 2010. The sample was poured into a 500 ml container located on the magnetic base of the viscometer. A paddle was started to rotate with a speed of 200 rpm which was automatically stopped after reaching a steady rotation by 10s giving the viscosity reading. The viscosity was calculated as the average of three experimental determinations. The adhesion of the coated WP and the prepared DP films to a glass substrate was evaluated according to ASTM D3359-09, 2009 using a cross-cut test method. The scratch hardness of the casted WP and the prepared DP films was estimated according to ASTM D3363-05, 2011 using Sheen pencil hardness tester. The bending of the coated WP and the prepared DP films was measured according to ASTM D522-08, 2008 using a cylindrical mandrel bending tester with different diameters. UV-resistance test was measured according to ASTM D4587-91 where the coated films were exposed to a short and a long-wave UV lamps; 4 watts, 245/312 nm wavelength. Heat resistance test was estimated by exposing the coated films to a temperature degree of 80^{o}C for 6 h in an air oven. Glass panels coated with the paints were used to assess their resistance to some chemicals according to ASTM D1647-89, 1996. The coated glass panels were immersed in 0.01 M NaOH solution, acetic acid, and water for 24 h at room temperature. The chemical resistance was estimated by measuring the loss in adhesion and gloss of the dried-films.

Results and Discussion

Various synthetic pigments were known for their harmful effects on the environment. Accordingly, many searches were carried out on the production of pigments from natural sources. To the best of our knowledge, we didn't find any published data on the application of the red pigments from *Penicillium purpurogenumred* on paints industry, and on the use of Nano technology for the improvement of the production of the pigment.

Investigation of the prepared nutrient powders

Generally, the repeated deformation, fracturing and welding processes, which occur during high energy milling, are responsible for considerable decrease in the particles' sizes with probable change in their morphology (Zawrah *et al.,* 2014, Taha *et al.,* 2016, Taha and Zawrah, 2017). The TEM images of milled nutrient powders for different milling times, *i.e.* 2, 5 and 10h are represented in Fig. 1. As obviously seen from this figure, the average particle size decreases with the increasing of milling times combined with appearance of particles agglomeration. The calculated average particle sizes of the milled

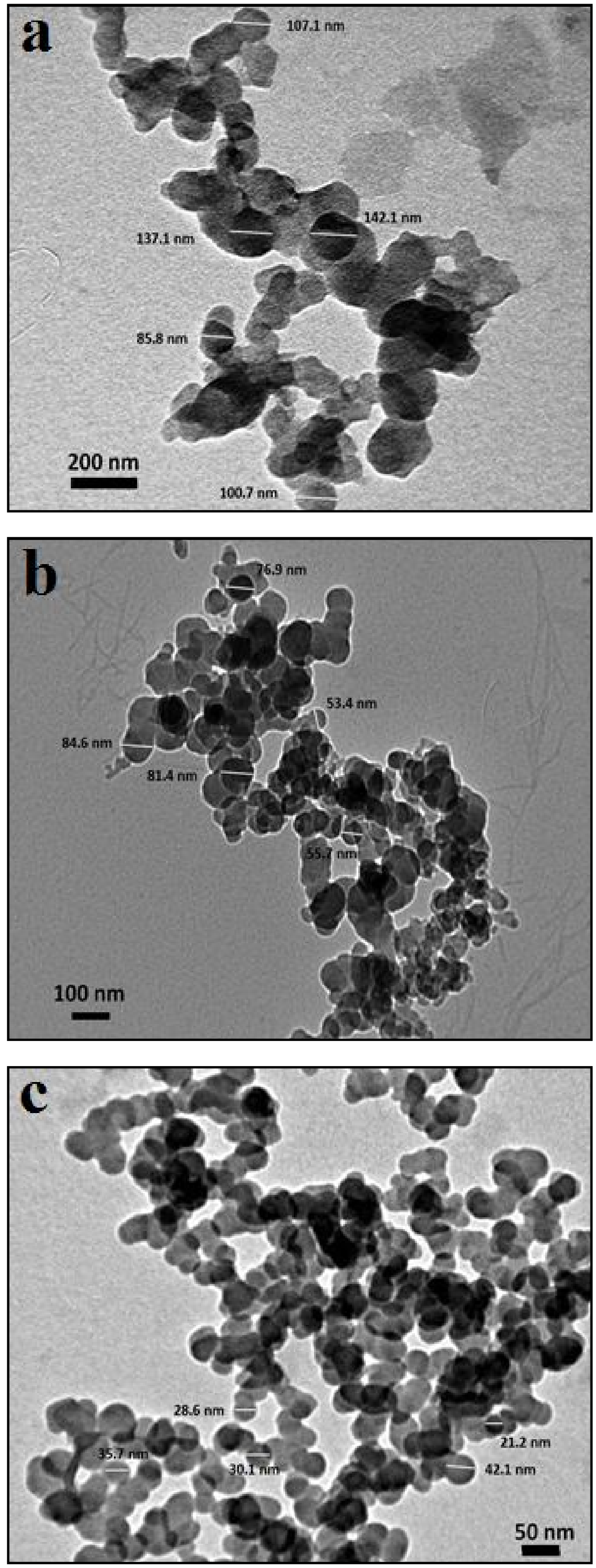

Fig. 1: TEM micrographs of milled powders different milling times (a) 2 h, (b) 5 h, and (c) 10 h.

powders are 114.5, 70.4 and 31.5nm for powders milled for 2, 5 and 10 h, respectively.

The milled nutrient powder was dissolved in 1L distilled water and autoclaved. The fungus was cultured

Table 1: Effect of nutrient Nano size particle on pigment output.

Particle size (nm)	Pigment concentration (mg/ml)
Control (normal size)	245
31.5	-
70.4	75
114.5	226

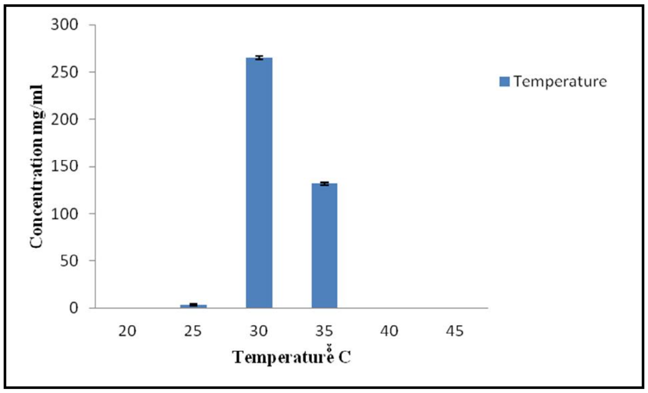

Fig. 2: Effect of temperature on pigment production by immobilized *Penicillium purpurogenum*

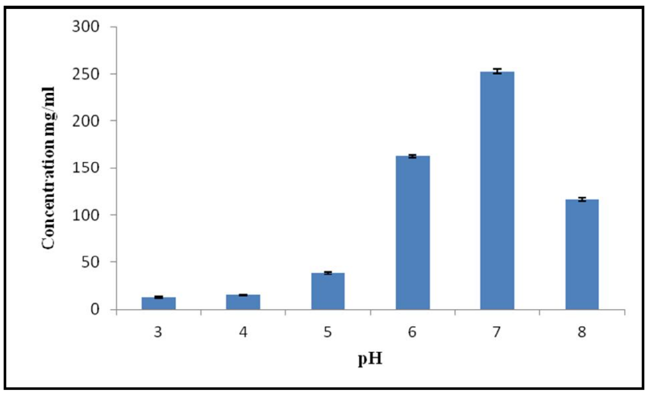

Fig. 3: Investigation of pigment production at different pH values

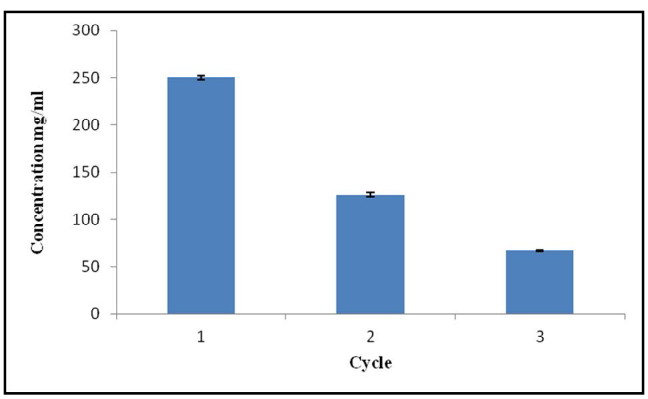

Fig. 4: Assessment of pigment concentration after Recycling of immobilized cells.

in the media with the different Nano scale particles and the concentration of the pigment was calculated as presented in Table 1. The optimum Nano scale for maximum production of pigment was obtained by use of 114.5 nm Powder nutrient in the fermentation medium of the fungus. Pigment concentration by this size (226 mg/ml) was close to that of the control (245 mg/ml).

Physiological studies on immobilized *P. purpurogenum*

Immobilized cells were incubated in the production medium at a range of temperatures and pH. Also, recycling of the beads was done for three cycles. From the date presented in Figs. 2, 3 and 4. It was clearly shown that 30°C was the optimum for maximum output of the pigment (264.6mg/ml) and the maximum yield was got in the neutral medium at pH 7(252.6)mg/ml. Also, the production of the pigment increased after the first incubation period (the first cycle) (250mg/ml). Our results were in accordance with those of Farzaneh, *et al*., 2014 who reported that 30°C was the optimum temperature for pigment production (Naghavi *et al*., 2014).

Coloring of white emulsion paint with the dye

Physical and mechanical properties of WP and DP are listed in Table 2. It is clear that S.C. gradually increased

Table 2: Physical and mechanical properties of the white emulsion paint and dyed-mixed paints.

Sample code	S.C.%	Viscosity cp	Densityg /cm³	Adhe-sion	Hard-ness	Bend-ing
WP	55.02	2107	1.2413	5B	1H	1 mm
1DP	55.45	2154	1.2396	5B	1H	1 mm
2DP	56.03	2185	1.2388	5B	1H	1 mm
3DP	56.51	2199	1.2369	5B	2H	3 mm
4DP	57.09	2221	1.2344	4B	2H	3 mm
5DP	57.65	2242	1.2325	4B	2H	3 mm
6DP	58.21	2275	1.2314	4B	2H	3 mm
7DP	58.57	2294	1.2288	4B	3H	3 mm
8DP	59.12	2323	1.2254	4B	3H	3 mm

Table 3: Chemical, heat, and weathering resistance properties of the white emulsion paint and dyed-mixed paints.

Sample code	Alkali	Acetic acid	Water	Heat	UV
WP	Pass	Pass	Pass	Pass	Failed
1DP	Pass	Pass	Pass	Pass	Failed
2DP	Pass	Pass	Pass	Pass	Failed
3DP	Pass	Pass	Pass	Pass	Failed
4DP	Pass	Pass	Pass	Pass	Failed
5DP	Pass	Pass	Pass	Pass	Failed
6DP	Failed	Failed	Pass	Pass	Failed
7DP	Failed	Failed	Pass	Pass	Failed
8DP	Failed	Failed	Pass	Pass	Failed

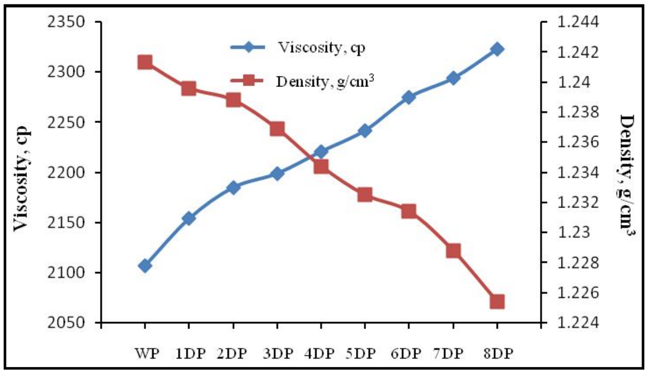

Fig. 5: Viscosity and density of the samples at ambient temperature.

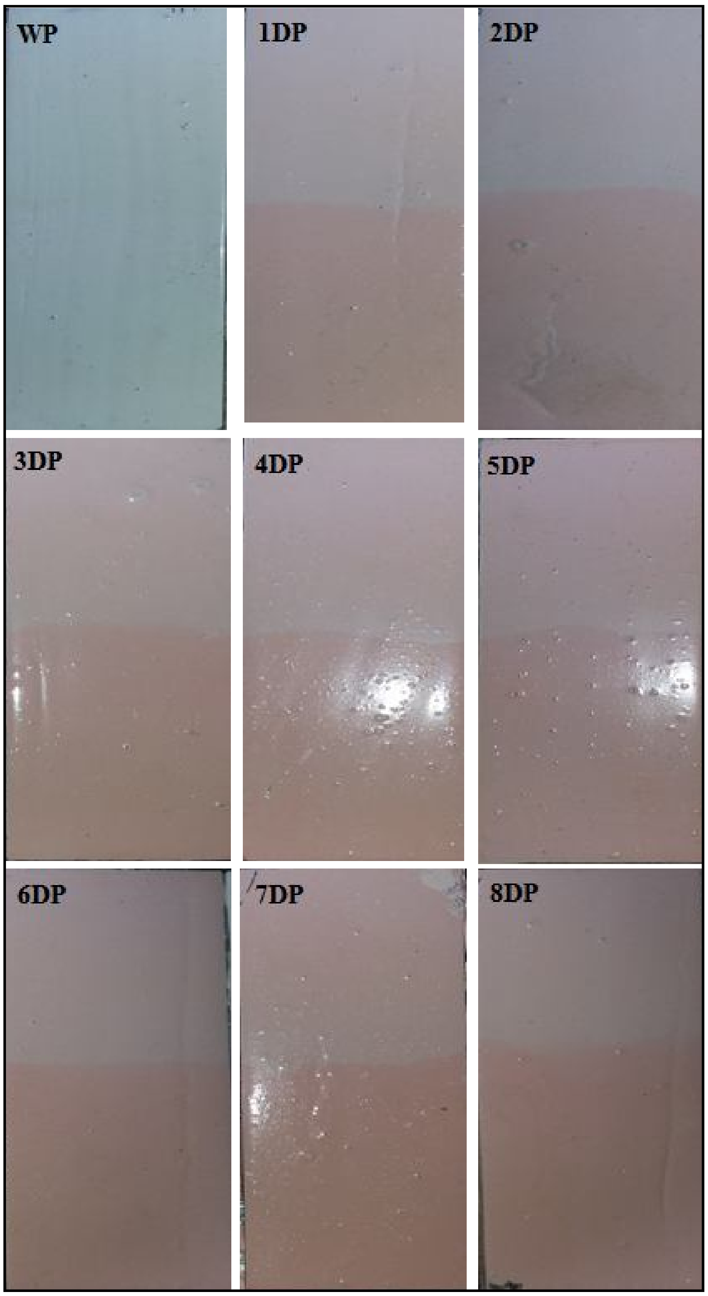

Fig. 6: The painted-samples before (down) and after (up) exposure to the UV-test.

with the amount of powder-dye added to reach an increase of 7.45% at 8DP. This logic increase is due to the addition of powder-dye to the WP. The viscosity and density of WP and DP samples are shown in Fig. 5. An increase in the viscosity of the paints can be observed with the amount of loaded-dye. This increase in viscosity may be due to the physical interaction originated between the emulsion resin and the dye such as H-bonding and Van Der Waals forces of attraction which increased the paint resistance to flow (Mangesana, *et al*., 2008, Davarcioglu, 2011, Leite, *et al*., 2012, Gaaz, *et al*., 2015). However, the decrease in density is due to the lower density of the added-dye. A trivial decrease in adhesion and an increase in hardness of the coated films with increasing the content of the dye in the paint can be detected from Table 3. This is a sequential result of the physical interactions formed between the polymeric chains of the binder in WP and the dye (Mohamed, *et al*., 2017). This caused an increase in the physical cross-linked chain structure of the paints with the added dye. The increase in the hardness of the paint films caused a very slight decrease in the flexibility of the paint films by increasing the amount of added-dye (Morsi and Mohamed 2017). The painted-films exhibited good chemical resistivity to dilute alkali, acetic acid, and water except for 6DP, 7DP, and 8DP. This indicates that the increase in the amount of the added-dye leads to a decrease in the chemical resistivity of the painted-films. All the samples passed the heat resistivity test; however, they failed in the UV-test. Fig. 6 shows the painted glass panels before their exposure to the UV test.

Conclusion

Penicillium purpurogenum is an intensive pigment- producer fungus. In this study, the fungus was immobilized and the produced pigment was optimized for maximum output. The nutrients in the fermentation medium were milled to different Nano scale particles to investigation the effect of the utilized milling ingredients on the yield. Finally, the application of the dried red powder was investigated where an increase in the viscosity and hardness of the paint film was recorded. Also, it provided a protection to the paint against different chemical factors e.g. Acids, alkali, water and heat. From the previous results and owing to the different benefits of this product. We recommend the use of this safe and natural pigments in paint industry.

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