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Potato Manufacturing Wastes – A Novel Substrate for the Production of Natural Pigments from *Monascus purpureus*

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Abstract

The aim of the present research was to investigate the feasibility of potato chips manufacturing wastes as a cheap substrate for the production of pigments by *Monascus purpureus* through solid-state fermentation. Maximum red, orange and yellow pigments of 126.5, 204.7 and 322.9 AU/g dry fermented material, respectively was achieved on potato wastes having 67% initial moisture content, 6.5 pH, 1.5mm particle size and supplemented with 2% ammonium sulphate. The optimum fermentation conditions were inoculation rate 140×10^3 spores /10g dry substrate, incubation period 15 days and fermentation temperature 30°C in complete darkness. To the best of our knowledge, this is the first report on pigments production from *M.purpureus* using potato wastes in solid-state fermentation.

***Keyword**: Potato manufacturing wastes, M.purpureus, pigments, food*

Introduction

In the recent years, coloring of food with pigments produced from natural sources is of worldwide interest and is gaining importance. These pigments are looked upon for their safe use as a natural food dye in replacement of synthetic ones because of undesirable toxic effects including mutagenicity and potential carcinogenicity. Although many natural colors are available, microbial colorants play a significant role as food coloring agent, because of its flexibility in production and easy down streaming process. Among the various pigment-producing microorganisms, *Monascus* is reported to produce non-toxic pigments, which can be used as food colorant. Besides a coloring agent it enhances the flavor of the food and acts as food preservative (Vidyalakshmi *et al.,* 2009).

The pigments produced from *Monascus* are classified into at least six types of pigments based on their color (1) red pigment (rubropunctamin, $C_{21}H_{26}NO_4$ and monascurubramin, $C_{23}H_{27}NO_4$); (2) orange pigment (rubropunctatin, $C_{21}H_{22}O_5$ and monascorubrin, $C_{23}H_{26}O_5$) and (3) yellow pigment (monascin, $C_{21}H_{26}O_5$ and ankaflavin, $C_{23}H_{30}O_5$) (Babitha, 2009). Monascus pigments are used traditionally for natural coloring of oriental foodstuffs in Asian countries and also in textile dyeing process (Santis *et al*., 2005; Velmurugan *et al*., 2010). Recent studies on Monascus pigments reported that it possess anti-tumor activity (Hsu and Pan, 2012).

Natural raw materials and by-products of industry have a wide use as culture media in fermentation processes because of their low cost

since the medium components can represent from 38 to 73% of the total production cost (Schmidell, 2001). So, there is a need to select cheap and efficient substrates for producing the bioproducts economically. Various agricultural by-products such as corn cob (Velmurugan *et al*., 2011), sugarcane bagasse (Silveira *et al*., 2011), grape waste (Silveira *et al*., 2008), jackfruit seed (Babitha *et al*., 2006), corn steep liquor (Hamano and Kilikian, 2006), wheat bran (Dominguez - Espinosa and Webb, 2003) and cassava (Yongsmith *et al*., 1993) were successfully utilized for the production of *Monascus* pigments.

In potato chips factories, during the slicing of potato tuber into thin round slices, so much amounts of potato fragments with unsuitable size and shape are produced. These fragments consider as a waste or by-product and take away with very low price value to use as a feed component for animals and poultry.

The present paper deals with the optimization of physical and nutritional conditions for the pigments production from *Monascus purpureus* Went NRRL 1992 by using of Potato chips manufacturing wastes as a basal medium under solid state fermentation conditions.

Materials and Methods

Culture

A culture of *Monascus purpureus* Went NRRL 1992 obtained from Microbiological Resources Center (MIRCEN), Ain Shames Univ. Cairo, Egypt, was used in the present study. It was maintained on Yeast Extract-Peptone-Dextrose agar (YEPD) medium, at 4°C and subcultured periodically every three weeks. This fungal strain was examined for its ability to produce the mycotoxin citrinin and found to be non-producing.

Inoculum preparation

M. purpureus Went NRRL 1992 was grown on YEPD slant agar in the dark at 30°C under static conditions. To fully sporulated (6-8 days old) agar slope culture, 10 mL of sterile distilled water was added and the spores were scraped under strict aseptic conditions. The spores suspension obtained was used as inoculum (approximately 7×10^5 spores per mL). As described by Babitha *et al*. (2007).

Fermentation medium

Wastes of potato chips manufacturing (Raw Pieces) were obtained from a factory of potato chips at Assuit Governorate, Egypt. After drying at 70°C, it was ground well to 1.0 - 2.0 mm particle size using an electrical mill and the produced potato flour (powder) was packed in polyethylene pages until use as the basal fermentation medium.

Solid-state fermentation procedures

Ten grams of potato powder was placed in a 250 mL Erlenmeyer flask and 2.0 mL of a nutrient salt solution (KH_2PO_4, 2; NH_4NO_3, 5; NaCl, 1; and $MgSO_4 \cdot 7H_2O$, 1 g/L) plus 1 mL zinc sulfate ($ZnS0_4.7H_2O$, 0.128 M) solution were added to each flask (Babitha *et al*., 2006 and Nimnoi and Lumyong, 2009). Initial moisture content was adjusted to approximately 41%, 44%, 47%, 50%, 52%, 55%, 57%, 58%, 60%, 62%, 63%, 64%, 66%, 67%, 68% and 69% with distilled water (V/W) in different flasks. The potato powder was then allowed to soak at 30°C for 1h.

Flask contents were mixed thoroughly, then covered with two layers of aluminum foil to prevent moisture loss and autoclaved at 121°C for 15 min. After cooling to room temperature, each flask was inoculated with 1 mL spores suspension (7×10^5spores / mL) and incubated at 30°C for 10 days in the dark. Unless otherwise indicated, these conditions were maintained throughout the experiment. All experiments were conducted in triplicate and means ± standard deviations were reported.

Determination of optimized conditions for pigments production

The effect of initial moisture content (41%, 44%, 47%, 50%, 52%, 55%, 57%, 58%, 60%, 62%, 63%, 64%, 66%, 67%, 68% and 69% (V/W)) was adjusted by adding distilled water. The fermentations ran for 10 days at 30°C in the dark. To study the effect of inoculation rate, 70×10^3, 140×10^3, 175×10^3, 350×10^3, 525×10^3, 700×10^3, 875×10^3, 1050×10^3 and 1225×10^3 (Number of Spores /10gram dry substrate(gds)) spore suspension was added under aseptic conditions after sterilization and cooling. The initial pH value (4.5, 5.5, 6.5, 7.5 and 8.5) was achieved by adjusting the pH of the added sterile distilled water with 0.5M HCl or 0.5M NaOH. To study the effect of particle sizes, Potato powder of different particle sizes was used to prepare different media, viz. M1 (particles 1.0 mm), M2 (particles 1.5 mm) and M3 (particles 2.0 mm). To study the effect of incubation period pigments were estimated after 5, 7, 9, 11, 13, 15, 17 and 20 days of incubation. Experiments were also performed to evaluate the influence of the addition of different organic and inorganic nitrogen sources such as monosodium glutamate, peptone, yeast extract, beef extract, ammonium nitrate, ammonium sulphate, ammonium coloride, potassium nitrate and urea at 1.0% concentration of each. The optimum concentration of the selected nitrogen source (ammonium sulphate) which stimulates pigments production was estimated by adding it at different concentrations (1.0, 2.0, 3.0, 4.0 and 5.0%) to the production medium.

Pigment extraction and quantification

At the end of incubation period, the contents of each flask was dried on aluminum foil at room temperature and ground to a fine powder using an electrical mill. 0.5 g of dried powdered fermented solids was extracted with 20 mL of 95% ethanol in an incubator shaker for 2 h at 180 rpm in a100 mL Erlenmeyer flask. The extract was then centrifuged at 10000 g/min. for 10 min to remove suspended solids. The supernatant was analyzed by a spectrophotometer using a 95% ethanol blank (Chiu and Poon, 1993; Johns and Stuart, 1991; Lin *et al.,* 1992). Pigments concentration was measured using a double beam spectrophotometer (UViline 9400 – SCHOTT Instruments, EU) at 400, 470 and 500 nm for yellow, orange, and red pigments, respectively, taking into consideration the dilution factor of the sample (Carvalho *et al*., 2003; Chiu and Poon, 1993). The results were expressed as absorbance unit (AU) per gram of dried solids (Lin and Iizuka, 1982). The pigment

absorbance was calculated using the equation:

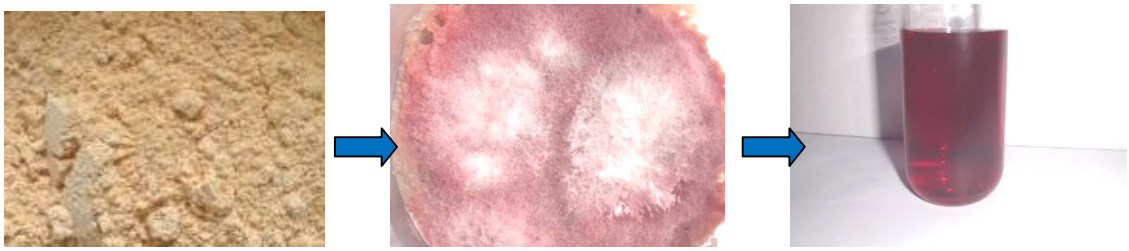
The absorbance unit of pigments $(AU\ g^{-1}) = Abs \times \frac{20}{0.5} \times df$

Analysis of citrinin

The presence of citrinin was determined by Thin Layer Chromatography as described by Rasheva, *et al*., (2003). Citrinin (Sigma) was used as a standard.

Results and Discussion

The obtained results show that potato powder served as a good substrate for the growth of *M. purpureus* NRRL 1992, which resulted in a considerable amount of pigments production. The results reported are the average of three sets of experiment values.



(A) (B) (C)

Figure 1: Changing color of potato powder by *Monascus purpureus* before and after fermentation by *M. purpureus* Went NRRL 1992. (A: raw potato powder; B: fermented potato powder; C: extracted pigment solution).

Effect of initial moisture

Data illustrated in Fig. (2) clearly showed that, negligible amounts of pigments were produced on potato powder containing 44-62% initial moisture content. Pigments production was markedly increased with increasing the moisture content reaching to the maximum at 67% moisture. The highest color intensity at 67% moisture of yellow, orange and red pigments estimated at 400, 470 and 500nm were 12.96, 7.36 and 7.24AU/g, respectively. At the same time the produced amount of all pigments was dramatically decreased at the higher moisture contents. The obtained results were similar to some extent with that reported by other investigators. Raimbault (1998) reported that the optimal initial moisture content for pigments production tends to be in the range of 40 to 70%. Babitha *et al*., (2007) found that pigment production was reduced at initial moisture content below 40%, while using jack fruit seed as substrate in solid state fermentation. More recently, Velmurugan, *et al*., (2011) reported an optimum moisture content of 60% for *Monascus purpureus* KACC 42430, while using corn cob powder as a substrate for production of pigments in solid state fermentation. The lower yield at high moisture content is due to agglomeration of substrate, reducing oxygen supply for *M. purpureus*. The decrease in pigment production at low moisture content is a result of low nutrient availability due to reduced nutrient salt dissolution, as well as less efficient heat exchange and oxygen transfer (Babitha, *et al*., 2007;

and Carrizales and Rodriguez, 1981). The optimum initial moisture content depends on the water holding capacity of the substrate and on the microorganism used.

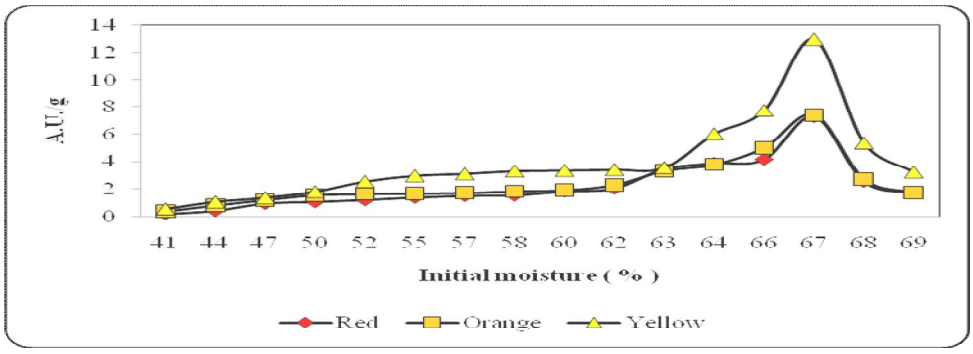


Figure 2: Effect of Initial moisture content on pigments production by *M. purpureus* Went NRRL 1992 grown on potato wastes using solid state fermentation.

Effect of inoculation rate

Inoculation of the pigments production medium with 140×10^3 (spores /10 gds) maximized yellow pigment yield (48.48 AU/gds), followed by orange pigment (27.64 AU/gds), and red pigment (23.76 AU/gds) as shown in Figure (3). The present results are rather differed from that described by (Velmurugan, *et al*., 2011; Babitha, *et al*., 2007; Pandey, *et al*., 2000; and Chakradhar, *et al*., 2009) who reported that, the optimal Inoculum size for pigments production was 4ml of spores (6×10^5 spores/mL) /gram of initial dried substrate (gds).

Numerous studies have shown the large influence of inoculum size on pigments production yield in SSF. Babitha, *et al*., (2007) demonstrated that, too little inoculum resulted in insufficient biomass and smaller amounts of the product, whereas too much inoculum produced excessive biomass and depleted the nutrients required for pigment formation.

So that, it is advantageous to achieve high level of the product by small inoculums size as obtained from our results. This may reflect a high potentiality of the studied fungal strain.

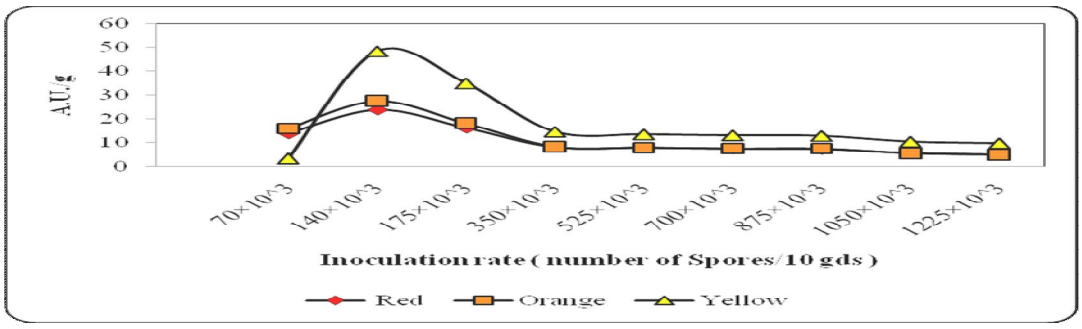


Figure 3: Effect of Inoculum sizes on pigments production by *M. purpureus* grown on potato wastes using solid state fermentation.

Effect of initial pH

Substrate pH is one of the most important factors determining microbial growth and metabolic activity in SSF. *M. purpureus* pigments yield were determined at different initial pH levels (4.5 to 8.5). The maximum yield of pigment was obtained at pH 6.5 (23.76, 27.64, and 34.86AU/g for red, orange, and yellow pigments respectively), close to the natural pH of potato powder samples soaked in distilled water. Production of yellow and orange pigments tends to decrease at both acidic (pH 4.5 - 5.5) and Alkaline (pH 7.5 - 8.5) values. These results were similar to that reported by previous investigators. Chen and Johns (1993); Lee *et al*., (2002); and Joshi *et al*., (2003) showed that the suitable pH for growth and *Monascus* pigments production was 5.5–6.5. On the other hand, production of red pigments proved to be not related with the changes of initial pH values (Fig.4). However this observation was closely consistent with Babitha, *et al*., (2007), who reported maximum pigments production by *M. purpureus* at pH 4.5 to 7.5, while using jack fruit seed as substrate in solid state fermentation.

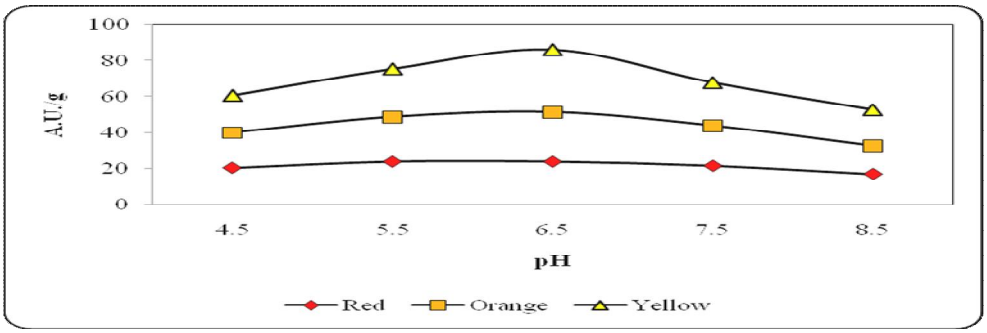


Figure 4: Effect of Initial pH on pigments production by *M. purpureus* grown on potato wastes using solid state fermentation.

Effect of potato particle size

Among the several factors in SSF processes which are important for microbial growth and activity, the substrate particle size is one of the most critical parameters (Zadrazil and Puniya, 1995; and Pandey, 1991). Data illustrated in Fig. (5) Clearly showed that *M. purpureus* produced the yellow pigment at high level when grown on potato particles of 1.5 - 2.0 mm size. At the same time, the fungus ability to produce orange and red pigments was enhanced on media containing potato particle size of 1.5 mm, but reduced dramatically with increasing the particles size to 2.0 mm. In the contarary of the present results, Babitha *et al*., (2006) found that *M. purpureus* LPB 97 produced the maximum pigments level on media consists of Jack fruit seeds with particles size between 0.4 and 0.6 mm. Generally, smaller substrate particles provide a larger surface area for microbial attack, and thus it should be considered as a desirable factor. However, too small particles may result in substrate agglomeration, which may interfere with aeration resulting in poor microbial growth. At the same time, larger particles provide better aeration efficiency but provide limited surface for microbial attack. Therefore, it may be necessary to provide compromised particle size (Pandey *et al*., 2000).

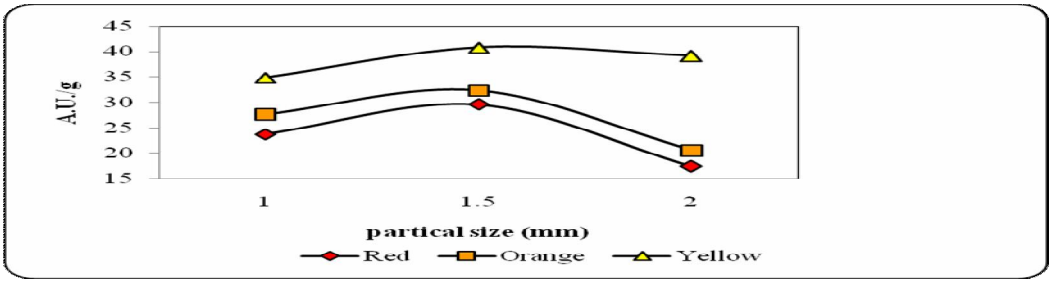


Figure 5: Effect of particle sizes on pigments production by *M. purpureus* grown on potato wastes using solid state fermentation.

Effect of incubation time

The amounts of pigments produced were increased gradually with increasing the incubation time (Figure 6). Maximum yellow, orange and red pigments production was obtained after 17 days (89.22 AU/g, 76.28 AU/g and 70.86 AU/g, respectively). Results are in agreement with Emon *et al*., (2007) who found that maximum pigments production was obtained after 2 weeks of incubation by *Monascus purpureus* CMU001, grown on Korkor 6 white glutinous rice as substrate in solid state fermentation. The present results are rather differed from this described by Velmurugan, *et al*., (2011) who reported that maximum pigments production were achieved by *Monascus purpureus* KACC 42430 within7 days, while using corn cob as substrate in solid state fermentation.

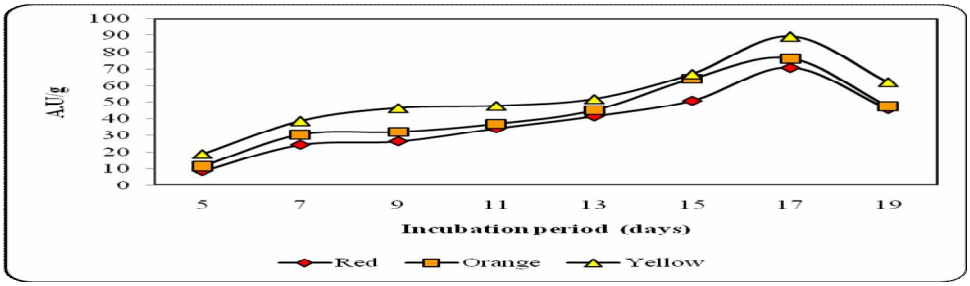


Figure 6: Effect of Incubation period on pigments production by *M. purpureus* grown on potato wastes using solid state fermentation.

Effect of nitrogen source on pigments production

Nine different organic and inorganic nitrogen sources were added separately at concentration of 1% to potato fermentation media as nutritional supplementary to elucidate its effect on pigments production by *M. purpureus* Went NRRL1992. From the spectral analysis observed for changes in different nitrogen sources, only ammonium sulphate showed stimulatory effect which duplicated the produced amount of yellow and orange pigments to reach 189.1 and 133.5AU/g, respectively when compared with control treatment. On the other hand, production of red pigment did not affected by adding of ammo-

nium sulphate at concentration of 1.0% (Fig. 7).

To estimate the optimum concentration of the best nitrogen sources, ammonium sulphate was added at different concentrations (1, 2, 3, 4 and 5%). Data illustrated in Fig. (8) indicated that 2% ammonium sulphate proved to be the optimum concentration for maximum pigments production. The obtained color intensity of red orange and yellow pigments increased from 70.86, 133.5 and 189.1 AU/g to 126.5, 204.7 and 322.9 AU/g, respectively by increasing ammonium sulphate concentration from 1.0 to 2.0%. The higher concentrations (3, 4 and 5%) of ammonium sulphate haven't the same stimulatory effect. There is no previous studies in the literature concerning nitrogen supplementation of potato for pigments production. However, by using of rice as production medium, some other investigators found that Mono sodium glutamate (MSG) was the best nitrogen source and the optimum concentration was 5% (Vidyalakshmi, *et al*., 2009; Rashmi and Padmavathi, 2011).

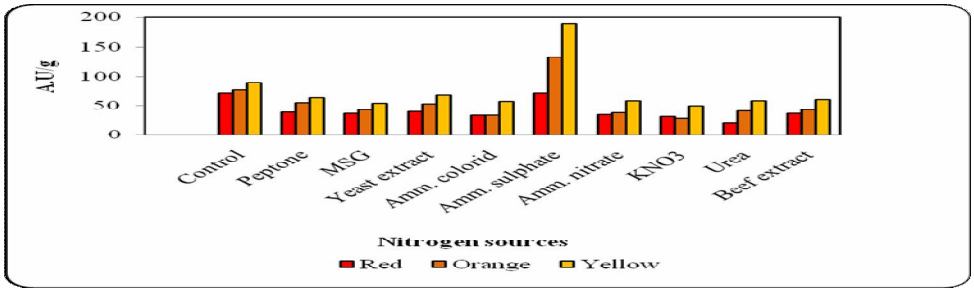


Figure 7: Effect of nitrogen sources on pigments production by *M. purpureus* grown on potato wastes using solid state fermentation.

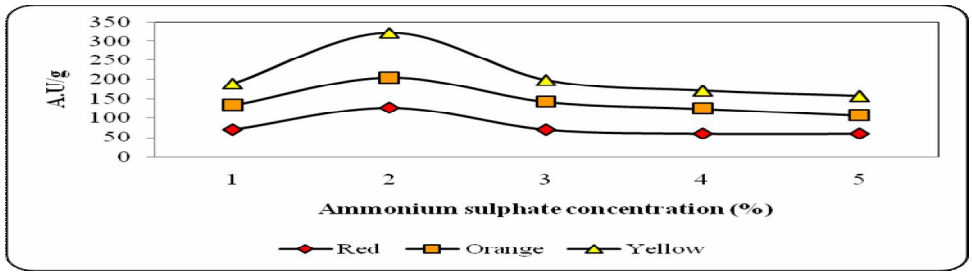


Figure 8: Effect of different concentrations of ammonium sulphate on pigments production by *M. purpureus* grown on potato wastes using solid state fermentation.

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مخلفات تصنيع البطاطس – مادة خام مبتكرة لإنتاج الصبغات الطبيعية من فطر المونـاسكس بوربوريوس

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الملخص

تهدف هذه الدراسة إلى إختبار إمكانية إستخدام المخلفات الصلبة لمصانع الشيبسى كمـادة خام رخيصة الثمن فى إنتاج الصبغات المفرزة من فطـر المونـاسـكس بوربوريـوس بطريقـة التخمير الصلبة. وقد تم تحقيق أعلى إنتاجية من الصبغات الحمراء والبرتقالية والصفراء بقيمـة ٥, ١٢٦ و٧, ٢٠٤ و٩, ٣٢٢ وحدة إمتصاص/ جم مادة جافة متخمرة ،على التـوالى ، وذلـك عند تنمية فطر المونـاسكس بوربوريوس علي بيئة مخلفات الشيبسي المحتوية علي ٦٧% رطوبة مبدئية والأس الهيدروجيني لها ٦،٥ وحجم جزيئات مادة التخمر ١,٥ مم مع تـدعيم البيئـة ب ٢% كبريتات أمونيوم وتلقيحها ب ١٤٠× ١٠[٣]جرثومة / ١٠جم مادة خام جافة وتحضينها علـي درجة ٣٠ م في ظروف مظلمة تماماً لمدة ١٥ يوم. وطبقاً للمعلومات المتوفرة فإن هـذه هـي أول دراسة عن إمكانية إستخدام المخلفات الصلبة لمصانع الشيبسي كمادة خام لإنتاج الـصبغات بواسطة فطر المونـاسكس بطريقة التخمير الصلبة.