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Separation of natural colorants from the fermented broth of filamentous fungi using colloidal gas aphrons

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ABSTRACT

There is a worldwide interest in the development of processes for producing colorants from natural sources. Microorganisms provide an alternative source of natural colorants produced by cultivation technology and extracted from the fermented broth. The aim of the present work was to study the recovery of red colorants from the fermented broth of *Talaromyces amestolkiae* using the technique of colloidal gas aphrons (CGA) comprising surfactant-stabilized microbubbles. Preliminary experiments were performed to evaluate the red colorants' solubility in different organic solvents, octanol/water partitioning, and their stability in surfactant solutions, namely hexadecyl trimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS), and polyoxyethylenesorbitan monolaurate (Tween 20), which are cationic, anionic and nonionic surfactants, respectively. The first recovery experiments were carried out using CGA generated by these surfactants at different volumetric ratios (V_R, 3–18). Subsequently, two different approaches to generate CGA were investigated at V_R values of 6 and 12: the first involved the use of CTAB at pH 6.9–10.0, and the second involved the use of Tween 20 using red colorants partially dissolved in ethanol and Tween 20. The characterization results showed that red colorants have a hydrophilic nature. The highest recoveries were obtained with Tween 20 (78%) and CTAB (70%). These results demonstrated that the recovery of the colorants was driven by both electrostatic and hydrophobic interactions. The V_R was found to be an important operating parameter and at V_R 12 with CTAB (at pH 9) maximum recovery, partitioning coefficient (K = 5.39) and selectivity in relation to protein and sugar (S_P = 3.75 and S_S = 7.20 respectively) were achieved. Furthermore, with Tween 20, the separation was driven mainly by hydrophobic interactions. Overall CGA show promise for the recovery of red colorants from a fermented broth. Although better results were obtained with CTAB than with Tween 20 the latter may be more suitable for some application due to its lower toxicity.

1. Introduction

Synthetic and natural colorants are extensively used in the food, cosmetic, and pharmaceutical industries [1,2]. However, natural colorants have recently gained popularity over synthetic ones, which, in some cases, are known to be potential carcinogens [1,3]. Therefore, there is a growing demand for ecofriendly/non-toxic colorants, specifically for the coloration of foods and pharmaceuticals, as well as for dyeing kids' cloths and leather garments [4,5].

Natural colorants can be produced by microorganisms such as fungi species [6]. Fungal colorants show diversity not only in their chemical structures but also in the color range of their colorants, possibly adding new or additional hues to the color palettes of the existing colorants derived from contemporary sources [7,8]. Most of the colorants produced by fungi are quinones, flavonoids, melanins, and azaphilones, which belong to the aromatic polyketide chemical group and have been widely described for medicinal applications and potential use as dyes [9]. The structures of polyketides are known to contain loose ð-electrons, as they often contain polyunsaturated functionality, i.e., ring systems, one or more

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carbonyl groups, carboxylic acid, and ester or amide functional groups exhibiting characteristic UV–vis spectra [8].

Monascus species is the most studied candidate for producing colorants [6]. However, alternative colorant-producing organisms are of great interest [2]. In this context, in the literature new producers of *Monascus*-like azaphilone colorants in liquid media such as *Talaromyces amestolkiae* (cited before as *Penicillium purpurogenum*) has been reported [6]. Furthermore, in studies performed by our research group, this microorganism showed the potential to produce natural colorants with significant antimicrobial activities, complete absence of toxicity against *Artemia salina* [10], and the ability to produce natural colorants stable under alkaline conditions, having good compatibility with polyethylene glycol (PEG) and sodium polyacrylate (NaPA) polymers to up to 70 °C [11].

Most of the research on natural colorants derived from microorganisms deals with their production. However, the extraction and/or purification of such colorants using new methods are of great interest, because most of the reported studies use organic solvents to extract the colorants from the fermented broth [4,7,12,13]. The use of organic solvents has some limitations, particularly in large-scale applications, because of their high cost, toxicity of solvents, potential environmental impact, and because organic solvents may lead to irreversible product degradation [14]. Thus, the development of more efficient and cost-effective separation and purification processes is crucial to improve the process efficiency and economics, while maintaining the required high quality standards for market approval [15,16].

In the search for alternative methods, there is a growing interest on the application of surfactants to separation processes [17]. Surfactants are amphiphilic molecules composed of a hydrophilic or polar moiety, known as the head, and a hydrophobic or nonpolar moiety, known as the tail. The surfactant head can be charged, dipolar (zwitterionic), or noncharged [18]. Sodium dodecyl sulfate (SDS), hexadecyltrimethylammonium bromide (CTAB), and polyoxyethylenesorbitan monolaurate (Tween 20) are examples of anionic, cationic, and nonionic surfactants, respectively.

Surfactants possess several unique characteristics such as the tendency to adsorb onto surfaces, to associate in solution to form micelles that dissolve nonpolar solutes [19,14], and the fact that most of them are nontoxic and biodegradable. Moreover, these systems usually have low energy requirements [20,14].

One application of surfactants in the extraction process is their use with colloidal gas aphrons (CGA). CGA are surfactant-stabilized microbubbles (10–100 μm) which can be generated by stirring a surfactant solution at high speeds (8000 rpm) [21,22] or by sonication and homogenization [23]. The core of the microbubble is composed of a gas surrounded by a thick multilayer shell, which is formed of an inner surfactant film enveloped by a viscous aqueous layer [24]. The double layer of surfactants provides rigidity and low permeability to the structure while imparting some hydrophilic character to it [24].

CGA can be generated by ionic and nonionic surfactants, which will influence the outer surface of the microbubble to be positive, negative, or noncharged, respectively, to which oppositely or noncharged molecules will adsorb, resulting in their effective separation from the bulk liquid [25,14]. Thus, the adsorption selectivity of the surfactant can be adjusted [26,14]. CGA exhibit unique characteristics, including high interfacial area and high stability compared to those of conventional foams; they can also be pumped and separated easily from the liquid phase without any mechanical aid. Furthermore, the use of biodegradable surfactants results in environmentally friendly processes, and the final product can also be safe for human consumption [14]. These properties help to reduce the number of operations required in product purification/recovery, making CGA a cost-effective separation technique compared to conventional methods such as centrifugation and supercritical fluid extraction [27].

In light of their favorable properties, researchers have considered various applications of CGA, with a particular focus on the separation processes [27]. They have been successfully used to recover proteins from whey [28], polyphenols from wine waste extracts [25], carotenoids from plant extracts [22], astaxanthin particles from a fermentation mixture containing yeast cells [29], and pulp fibers from paper machine backwater [30].

As red colorants are one of the most important dyes used in many industries [3], the aim of this study was to investigate the recovery of red colorants from the fermented broth of *T. amestolkiae* using CGA generated by different surfactants under several conditions.

2. Material and methods

2.1. Materials

CTAB, SDS, Tween 20, octanol and bicinchoninic acid assay kit was obtained from Sigma Chemicals (St. Louis, MO). All the other reagents were of analytical grade and were used as received. The laboratory mixer (SL2T) fitted with four bladed impeller (D = 30 mm) surrounded by a high shear screen and with a digital readout of the impeller speed was supplied by Silverson (Waterside, Bucks, UK). The spectrophotometers used were an Ultrospec 1100 pro purchased from Amersham Pharmacia Biotech (Biochrom, Cambridge, UK) and a PerkinElmer lambda 20 UV–vis Spectrophotometer coupled with a microcomputer. The pH was measured with a bench-top pH meter from Whatman (PHA 320, Kent, UK).

2.2. Fermentation process

T. amestolkiae was the microorganism used to produce the red colorants. It was kindly provided by the Culture Collection by Federal University of Amazon, DPUA, AM, Brazil. The cultures preserved in distilled water were reactivated in Czapeck Yeast Extract Agar (CYA) [31]. The cultures were maintained at 25 °C for 7 days and after maintained in fridge at 4 °C. The inoculum was prepared in CYA plate and the cultures were maintained at the same conditions of reactivation.

For fermentation, 5 mycelial agar discs (8 mm diameter) from inoculum were obtained by a self-designed cutter and transferred to 25 mL of fermentation medium in 125 mL Erlenmeyer flasks and incubated in orbital shaker incubator under 30 °C, 150 rpm for 15 days. The fermentation medium composition was liquid Czapeck Yeast Extract with modifications as described by Santos-Ebinuma et al. [11]. The media had the pH adjusted to 4.5 with HCl (5 M). After fermentation, the fermented broth was filtrated. The supernatant containing the red colorants was frozen in ultra-freezer at −70 °C to be used in the partitioning studies. All culture media prepared was autoclaved at 121 °C for 15 min.

2.3. Generation of CGA

The SDS, Tween 20 and CTAB pH 6.9 solutions were prepared in deionised water. The CTAB pH 8.0 was prepared in McIlvaine's buffer and the CTAB pH 9.0 and 10.0 solutions were prepared in Sodium Carbonate-bicarbonate Buffer. The SDS and CTAB solutions were prepared at concentration 2 mM while the Tween 20 solution at concentration 20 mM. CGA were generated by stirring 300 mL of CTAB, SDS or Tween 20 solution, at 8000 rpm for 5 min at room temperature using a high-speed impeller (Silverstone SL2T).