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Recovering violacein from Yarrowia lipolytica cells using alternative solvents

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PURPOSE OF THE ABSTRACT

Violacein is an indole derivative pigment [1], well-known by its promising biological activities, namely as an antibacterial, antiviral, antioxidant and antitumoral compound [2]. As pigment, it is expressed intracellularly, and thus efficient downstream processes are of utmost importance. There are a number of mechanical methods available to achieve extraction of intracellular biomolecules. Nevertheless, several pros and cons (such as, the energy consumption, compatibility, high costs, non-selective character and effects on the biological activity of the target compounds) can be debated. Non-mechanical techniques are more selective and gentler on the desired molecule. Surfactants are amphiphilic molecules, that above their critical micellar concentration (CMC), start to aggregate forming micelles. Moreover, the spontaneous insertion of cations in the lipid bilayer causes the swelling of membranes and, at concentrations above the CMC, can lead to an easier cell disruption [3]. Aqueous biphasic systems (ABS) have been widely used for the recovery and purification of biomolecules [4]. Ionic liquids are a specific class of salts, which combined with the advantages of ABS allow high performance extractions in addition to presenting other advantages like their quick phase separation, and low viscosity, favouring the mass transfer [5].

In this work, the extraction of violacein was optimized and its purification was pursued. The pigment was recovered from genetically modified Yarrowia lypolitica cells. With this goal, both solid-liquid and liquid-liquid extractions were optimized using aqueous solutions of alternative solvents. The effect of different classes of solvents, including surfactants, tensioactive and non-tensioactive ILs, on the yield of extraction of violacein was studied. Additionally, the effect of the ILs' chemical structure in the cell membrane disruption and therefore, the violacein removal were also assessed. Operational conditions such as the solid-liquid ratio, the effect of consecutive extractions, the time of extraction and the concentration of the solvent were accessed. It was found that only the aqueous solutions of tensioactive compounds were able to extract violacein, the solution of surfactant A being the most efficient.

After the optimization step, the effort was directed to the purification of the pigment from the proteins of the raw extract through the application of ABS. Firstly, the phase diagrams for different ABS composed of surfactant - Tween 20 and five different cholinium-based ILs were characterized. Lastly, the violacein purification was defined with these ABS. For all tested systems about 100% of the violacein content was concentrated in the surfactant A-rich phase, while a partial separation of the proteins among the two phases was observed. In the end, the integrated downstream process able to extract and purify violacein was defined, considering all the conditions optimized in both steps, solid-liquid extraction (i) and purification (ii).

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FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Violacein | Solid-liquid extraction | Aqueous biphasic systems | Ionic Liquids

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