Design of Environmental Processes Laboratory, School of Chemical and Environmental Engineering, Technical University of Crete

Proceedings of

1st International Conference on Sustainable Chemical & Environmental Engineering

SUST
E N G
2022

31st Aug - 4th Sep 2022

Rethymno, Crete, Greece

hybrid event

www.susteng.eu

ISBN: 978-618-86417-0-9

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Application of ionic liquids for bacterial carotenoid extraction

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keywords: *bacterial carotenoids; ionic liquids; extraction method.*

Introduction

One of the ways to make microbial bioprocesses more economically viable is to enhance valorization of high added value products resulting from the biomass, like carotenoids, which have a high market value. To recover these pigments from microbial biomass a good extraction method is required. Solvent extraction is one of the methods commonly used to extract carotenoids, however, solvent extractions are both material and time-consuming, and moreover also present some health and safety concerns [1].

Ionic liquids (ILs) are a promising step forward to tackle some of these problems, even with their high price, and has been tested for the extraction of microorganism's components [2]. These "molten salts" are a group of compounds that have been known for a long time, but only in the last decades they have been attracting more attention from both researchers and industry. ILs are solvents that have a high solvation power for a wide range of molecules [3]. ILs are salts with a melting point below 100°C, which possess unique properties that depend on both the cation and anion present, high thermal and chemical stability, a large electrochemical window, great solvent power, non-flammability, and a negligible vapor pressure. Their versatility is one of their most attractive features, making them adaptable to many technologies [2]. Therefore, ILs can be used to facilitate chemical reactions, extraction and separation, biotransformation, and can be used in biorefineries and other processes.

As shown in previous works, *Gordonia alkanivorans* strain 1B has the capacity to produce carotenoids, however, since it was originally isolated form hydrocarbon rich environments, it is highly resistant to different organic solvents commonly used in extraction protocols. This makes the process slow and laborious, lowering yields and increasing solvent spending [4, 5]. As such, new extraction protocols must be developed and tested to obtain higher pigments yield. So, herein, the potential of ILs for carotenoids extraction was evaluated, since these compounds have been described as a good option to extract pigments produced by microorganisms.

In this context, a preliminary screening of 19 ILs, chosen based on their properties, was performed to ascertain which, if any, had potential to be used for carotenoid extraction from cells of *G. alkanivorans* strain 1B, in combination with ethyl acetate (EAc) as co-solvent. After the selection of an IL that highly increases extraction efficiency, the novel extraction process was optimized through a surface response methodology based on a Doehlert distribution for two factors (volume of IL and EAc)

Materials and methods

Ionic liquids – #1: 1-Ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; #2: 1,3-Dimethyl imidazolium Dimethyl Phosphate; #3: Trihexyltetradecylphosphonium bis(trifluoromethylsulfonyl) amide; #4: 1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; #5: 1-Hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; #6: 1-Ethyl-3-Methylimidazolium hydrogensulfate; #7: Triisobutyl methyl phosphonium tosylate; #8: 1-Ethyl-3-Methylimidazolium thiocynate; #9: 1-Butyl-3-Methylimidazolium thiocynate; #10: 1-Butyl-3-Methylimidazolium tetracholoroferrate (III); #11: 1-Butyl-3-Methylimidazolium dicyanamide; #12: 1-Butyl-3-Methylimidazolium triflate; #13: 1-Ethyl-3-Methylimidazolium dihydrygeno phosphate; #14: 1-Ethyl-3-methylimidazolium trifluoromethanesulfonate; #15: 1-Ethyl-3-Methylimidazolium methanosulfonate; #16: Didecyl-dimethyl-amonium nitrate; #17: 2-hydroxyethyl ammonium formate; #18: Tributyl(ethyl)phosphonium Diethyl Phosphate; #19: Methylimidazolium bis(trifluoromethylsulfonyl)imide.

Bacterial biomass - The biomass used in this study was obtained from the cultivation of *Gordonia alkanivorans* strain 1B [6] in a sulfur free mineral medium [4, 5] and fructose as C-source. Afterwards, cells

were centrifuged (8600 x *g* at 4°C, 20 min) and concentrated until obtaining the quantity of cells necessary (cells concentration: ≈50 g/L dry cell weight) for further extraction.

Preliminary Carotenoid extraction method - a protocol was adapted from Ruiz et al. [7], in which biomass of strain 1B was extracted with EAc mixed with each tested IL (#1 to #19). For each extraction, 400 µL of biomass, 300 µL of IL and 300 µL of EAc were put in a 1.5 mL Eppendorf tube and mixed in an overhead shaker, at room temperature, overnight. After centrifugation (15000 x g, 15 min), the results of carotenoid extraction were evaluated, visually and/or quantitatively through spectrophotometer analysis [4, 5].

Results and discussion

To evaluate the potential of ILs to increase the efficiency of carotenoid extraction from strain 1B biomass, a set of assays was carried out to test 19 ILs combined with EAc, a solvent known for its high extraction capacity. Out of the 19 ILs tested (#1 to #19), only 10 were able to form a biphasic system and extract carotenoids. Total carotenoids in the more colorful extracts were quantified using a spectrophotometer. Based on the value of total carotenoids obtained, the two ILs that generated the highest extractions were selected: IL#2, which extracted 2.79 µg, and IL#18, which extracted 3.43 µg. These results contrast with the value of the extracted carotenoids using EAc as sole solvent (0.92 mg). Moreover, subsequent tests confirmed the greater efficiency of IL#18 as a co-solvent to extract carotenoids demonstrating the advantages of this novel method towards a faster and enhanced extraction. The good results obtained for IL#18 were expected, since the IL#18 belongs to the group of ILs which could be acting as lipidic membrane permeabilizer. IL#18 permeates the cell membrane facilitating ethyl acetate penetration thus increasing carotenoid extraction. Further optimization of the extraction method using IL#18/AEc was carried out using a surface response methodology, based on the Doehlert uniform shell design [9] for two factors (X1 = volume IL#18 volume; X2 = volume EAc). The results obtained point out for 50 µL of IL#18 with 1125 µL of AcE, as the best condition for high carotenoid extraction from biomass of *G. alkanivorans* strain 1B.

Acknowledgments: This work was financed by national funds through FCT (Fundação para a Ciência e a Tecnologia) in the scope of the project GreenFuel (PTDC/EAM-AMB/30975/2017). Tiago P. Silva also acknowledges FCT for his PhD financial support (SFRH/BD/104977/2014).

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