Chlorella vulgaris cultivation in food industry wastewater for alginate beads encapsulation

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Microalgae, recognized for their microscopic size and photosynthetic capabilities, have gained increasing recognition across various sectors, including cosmeceutical and pharmaceutical products and food supplements (Markou et al., 2018). With their capacity to produce an extensive range of valuable biomolecules, such as lipids, proteins, polysaccharides, carbohydrates, amino acids, and pigments, they offer a multifaceted solution to various industries (Mat Aron et al., 2021). Notably, pigments like chlorophylls and carotenoids, vital for photosynthesis, renowned for their vibrant colors and rich in antioxidant properties, have sparked interest in functional food and cosmetic formulations (Aziz et al., 2020; Sun et al., 2022). Yet, the extraction of these pigments presents a challenge due to their susceptibility to degradation. Encapsulating them in materials like alginate beads can mitigate this issue (Gajic et al., 2021). Furthermore, microalgae can play a crucial role in the circular bioeconomy by efficiently treating industrial wastewater, including that from the food industry. These wastewater sources serve as sustainable water and nutrient inputs for microalgae cultivation, resulting in the simultaneous nutrient removal and biomolecule production, thus fostering a sustainable approach to wastewater treatment and resource utilization (Melo et al., 2022).

The objective of the present study was to cultivate the microalgae *Chlorella vulgaris* in a mixture of food industry wastewaters comprising brewery wastewater, cheese-whey and expired orange juice along with water for dilution. The goal was to produce high-value compounds enriched in alginate beads. A solvent screening strategy was crucial in determining the most effective extraction technique for efficiently extracting the bioactive compounds for the encapsulation process.

Chlorella vulgaris was first cultivated in 5 L photobioreactor at a batch mode under a mixture of the food industry wastewaters whose optimal composition was determined in a previous study (study is under publication). The evaluation of microalgal growth consisted of ongoing measurement of optical density (OD) via absorbance readings at 680 nm obtained with a microplate reader, along with daily assessments of dry weight. In addition to monitoring growth, the study also evaluated the ability of the microalgae to bioremediate the wastewater mixture by consuming both inorganic and organic pollutants. At the end of the cultivation, the microalgae were harvested and underwent lyophilization. The microalgal biomass was characterized by quantifying primary metabolites, including total carbohydrates, proteins, and lipids.

After lyophilization, the process of identification, quantification, and extraction of the bioactive molecules was conducted. A different selection of solvents was employed to maximize the overall yield of the process. Prior to extraction, the microalgae were subjected to disruption with vigorous usage of mortar and pestle. Four organic solvents, namely, ethanol (EtOH), acetone, ethyl acetate, and hexane, which are approved for use in the food industry, were examined to assess their effectiveness in extracting high-value compounds produced by *Chlorella vulgaris* cultivated in the optimal wastewater mixture. The chemical characterization profile of the extracts from each trial was analyzed using Ultra High-Performance Liquid Chromatography (UHPLC), focusing on three identified compounds: lutein, chlorophyll a and chlorophyll b.

As it can be seen in figure 1, the results of the solvent screening indicate ethanol as the most effective for pigment extraction. For the preparation of the alginate beads, extraction was performed with ethanol from 10 g of lyophilized microalgae. The final extract obtained from upscaling the extraction process with 10 g of lyophilized microalgae was identified and quantified using UHPLC. Subsequently, it was dissolved in sunflower oil, resulting in 197 μg of lutein per mL of oil. This enriched sunflower oil, underwent encapsulation through ionotropic gelation with alginates as the carrier, enabling its application as a food supplement, with an encapsulation yield of 93.3%. Lutein, the primary compound found in the microalgae biomass, demonstrated a satisfactory encapsulation efficiency of 55%. The adequacy of encapsulating the microalgae biomass in alginate beads was also confirmed by observing changes in the colorimetric properties of these beads, distinguishing them from those containing only pure sunflower oil or being empty.

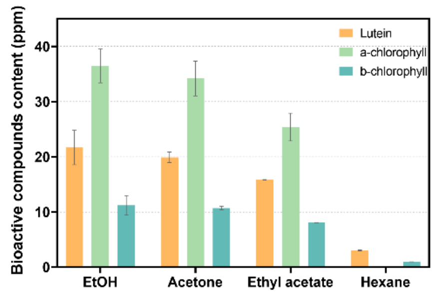

Figure 1. Assessment of different solvents for extracting bioactive compounds from *Chlorella vulgaris*.

Overall, the findings demonstrate the significant role of microalgae in converting food industry wastewater into valuable products through extract encapsulation in alginate beads. This aligns with the principles of a sustainable, circular bioeconomy, emphasizing the management of resources and the utilization of food industry by-products for the creation of marketable products.

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