



Valorisation of microalga *Tetrademus obliquus* grown in brewery wastewater using subcritical water extraction towards zero waste

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ABSTRACT

In this study, green technology was applied for extraction of compounds from wastewater-grown microalga biomass with the final goal of obtaining microbiologically safe products within a sustainable biorefinery process with zero waste. *Tetrademus obliquus* biomass resulting from brewery wastewater treatment, with (To-CO₂) and without CO₂ supplementation (To), was submitted to subcritical water extraction (SWE) at temperatures 120–220 °C for 10 min. The impact of the different SWE conditions in the obtained liquid extracts and solid residues were investigated for metal content and chemical and microbiological profiles. Gas chromatography–mass spectrometry analysis indicated that of *T. obliquus* extracts and residues are valuable sources of aliphatic saturated, unsaturated, and alkylated (mostly methylated) hydrocarbons, phenols, esters, and ketones. Polyphenolic content and antioxidant activity were enhanced approximately 4 times by increasing the temperature from 120 to 220 °C. Also, the content of polyphenols doubled when *T. obliquus* was supplemented with CO₂ for all the tested temperatures (To: 0.249–1.016 mg GAE mL⁻¹; To-CO₂: 0.437 – 1.767 mg GAE mL⁻¹). The microbiological analysis determined that liquid extracts and residues represent safe sources of bioactive components that can be used in different industries. In addition, the lower content of heavy metals in residues suggests the possibility of using the solid waste as animal feed or soil conditioner in agricultural applications.

1. Introduction

Microalgae have proven to be an excellent alternative to conventional secondary and/or tertiary wastewater treatments (WWT) due to their numerous benefits, including replacement of mechanical aeration for photosynthetic O₂ for bacterial activities with a consequent reduction of greenhouse gases emissions, avoidance of toxic solid sludge formation, lower energy consumption, and overall costs. Furthermore, the biomass generated can be successfully applied into different areas considering their enriched composition and the potential presence of important bioactive components in microalgae [1].

Most microalgal bioactive metabolites are produced and entrapped within the cells, needing effective extraction technologies to release them. Commonly used extraction techniques include expellers, solid–liquid extraction, supercritical fluid extraction, and ultrasound

techniques [2–7]. One of the recent advancements in extraction is the use of subcritical water extraction (SWE) technology. This technology has been demonstrated to offer higher efficacy and selectivity, lower production costs, and shorter extraction times compared to the aforementioned methods [8,9].

In SWE, water is used as the extraction solvent and catalyst to convert biomass into value-added products. Subcritical water is found at the temperature range between 100 (boiling point) and 374 °C (critical point), during operation under pressures up to 22.1 MPa (critical point), above the vapor pressure, to preserve the water within the liquid state. Under these conditions, water has unique properties, the viscosity and density decrease, while the dielectric constant increases, therefore lowering its polarity. As a result, the hydrogen bonding structure is weakened, and extraction of non-polar components is possible. The adjustment of SWE parameters, namely temperature as the most

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