Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-024-05279-w

ORIGINAL ARTICLE

Evaluation of different fractionation methods for the simultaneous protein and carbohydrate extraction from microalgae

Pedro L. Martins[1,2] · Luís C. Duarte[1] · Helena Pereira[2] · Alberto Reis[1] · Florbela Carvalheiro[1]

Received: 5 September 2023 / Revised: 28 December 2023 / Accepted: 1 January 2024

Abstract

The production of high-value products from microalgae, one of the preferred emerging biorefineries' feedstocks, relies on the crucial step of biomass fractionation. In this work, the fractionation of *Chlorella vulgaris* and *Scenedesmus obliquus* biomass was tested for protein extraction using a wide range of physical, chemical, and enzymatic treatment combinations, including ultrasound, cell homogenizer, cellulase, and alcalase combinations in aqueous and alkali extraction conditions. The impact of these processes on biomass carbohydrates was also evaluated. Alkaline-assisted ultrasound treatments using alcalase presented the highest protein extraction yield, reaching 90 g/100 g protein on *C. vulgaris*, closely followed by the same treatment in aqueous conditions (85 g/100 g protein). The same aqueous treatment achieved the best performance on *S. obliquus*, reaching 82 g/100 g protein. All treatments on both microalgae partially solubilized the polysaccharide fraction with all alkaline treatments solubilizing over 50 g/100 g sugars for all conditions. Overall, all the treatments applied were effective methods for biomass fractionation, although they showed low selectivity regarding the individual extraction of protein or carbohydrates.

Keywords Biorefineries · Blue bioeconomy · Cell disruption · Pre-treatments · *Chlorella vulgaris* · *Scenedesmus obliquus*

1 Introduction

Microalgae are one of the most innovative and promising biomass sources that have been suggested for biorefinery applications, namely for the production of biofuels, e.g., biodiesel from microalgae oil [1]. However, microalgae potential is much wider, as they have also been reported as a viable source of bioproducts [2], namely of pigments [3–5], protein [6, 7], amino acids [6], and fatty acids [8] for food and feed applications. The microalgae sector has gained increasing relevance in the EU, as part of the European Green Deal that includes the use of microalgae as an essential facet of the Blue Bioeconomy in the European space [9].

Microalgae have a large range of potential high-value commercial applications, in sectors such as bioplastics and biomaterials [10] with nutraceutical applications, due to their high nutritional value [11]. Applications for the production of advanced biofuels have also gained increased interest, thereby granting microalgae an even more important role in climate change mitigation [12]. The first approaches to use microalgae in biorefineries were based on solvent extraction for isolation of their lipid fraction and subsequent conversion of bio-oil to biodiesel, while a protein and sugar-rich solid residue was left with little use. By itself, biofuel production from microalgae is not economically viable [13], and needs to be coupled with the production of other high-value products for the economic feasibility of the approach. Therefore, the most recent strategy is to target microalgae biomass towards the production of added-value products such as platform chemicals, biomaterials, and food and feed products, which may also be connected to biofuel production.

Microalgae have an incredible ability to accumulate different types of macromolecules, depending on their growth conditions, thereby presenting a versatility in composition that is virtually unparalleled elsewhere in the biosphere. Due to this versatility, growth conditions are very important as,

✉ Florbela Carvalheiro
florbela.carvalheiro@lneg.pt

1 Unidade de Bioenergia e Biorrefinarias, LNEG - Laboratório Nacional de Energia e Geologia, Estrada do Paço do Lumiar, 22, 1649-038 Lisbon, Portugal

2 Centro de Estudos Florestais, Laboratório Associado TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, 1349-017 Lisbon, Portugal