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Downstream Processing of *Isochrysis galbana* using Wet Biomass

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ABSTRACT

In the present work, wet *Isochrysis galbana* biomass has been processed directly after harvesting to obtain high added-value compounds. A three-step sequential process has been designed to extract soluble proteins, lipids and pigments, leaving an exhausted residue. With this process, based on the use of compressed fluids, we were able to lower the energetic requirements, obtain higher extraction efficiency and lower generation of residues (while using environmentally benign solvents), compared to a similar procedure developed using dry microalgae biomass.

The three-step sequential process started using subcritical water (employing mainly the residual water contained in the wet biomass) at 10-100 bar and 30-50 ºC to recover soluble proteins; the second step consisted on a pigments extraction using carbon dioxide expanded ethanol (CXE) (pressures between 50-100 bar, 40-60 ºC and ethanol percentages 40-80%); later on, a supercritical fluid extraction using pure CO_2 is used to recover lipids (conditions between 250-400 bar and 40-70 ºC). By using this integrated process, we were able to recover around 70-80% of valuable lipids and pigments while proteins and sugars were mainly left in the residue. Life Cycle Assessment (LCA) has been measured and compared with results previously obtained in our research group working with *Isochrysis galbana* after freeze drying of the biomass. Results obtained demonstrated that energy requirements and associated costs of the developed process were much lower, therefore increasing the possibilities for biorefinery development at large scale.

INTRODUCTION

A biorefinery process involves biomass conversion into fuel, power, and added-value chemicals, being microalgae suggested as a promising and sustainable feedstock for various food and non-food products [1]. One of the most important challenges in microalgae biorefinery is the improvement of processes that allow the complete recovery of high added-value products from algal biomass (proteins, carbohydrates, lipids, pigments, etc.). Although important advancements have been done in this field, most of

the processes start with the drying of the biomass after collection by using different drying procedures that require high energetic and operational costs.

Among the technologies available for biorefining, the use of compressed green solvents such as supercritical fluid extraction (SFE) with CO_2, carbon dioxide expanded ethanol (CXE) or subcritical water extraction (SWE) offer several advantages over conventional solvent extraction. These processes are faster and present higher extraction efficiency, since working a high pressure and temperature solvent diffusivity is increased at the same time that viscosity is reduced [2].

Taking advantage of these technologies, a downstream platform for the fractionation of *I. galbana* has been proposed recently, using dry algae biomass [3]. The aim of the present work is to go a step further by developing a more sustainable process starting from wet biomass. This new process involves the following sequence: SWE > CXE > SFE [4]. Life Cycle Assessment (LCA) has been measured and compared to results obtained for the process starting from dry biomass.

MATERIALS AND METHODS

Samples

Frozen samples of *I. galbana* (T-ISO) were obtained from Fitoplancton Marino S.L. (Cadiz, Spain), and stored at -20 ºC under dark conditions until further use.

Compressed fluids processing platform

Extractions were carried out in a "Spe-ed Helix" supercritical fluid extractor from Applied Separations (Allentown, PA, USA). This versatile equipment can be used to perform SFE (with or without a co-solvent) and PLE. Extractions were performed in three sequential steps using (i) pure water (PLE), (ii) $ScCO_2$/ ethanol (CXE) and (iii) supercritical CO_2 ($ScCO_2$) as solvents, respectively, in order to exhaust the microalgae biomass of extractable compounds, fractionating its components in order to give valuable isolated fractions.

Chemical analysis

Methodologies employed for analyzing the different fractions obtained have been described elsewhere [3]. Total carotenoides and chlorophylls, were determined by spectrophotometry using analytical standards, and were expressed as mg g^{-1} extract. Antioxidant capacity (TEAC) was determined by the inhibition of ABTS radical, and was expressed as mmol trolox equivalents g^{-1} extract. Total lipids were estimated by gravimetry, and the result was expressed as percentage (%w/w). Total protein content, expressed as percentage (%w/w), was calculated from total nitrogen obtained by Dumas method, using a N-to-protein conversion factor of 4.68. Neutral sugar composition was performed by hydrolysis and chromatographic analysis of the monomers; the total content was expressed as percentage (%w/w).
Recoveries were determined by measuring the initial content in the microalgae biomass and were expressed as percentage (%w/w).

Life Cycle Analysis (LCA)

Life Cycle Analysis calculations were done using SimaPro software PRé 8.2 (PRé Consultants, Amersfoort, The Netherlands, 2010. Available at: www.pre.nl.). The functional unit (FU) to which the environmental impact categories are normalized is here

defined as 100 g of equivalent dry biomass. The calculation method followed was ReCiPe endpoint H (v1.12 http://www.lcia-recipe.net/).

RESULTS

Downstream processing platform

Extractions were performed in three sequential steps using (i) pure water (PLE), (ii) $ScCO_2$/ ethanol (CXE) and (iii) supercritical CO_2 ($ScCO_2$) as solvents, respectively. The aim of the work was to invert the order of the steps composing the sequential process developed for *I. galbana* [3], in order to obtain fractions of similar composition, but using wet biomass, and thus making the process energetically more efficient.

Chemical characterization of extracts

The main pigment found in the extracts was the xanthophyll fucoxanthin (see **Figure 1**), which was quantified in the three extracts of the process by HPLC. This pigment is very appreciated for its biological activities.

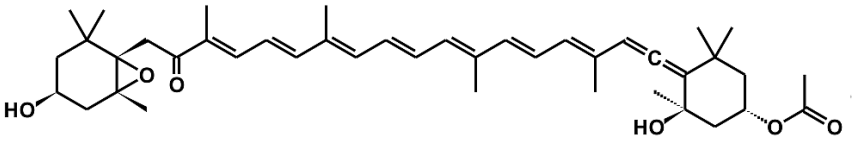

Figure 1. Structure of fucoxanthin ($C_{42}H_{58}O_6$)

Lipid fractionation is similar to that obtained in the process starting from dry biomass [3], so that non polar triacylglycerols are preferentially extracted by SFE while polar lipids are mainly extracted by CXE.

Finally, a quantitative evaluation on the recoveries of added value compounds achieved using one or the other approach was also performed. Recoveries were defined as the percentage of compound extracted from the initial biomass (dry weight) and those obtained from wet biomass downstream processing are detailed in **Figure 2.**

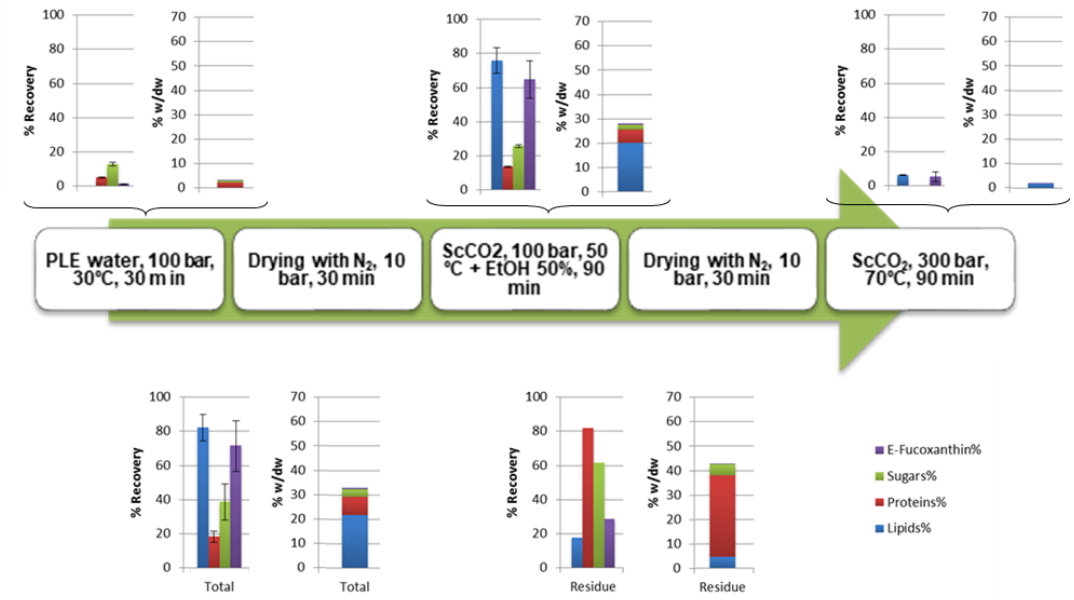

Figure 2. Percentage recovery and composition (% weight extract/dry weight algae) obtained considering the 3 step sequential process for *I. galbana* wet biomass (reverse process).

Recoveries obtained from wet biomass were superior to those obtained from dry biomass. It is remarkable the recovery of around 80% of lipids and 60-80% of fucoxanthin present in the initial biomass when using the process developed for wet biomass. Recoveries of proteins and sugars were below the 50%, so they mainly remain in the residue.

LCA

The environmental impacts of the proposed process [4] were compared to a previously published biorefinery process, but starting from dry biomass [3]. **Figure 3** depicts the flow chart of the compared processes.

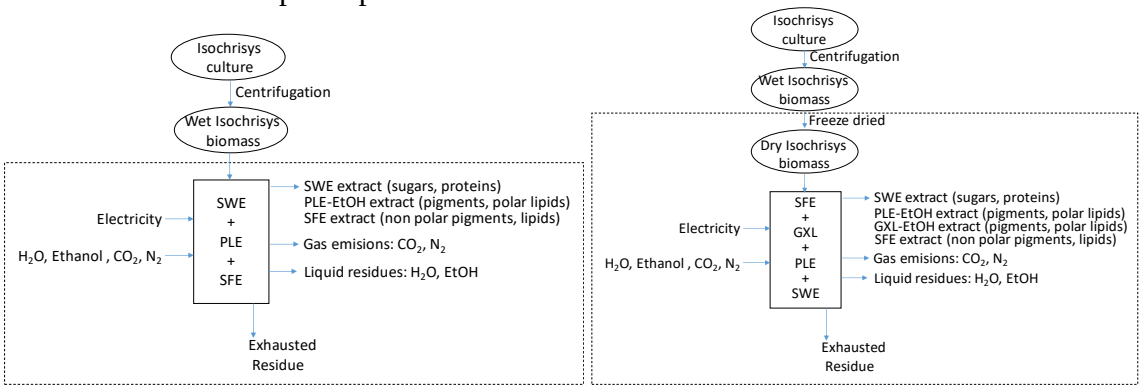

Figure 3. Flow chart of biorefinery processes used for comparison. System boundaries for LCA limited by dotted lines.

Basically in both processes the same kind of extract can be obtained, but in different order. Considering the environmental impact indicators studied, it is clear that the process that need the freeze drying step has greater environmental impacts, mainly because of energy consumption of this operation.

CONCLUSION

A downstream processing platform is described for the first time to extract bioactive compounds from the microalga *I. galbana* using wet biomass and pressurized green solvents. Extractions were performed in four sequential steps using (1) SWE, (2) CO_2 expanded ethanol (CXE), and (3) $ScCO_2$, considering as raw material of extraction the residue of the previous extraction step. The extracts were chemically characterized by HPLC analysis with different detectors (DAD, ELSD, MS). The chemical composition of the extracts obtained from wet biomass does not differ from the composition of the extracts obtained from dry biomass. The main pigment found in the extracts was the xanthophyll fucoxanthin, which was quantified in the three extracts of the process. Lipids were selectively extracted according to their polarity, since polar lipids were found in CXE extracts, while triacylglycerols were extracted only by $ScCO_2$. Some valuable sugars and proteins are obtained in the SWE extract although most of them remain in the residue and therefore, can be submitted to other sequential processes for their fractionation.

LCA results demonstrated that this process is more sustainable than the downstream process involving a freeze drying step of the biomass.

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