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Assessing microalgae biorefinery routes for the production of biofuels via hydrothermal liquefaction

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HIGHLIGHTS

- HTL of two algae species was studied after extracting high value products.
- Extracting lipids has no beneficial effects in the biofuel quality and quantity.
- Extracting proteins increases the biofuel yields, reducing its nitrogen content.
- A high recovery of nutrients can be achieved in the aqueous by-product.

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ABSTRACT

The interest in third generation biofuels from microalgae has been rising during the past years. Meanwhile, it seems not economically feasible to grow algae just for biofuels. Co-products with a higher value should be produced by extracting a particular algae fraction to improve the economics of an algae biorefinery. The present study aims at analyzing the influence of two main microalgae components (lipids and proteins) on the composition and quantity of biocrude oil obtained via hydrothermal liquefaction of two strains (*Nannochloropsis gaditana* and *Scenedesmus almeriensis*). The algae were liquefied as raw biomass, after extracting lipids and after extracting proteins in microautoclave experiments at different temperatures (300–375 °C) for 5 and 15 min. The results indicate that extracting the proteins from the microalgae prior to HTL may be interesting to improve the economics of the process while at the same time reducing the nitrogen content of the biocrude oil.

1. Introduction

The interest in microalgae as a source of third generation biofuels has increased during the last few years, due to the concerns related to the climate change and an increasing world population with higher energy demands. The advantages and disadvantages of microalgae have been highlighted in several papers (Patil et al., 2008; Malcata, 2011; Khoo et al., 2013; Liu et al., 2013), and the different techniques to convert them to biofuels widely reviewed (Brennan and Owende, 2010).

In this context, hydrothermal liquefaction (HTL) has recently gained momentum as conversion technique, with a growing number of publications (Zou et al., 2010; Yu et al., 2011; García Alba et al., 2012; Valdez et al., 2012). If an algae-based liquid fuel is pursued, HTL appears to be the best technology to achieve this, as it avoids the high energy costs of drying the algae, while converting all the algae fractions into biofuel by using the water naturally accompanying the feedstock. HTL benefits from the special properties of hot compressed water at near critical conditions (Peterson et al., 2008) that enhance the conversion of biomass to biofuel.

Although much has been discussed about the optimal configuration for an HTL-based algal biorefinery, it is still not clear how it should look like. There are various approaches which can be divided into two main pathways: either directly using the whole microalgae biomass for HTL; or previously extracting valuable compounds from the algae cells and then converting the residual biomass into biofuel. A consensus seems to have been established

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in support of the second pathway. Through this second approach it is possible to widen the portfolio of products that can be obtained in an algae biorefinery and improve its economics. Vanthoor-Koopmans et al. (2013) and Draaisma et al. (2013) have recently reviewed the possibilities of coupling the production of algae biofuels with the extraction of higher value compounds for food, feed and chemicals, and explored the market opportunities of these algae co-products.

The extraction of lipids from algae has been extensively studied, due to the interest in obtaining algae biodiesel (Mata et al., 2010). Algal lipids can also be used for the production of omega 3 fatty acids for food (such as eicosapentanoic acid and docosahexaenoic acid) or building blocks for chemicals (Vanthoor-Koopmans et al., 2013). Some researchers have already investigated the performance under hydrothermal conditions of lipid-extracted algae (Vardon et al., 2012; Zhu et al., 2013).

There is also some work available with regard to microalgal proteins. Huo et al. (2011) investigated the production of alcohol by using metabolic engineering of *Escherichia coli* to convert proteins into C_4 and C_5 alcohols. García-Moscoso et al. (2013) investigated the flash hydrolysis of *Scenedesmus* sp. to extract proteins and produce under mild hydrothermal conditions a biofuel intermediate with low nitrogen content (the presence of nitrogen in the biofuel is undesired as it would lead to NO_x emissions). Proteins can be used for the production of fertilizers and antioxidants, and also for human and animal nutrition (Romero García et al., 2012; Vanthoor-Koopmans et al., 2013; Draaisma et al., 2013). Moreover, the use of amino acid based fertilizers has been indicated as beneficial for metabolic energy savings, since the nitrogen from amino acids does not need to be reduced in order to be uptaken by the biomass like in the case of nitrates (Huo et al., 2011; Romero García et al., 2012).

Although the extraction of valuable co-products would positively impact the economics of the process, it is not clear what effect does the extraction of a certain fraction of the algae have on the biofuel yield and quality.

Previous work carried in our group on a set of eight strains (López Barreiro et al., 2013) showed that via HTL the marine species *Nannochloropsis gaditana* and the freshwater species *Scenedesmus almeriensis* produced biocrude oil with a low amount of nitrogen. These two strains were selected for this study. A comparison has been made while considering the hydrothermal conversion of three different algae materials: in their raw state, after extracting lipids and after extracting proteins. Temperatures from 300 to 375 °C and reaction times of 5 and 15 min were tested in microautoclave experiments, with the objective of investigating the role of the presence/absence of lipids or proteins in the yield and quality of the biocrude oil.

Currently many studies are being carried out analyzing the potential of HTL for several algae strains. But to the best of our knowledge, this paper is the first one reporting a systematic comparative study of the influence on the yields of HTL and the quality of the biocrude oil of the extraction of two distinct algae fractions (lipids and proteins) to obtain valuable co-products from marine and freshwater strains.

2. Methods

2.1. Chemicals

All solvents and chemicals used in this study were obtained from Sigma–Aldrich and Merck (purities ⩾98%) and used without any further purification.

2.2. Raw microalgae (RA)

N. gaditana (CCAP 849/5) and *S. almeriensis* (CCAP 276/24) were obtained in a dry state from the cultivation facility in Las Palmerillas (University of Almería, Spain). They will be called NG and SA respectively from now onwards. The lipid content was determined gravimetrically by the Bligh & Dyer method (Bligh and Dyer, 1959), and the protein content was determined following González López et al. (2010).

2.3. Lipid-extracted algae (LEA)

The lipid-extracted algae were obtained by subjecting 5 g of the dry raw algae to a Soxhlet extraction with n-hexane (VWR®, >99% purity) for 5 h, according to the DIN EN ISO 734–1 Norm (2006). The extraction was done in triplicate to check the reproducibility of the data.

2.4. Protein-extracted algae (PEA)

The protein-extracted algae were obtained by subjecting approximately 200 g of the dry raw algae to the procedure developed by Romero García et al. (2012). This method consists of an enzymatic hydrolysis that yields amino acid concentrates. The algae cells were first disrupted via high-pressure homogenization, and then subjected to enzymatic hydrolysis by using the endoprotease Alcalase 2.5 L and the exoprotease Flavourzyme 1000 L to obtain the amino acids. Viscozyme L was added to the medium as well to reduce the viscosity of the mixture in order to enhance the mass transfer and thus improve the yield of the enzymatic hydrolysis.

The success in the production of amino acid concentrates is measured by the degree of hydrolysis, which represents the amount of free amino acids hydrolyzed, compared to the total amount of proteins available for hydrolysis. For further details about the extraction method, the reader is referred to Romero García et al. (2012).

2.5. Algae paste characterization

The dry weight of the algae feedstock was determined by subjecting the sample to 105 °C overnight, and the ash content was obtained at 550 °C for 5 h. The elemental composition (in weight percentage) was measured by a CHNS Analyzer Flash 2000 (Thermo Scientific) and used in Boie's formula (Annamalai et al., 1987) (Eq. (1)) to calculate the Higher Heating Value (HHV) of the biocrude oil.

$$\mathrm{HHV_{Boie}}\ (\mathrm{MJ\ kg^{-1}}) = 0.3516 \cdot \mathrm{C} + 1.16225 \cdot \mathrm{H} - 0.1109 \cdot \mathrm{O} + 0.0628 \cdot \mathrm{N} \quad (1)$$

The composition of the inorganic matter was investigated by means of inductively coupled plasma optical emission spectroscopy (ICP-OES) on an Agilent 7025 instrument.

The salts from the cultivation medium need to be removed from the algae pellet before determining its organic content to avoid overestimations caused due to the presence of hydrated forms of these salts in the dry mass of the algae pastes (Zhu and Lee, 1997). The organic content was determined in a different way for each type of feedstock: for RA an algae pellet was centrifuged two times (10,000 rpm, 10 min) with 40 mL of de-ionized water, so that the salts in the slurry could dissolve and be removed with the supernatant after centrifugation. The remaining pellet was then dried overnight at 105 °C to determine the bio-dry weight (free of salts). The ash content of the pellet was further analyzed by subjecting the sample to 550 °C for 5 h in a muffle furnace.