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Influence of strain-specific parameters on hydrothermal liquefaction of microalgae

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HIGHLIGHTS

- Eight different algae species were subjected to hydrothermal liquefaction.
- The influence of strain-specific parameters on the HTL process were investigated.
- The inorganic material in the feedstock directly affects the HTL process.
- Strain-specific parameters play less of a role at high hydrothermal temperatures.

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ABSTRACT

Algae are an interesting feedstock for producing biofuel via hydrothermal liquefaction (HTL), due to their high water content. In this study, algae slurries (5–7 wt% daf) from different species were liquefied at 250 and 375 °C in batch autoclaves during 5 min. The aim was to analyze the influence of strain-specific parameters (cell structure, biochemical composition and growth environment) on the HTL process. Results show big variations in the biocrude oil yield within species at 250 °C (from 17.6 to 44.8 wt%). At 375 °C, these differences become less significant (from 45.6 to 58.1 wt%). An appropriate characterization of feedstock appeared to be critical to interpret the results. If a high conversion of microalgae-to-biocrude is pursued, near critical conditions are required, with *Scenedesmus almeriensis* (freshwater) and *Nannochloropsis gaditana* (marine) leading to the biocrude oils with lower nitrogen content from each growth environment.

1. Introduction

The concerns about the climate change and an increasing energy demand, together with a growing world population and the development of new economies demand new, cleaner energy sources. In this context, biomass is expected to play an important role, as it represents the single renewable energy source that contains carbon, making it suitable for the production of biofuels and chemicals within a zero net CO_2 emission technology.

Amongst the various types of biomass, microalgae appear as an interesting biomass feedstock for energy production. They have relatively simple growth requirements (water, light, a carbon source and nutrients) and higher photosynthetic efficiency, faster growth rate and higher area-specific yields than terrestrial biomass (Patil et al., 2008).

Microalgal research is focused mainly on optimization of growing systems, with less effort spent to their downstream processing. The production of microalgae for biofuel in these systems is far from being economically competitive with fossil fuels. They require still a high degree of optimization in aspects like light requirements and harvesting methods. Moreover, water requirements or nutrient recovery within the HTL process are critical aspects that still need to be properly addressed to analyze its sustainability.

With regard to the energy use of microalgae, the main focus has been subjecting lipid-rich strains to solvent extraction plus subsequent transesterification, in order to produce biodiesel (Chisti, 2007). This process has some drawbacks: usually lipid-rich strains are slow-growing organisms; and when converting only the lipids, a large amount of the biomass remains unused, thus generating an

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important quantity of residual algal mass. Hydrothermal liquefaction is an interesting alternative to process the whole microalgae biomass (not only the lipids) in a wet state to produce a liquid viscous energy carrier, similar in nature to crude petroleum. Thus, the potential of producing large amounts of biocrude is higher for this technology (López Barreiro et al., 2013). It also benefits from the high water content of microalgae, thus avoiding the energy costs of drying them.

Recent studies on microalgal HTL have investigated several parameters of the process, such as the type of strain, temperature, reaction time, microalgae concentration in the feed, application of catalysts, or further upgrading of the produced biocrude oil (Biller and Ross, 2011; Biller et al., 2011; Brown et al., 2010; Duan and Savage, 2011; Duan et al., 2013; Elliott et al., 2011, 2012; García Alba et al., 2012; Torri et al., 2012; Zou et al., 2010a,b). Batch reactors have been commonly used, with temperatures varying from 200 to 500 °C and reaction times of 60 min. However, some recent studies are shifting towards the use of reduced reaction times of around 5 min (García Alba et al., 2012).

This work is the most extensive study available to date in terms of number of strains tested for HTL. The aim of this research was to study the influence in the process of strain-specific parameters, such as biochemical composition, cell structure and growth environment, in view of proposing guidelines for the strain selection for an algae biorefinery.

In the algae biorefinery concept that we envisage, the cultivation under axenic conditions of large amounts of microalgal biomass appears unlikely, due to economic constraints. Most of the studies about microalgae HTL have used experimental conditions that will never be achieved at large industrial scales (i.e., use pulverized algae cells mixed with de-ionized water (Zou et al. 2010a,b)). The strains that have been tested in this study were cultured under non-axenic conditions and subjected to HTL together with the water, salts and nutrients present in the culture medium. By means of this, some practical issues were faced (i.e., interferences of salts in the mass balances) that were not reported in other studies related to this topic, although they may be critical for a practical implementation of this technology.

2. Methods

2.1. Microalgae strains

Eight algae strains were used in this study. The freshwater species selected were *Scenedesmus obliquus* (UTEX 2630), *Scenedesmus almeriensis* (CCAP 276/24), and *Chlorella vulgaris* (SAG 211-11b). The marine strains were *Phaeodactylum tricornutum* (CCAP1055/1), *Tetraselmis suecica* (CCAP 66/4), *Nannochloropsis gaditana* (Lubián CCMP 527), *Porphyridium purpureum* (SAG 113.79) and *Dunaliella tertiolecta* (SAG 13.86).

These species covered a wide range of strain-specific parameters: marine and freshwater environments were tested; the biochemical and elemental composition was notably different amongst the different strains, as well as the type of cell wall (Table 1).

Table 1
Feedstock biochemical and elemental composition (wt%, dry basis).

Feedstock	Proteins	Lipids	Ash	N	C	H	S
Scenedesmus obliquus	28.0	16.8	28.3	5.8	44.4	5.4	0.3
Phaeodactylum tricornutum	37.5	21.9	24.6	5.2	38.0	4.8	0.7
Nannochloropsis gaditana	43.9	25.1	11.8	6.9	51.0	6.6	0.4
Scenedesmus almeriensis	51.7	21.8	12.0	6.8	50.6	6.4	0.4
Tetraselmis suecica	43.6	19.5	15.9	6.3	45.0	5.9	1.1
Chlorella vulgaris	41.2	20.4	22.3	6.2	42.3	5.1	0.4
Porphyridium purpureum	45.6	12.1	9.8	6.0	45.6	6.1	1.1
Dunaliella tertiolecta	50.8	23.4	6.4	8.6	51.9	7.5	0.5

2.2. Photobioreactors (PBR) and culture conditions of microalgae strains

Strains were cultured in bubble column PBRs made of transparent acrylic tube, with a diameter of 0.2 m, a height of 1 m and a working volume of 25 L. The PBRs were operated under non-axenic conditions in a greenhouse at environmental temperature with a light intensity of approximately 250 μmol m^{-2} s^{-1} at the surface of the PBR. Light was continuously provided by metal halide lamps (Philips HPI-T). The pH was not controlled, and bubbling air was continuously supplied through diffusers at an aeration rate of 0.5 vvm (volume gas per volume of mixed culture per minute). The absence of biological contamination in the culture was visually checked with light microscopy. Strains were supplied with $NaNO_3$–N and KH_2PO_4–P, plus the micronutrients and vitamins from a modified F/2 medium (Andersen, 2005). For marine strains, artificial sea water was used (Instant Ocean, Spectrum Brands, Atlanta, US) with a salt concentration of 25 g L^{-1}, except for *P. purpureum*, that required a concentration of 15 g L^{-1}.

2.3. Harvesting

The harvesting of fresh microalgae from the culture was done every 2–3 days using a Westfalia centrifuge (Model: OTC 3-03-107) operated at about 12,300*g* (10,000 rpm) and recovered manually from the inside vessel.

2.4. Algae paste characterization

The algae paste obtained after centrifugation was fully characterized. Its organic content was determined following the method proposed by Zhu and Lee (1997), which consists of washing the algae paste with distilled water in a filter and drying the filter cake for 24 h at 105 °C. The dry washed samples are then subjected to 550 °C for 5 h under oxidizing conditions to calculate the ash content. The washing step is related with the removal of water soluble inorganic matter present in the algae pastes, and is schematically shown in Fig. 1. The effect of this step will be discussed in following sections.

For the rest of the analyses of the algae paste, dry samples of the raw paste (without removing the inorganic matter) were used. The elemental composition (CHNS) was measured using an elemental analyzer (Thermo Scientific Flash 2000). With regard to the biochemical composition, the total lipid content was analyzed by the method described by Ryckebosch et al. (2012), extracting the lipids from microalgae lyophilized samples with a mixture of chloroform–methanol 1:1. For the protein analyses, freeze-dried samples were pre-weighted (ca 4 mg) and diluted in a lysis buffer (10 mL) during 20 min at chamber temperature, according to González López et al. (2010), to facilitate the extraction of proteins. The BCA Protein Assay Reagent Kit (Pierce®) was used according to the manufacturer's recommendations. For the lipid and protein analyses, each sample was tested in duplicate and the results were averaged.

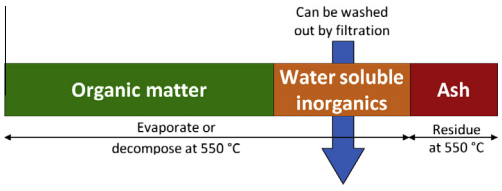

Fig. 1. Schematic representation of the dry algae paste composition pointing at the effect of washing it in a filter before determining its organic content.