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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.

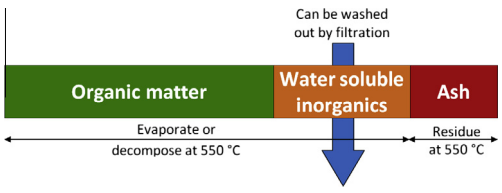

Fig. 1. Schematic representation of the dry algae paste composition pointing at the effect of washing it in a filter before determining its organic content.

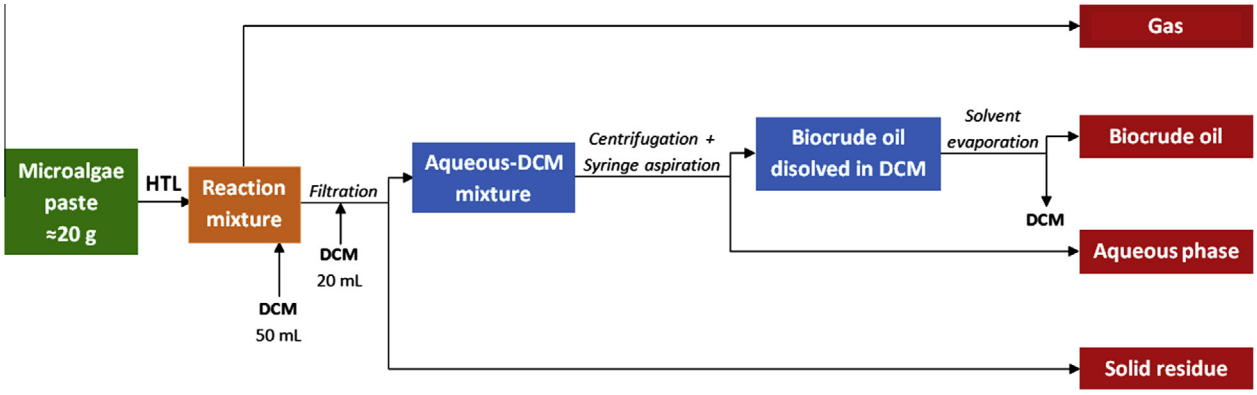

Fig. 2. Scheme of the experimental procedure including the product separation.

2.5. Hydrothermal liquefaction (HTL) and product separation

Algae slurries containing 5–7 wt% of organics were used. The concentration of the pastes obtained after centrifugation was adjusted by adding de-ionized water to bring them to a similar concentration. Two temperatures (250 and 375 °C) were tested for each species, with reaction times of 5 min. An average of three HTL experiments were performed for each species at 250 °C, while at 375 °C the experiments were repeated five times on average. Only liquefaction of *D. tertiolecta* at 250 °C could be performed just once, due to a lack of material.

A scheme of the HTL experimental procedure and the product separation is presented in Fig. 2. The experiments were carried out in a 43 mL (internal volume) cylindrical stainless steel autoclave, with top and bottom covers. Typically, 20 g of algae slurry (1.0–1.4 g of algae organics, daf) were loaded into the reactor. A leakage test was done prior to each experiment with 80 bar of He, which also helped to flush the air initially present inside the autoclave. Following this, the reactor was loaded with 5 bar of He to facilitate the product gas collection after the experiment, especially for the experiments at 250 °C, in which not much gas was produced.

The autoclave was heated during the experiment by immersion in a hot fluidized bed of sand that used preheated air as fluidizing agent. The inner temperature in the autoclave was measured by a thermocouple. The heating time to the desired temperature appeared to vary within species, being 3–4 min for experiments at 250 °C, and 5–7 min for experiments at 375 °C. Once the autoclave achieved the desired reaction temperature, it was maintained for 5 min. After completing the reaction time, the autoclave was quenched by submerging it into a water bath for 3–4 min. As the heat up and cool down periods are in the same order of magnitude as the time at temperature, these batch experiments do not allow to determine reaction kinetics.

When the inner temperature of the autoclave achieved ambient conditions, the gas pressure was recorded and a sample was taken for analysis. The autoclave was then opened and the product collected in a vessel. Part of the biocrude oil produced usually adhered to the reactor walls. To maximize the collection of product, dichloromethane (DCM) (Sigma–Aldrich, 99% purity) was used as solvent. A total of 50 mL was added to rinse the autoclave, in steps of 20, 10, 10, 5 and 5 mL, shaking it vigorously between each addition.

The bi-phase mixture obtained (water on the top, biocrude oil dissolved in dichloromethane on the bottom) contained solids (unconverted cells and/or char-like material, depending on the process conditions), which were recovered by vacuum filtration (glass microfiber filter, Whatman GF/B, 1 μm pore size). This residue was additionally washed with 20 mL of dichloromethane to ensure that the filter cake only composed of insoluble solids.

The filtrate was then centrifuged at 7232*g* (Eppendorf 5804, 15 min, 7500 rpm) to maximize the separation of the dichloromethane and aqueous phases, the latter being recovered by aspiration with a syringe.

The remaining phase contained the biocrude oil dissolved in dichloromethane. The dichloromethane was evaporated and recovered with a cold trap system. It was always transparent, indicating that the loss of biocrude oil during this step was minimal. To ensure the removal of any water or dichloromethane that could still be present in the biocrude oil obtained, N_2 was flushed through the sample vessel for 24 h. This process is likely to cause the loss of some light compounds initially present in the biocrude, thus potentially reducing the yield of this phase.

2.6. Definitions and analysis of HTL products

The yields of biocrude oil, gas, organics dissolved in the aqueous phase and solids were calculated as the percentage by weight of the organic mass from each of them (m_i) and the mass of microalgae (dry, ash free) initially loaded to the reactor ($m_{microalgae}$), according to Eq. (1):

$$\text{Yield}_i(\text{wt\%, daf}) = (m_i/m_{microalgae}) \times 100 \quad (1)$$

The biocrude oil was subjected to elemental analysis to measure the C, H, N, S and O content (Thermo Scientific Flash 2000). It needs to be highlighted that the oxygen content of the biocrude was measured directly, and not by difference. The Higher Heating Value (HHV) of the biocrude oil was calculated according to Boie's formula. The formula considers the content of C, H, O and N, not accounting for S. This equation was also applied for the HHV calculation of the microalgae feedstock.

$$\text{HHV}_{Boie}(\text{MJ kg}^{-1}) = 0.3516 \cdot \text{C} + 1.16225 \cdot H - 0.1109 \cdot \text{O} + 0.0628 \cdot \text{N} \quad (2)$$

Gel Permeation Chromatography (GPC) analyses were carried out to determine the molecular weight distribution of the biocrude oils, using an Agilent 1200 series HPLC system with 3 GPC PLgel 3 μm MIXED-E columns connected in series, with a refractive index detector. The column temperature was 40 °C, and tetrahydrofuran (THF) was used as solvent, at a flow of 1 mL min^{-1}. It is important to mention that this separation mechanism is based on volume exclusion and not weight, and calibrated with polystyrene standards with a molecular weight ranging from 162 to 29,510 g mol^{-1}.

To determine the dry organic matter present in the aqueous phase, an aliquot of 2 mL was dried at 70 °C for 24 h. The ash content was determined by treating the dry residue at 550 °C for 5 h. It needs to be emphasized that, contrary to many previous studies, in this work the yield of organics dissolved in the aqueous phase was

measured gravimetrically, instead of assuming a 100% mass balance closure and calculating it by difference (which is a common practice in earlier studies). This allowed checking the mass balance closure accurately.

For the gas phase analyses, a gas sample was taken after each experiment, and analyzed using a gas chromatograph (Varian Micro GC CP-4900), containing two analytical columns: Molsieve 5A (10 m) and PPQ (10 m). The device used helium as carrier gas. The total mass of gas produced was calculated considering the final pressure of the gas after the HTL experiment and its composition, by applying the ideal gas law. The mass obtained was used in Eq. (1) to calculate the gas yield.

The filtrated solids were quantified by drying them at 105 °C for 24 h to remove any residual water and dichloromethane after the filtration. Their ash content was determined by treating them at 550 °C for 5 h.

Additionally, samples of the algae feedstock and the solid residue remaining after HTL were analyzed with a field emission scanning electron microscope (FE-SEM, type DSM 982 Gemini, Carl Zeiss Ltd). The FE-SEM was equipped also with an energy dispersive X-ray unit (EDX) to analyze the chemical elements present in the sample.

3. Results and discussion

3.1. Feedstock characterization

The ultimate analyses for the various microalgae pastes, as well as their biochemical composition, are shown in Table 1. The variation between strains indicates that they were adequately selected to assess the influence of the biochemical composition in the biocrude oil formation.

As previously mentioned in Section 2.4, the algae pastes needed to be washed with distilled water to remove the water soluble inorganic matter present in the algae pastes, in order to accurately determine their organic content. This inorganic matter is mainly constituted by water soluble salts (Zhu and Lee, 1997) that accumulate in the algae paste during the cultivation and harvesting steps. These salts stay in the dry residue of the algae paste at 105 °C, but decompose at 550 °C during the ash determination. This decomposition might be related to its thermal degradation, or to the presence of hydrated forms of the salts that are thermally stable at 105 °C, but not at 550 °C (Ramsurn and Gupta, 2012). Therefore, if this inorganic matter is not removed, it results in an overestimation of the organic content of the pastes. By previously washing a slurry sample in a filter, the salts are entrained with the filtrate, leaving in the filter just the pure algae cells, and thus circumventing disturbances during the determination of the organic content. This process might also cause the loss of some organic matter, although this effect appears to have a minimal influence in the assessment of the overall process, especially if compared with the amount of salts present in the algae paste.

The type of salts present in the culture can be very broad, especially for marine species, and their solubility in water is affected by the pH of the culture. Over the period of cultivation of the algae the pH tends to increase, thereby decreasing the solubility of salts and thus leading to precipitation. The water from the region of Ghent, used for cultivation in this work, is a hard water with a high alkalinity (Water-link, 2013), and rich in ions like Na^+, Ca^{2+}, Cl^-, NO_3^-, SO_4^{2-}. The F/2 medium used for cultivation is rich in $NaNO_3$ as well. Both circumstances would explain the accumulation of salts while growing and harvesting algae. By controlling the pH and/or using other harvesting methods (i.e., flotation), the accumulation of these salts could be avoided or, at least, significantly reduced, thus circumventing their disturbances in the subsequent hydrothermal process.

3.2. Biocrude oil

Table 2 shows the biocrude oil yields obtained for all the strains, as well as their elemental composition and the Higher Heating Value (HHV) at 250 and 375 °C. The oxygen content is rather low (especially for the biocrude oils obtained at 375 °C) when compared to the HTL oils from other types of biomass (Toor et al., 2011). Compared to the original feedstock, the nitrogen content is reduced in the oils obtained at 250 °C, but not at 375 °C, mainly due to the higher degradation of proteins at those conditions (García Alba et al., 2012; Torri et al., 2012). With respect to carbon and hydrogen, a densification takes place in the biocrude. It is also noticeable that, in contrast to the reaction time commonly applied in literature (60 min) (Biller et al., 2011; Brown et al., 2010), 5 min appear to be sufficient to obtain a high biocrude oil yield when working at 375 °C. High temperatures also seem to promote the presence of sulfur in the oil, although its content is usually below 0.5 wt% for most of the oils.

The elemental composition indicates that the biocrude oils are not yet directly applicable as transportation fuel for any of the species tested. That would require further upgrading steps to reduce the N, S and O content and hence change the biocrude chemical composition. Several research groups are actively working on oil upgrading (Yeh et al., 2013), with satisfactory results in terms of deoxygenation and denitrogenation, especially for heterogeneous catalysts, in situ during the hydrothermal conversion (Duan and Savage, 2011; Biller et al., 2011), or as a post-treatment to upgrade the biocrude oil (Duan et al., 2013; Li and Savage, 2013). Especially inter-

Table 2
Yield (wt%), elemental composition (wt%), molar ratios and HHV (MJ kg^{-1}) of the biocrude oil from different strains at 250 and 375 °C.

Strain	T (°C)	Yield	N	C	H	O	S	Others*	O/C	H/C	N/C	HHV
Scenedesmus obliquus	250	17.6	5.1	69.3	9.1	12.9	0.2	3.4	0.14	1.58	0.06	33.8
	375	50.6	6.3	73.2	8.9	8.1	0.3	3.1	0.08	1.46	0.07	35.6
Phaeodactylum tricornutum	250	40.8	4.7	62.9	8.0	12.0	0.3	12.2	0.14	1.53	0.06	30.3
	375	54.3	5.8	73.4	9.1	7.8	1.0	2.9	0.08	1.49	0.07	35.9
Nannocholoropsis gaditana	250	34.4	3.7	71.5	9.7	11.5	0.2	3.4	0.12	1.63	0.04	35.4
	375	54.3	5.2	74.7	9.9	8.5	0.4	1.3	0.09	1.59	0.06	37.2
Scenedesmus almeriensis	250	35.7	4.1	72.6	9.4	12.5	0.3	1.2	0.13	1.55	0.05	35.3
	375	58.1	6.1	74.3	9.1	8.4	0.4	1.7	0.08	1.47	0.07	36.2
Tetraselmis suecica	250	29.4	4.8	62.6	7.4	14.0	0.4	10.9	0.17	1.42	0.07	29.3
	375	45.6	6.1	74.0	9.0	7.7	0.9	2.4	0.08	1.46	0.07	36.0
Chlorella vulgaris	250	33.0	5.5	70.6	9.2	12.3	0.4	2.2	0.13	1.56	0.07	34.4
	375	55.3	7.1	72.5	8.7	8.6	0.5	2.6	0.09	1.44	0.08	35.0
Porphyridium purpureum	250	24.7	5.0	69.1	8.4	15.2	0.5	1.7	0.16	1.46	0.06	32.7
	375	47.1	6.8	73.9	8.2	8.7	0.7	1.7	0.09	1.33	0.08	35.0
Dunaliella tertiolecta	250	44.8	5.3	71.3	9.1	12.2	0.4	1.7	0.13	1.53	0.06	34.6
	375	55.3	6.2	72.0	8.8	9.9	0.3	2.7	0.10	1.47	0.07	34.9

* By difference (100 − N − C − H − O − S).

esting are the results provided by Elliott et al. (2011) for the upgrading of algal biocrude obtained via HTL. By hydroprocessing the biocrude in a continuous-flow fixed-bed catalytic reactor with a $CoMoS/Al_2O_3F$ catalyst, they significantly reduced the concentration of O (0.85%), N (<0.05%) and S (<0.005%) in the hydrotreated biocrude.

At 250 °C, the differences between yields are quite broad from one species to another, varying from 17.6 wt% for *S. obliquus* to 44.8 wt% for *D. tertiolecta*. This is likely related to the biochemical composition of the algae, or to the cell configuration (i.e., cell wall strength). The strains leading to the highest oil yields at 250 °C are those with no or reduced cell wall (*D. tertiolecta* and *P. tricornutum* cultured in a silicon-free medium, respectively). It can be hypothesized that this lack of strong cell walls enhances the extractability of the inner compounds at these mild conditions. In contrast, *S. obliquus*, reported to have a very resistant and thick cell wall (Mussgnug et al., 2010), shows a very low oil yield at those conditions. These observations indicate that at low hydrothermal temperatures, the type of strain used affects the liquefaction process.

In contrast, at 375 °C the biocrude oil yields from all the species get closer to each other, varying from 45.6 wt% for *T. suecica* to 58.1 wt% for *S. almeriensis*. When working at 375 °C, the hydrothermal conditions appear to be so harsh that the influence of the strain used appears to have less influence in the process. Only *P. purpureum* and *T. suecica* have biocrude oil yields below 50 wt%. The reason for that behavior from *P. purpureum* could be its typical high content of carbohydrates, which has been reported to reduce the production of biocrude oil via HTL (Biller and Ross, 2011). In the case of *T. suecica*, the explanation for this reduced yield is uncertain, but it could be related to the fact that the mass balance closure is the lowest obtained for all the species (90.3 wt%, see Section 3.3), possibly indicating an overestimation of the organic content of the algae paste from that species.

The HHV of the biocrude does not vary significantly amongst the various species at 375 °C. This indicates that near critical conditions produce oil similar in energy content, independently of the strain used. In contrast, large differences can be found at 250 °C. For *P. tricornutum* and *T. suecica*, the carbon content of the biocrude oil obtained at 250 °C is quite low (below 63%), compared to the typical 69–72% for the other species. These two species also exhibit a large content of non-CHONS material. Apparently the biocrude oil still includes some inorganic material from the algae slurries. The reason why some biocrude oils produced at 250 °C have more inorganic matter than others is uncertain at this stage, but it seems in agreement with the various species showing different behavior at mild hydrothermal conditions.

3.3. Mass balances

The organic mass of the other product phases was analyzed as well, in order to close the mass balances. At 250 °C there was an excess of mass measured for all the species (more than 100% mass balance closure). This excess was identical for each species to the overestimation in its organic content when the algae cells were not washed prior to the organic content determination, and it was therefore found that the water soluble inorganic matter from the algae paste was mixed with the biocrude and aqueous phases at 250 °C, causing an effect analogous to the one reported in Section 3.1 (overestimation of organic mass). At 375 °C this effect is not observed and salts content was strongly reduced, generally leading to biocrude and aqueous products with less inorganic mass.

For the experiments at 250 °C, the challenge was to determine for each species the distribution of the water soluble inorganic matter originally present in the feedstock over the aqueous and biocrude oil phases. The presence of part of them in the biocrude oil was evident from the elemental analysis (see Table 2). The remaining part of these salts was assumed to be in the aqueous phase, and therefore subtracted from the gravimetric yield. Consistently with this assumption, detailed analyses of the aqueous phase (data not shown) revealed a strong reduction of the content of Ca and Mg in the aqueous phase by increasing the temperature of the process from 250 to 375 °C. In contrast, the content of K and Na stayed constant, regardless of the process temperature. Fig. 3a shows the presence of these salts in the aqueous phases from HTL at 250 °C after centrifugation at 10,414*g* (Eppendorf 5804, 10 min, 9000 rpm). Such a deposition cannot be found in aqueous phases from experiments carried out at 375 °C (Fig. 3b). This supports the validity of our explanation for the fate of salts. Further discussion about the behavior of inorganic compounds follows in Section 3.4.

Applying this correction resulted in a mass balance closure for all the species in the range of 90.3 to 103.6%, which was considered acceptable for the purposes of this study. The reproducibility of the data was very good (average standard deviations of the yields: ±1.3%). The mass balances are presented in Fig. 4 for 250 °C (a) and 375 °C (b). Average yields are reported for each product phase and species.

The yields of aqueous products were showing large variations at 250 °C for the different species (yields vary from 17.5 to 38.3 wt%) but not at 375 °C (from 11.3 to 18.9 wt%). It needs to be highlighted that these yields were measured after extraction the mixture water-biocrude oil with dichloromethane. A gravity separation of the products for microalgal HTL (without solvent extraction) is still to be assessed, but could hypothetically lead to higher yields of organics in the aqueous product.

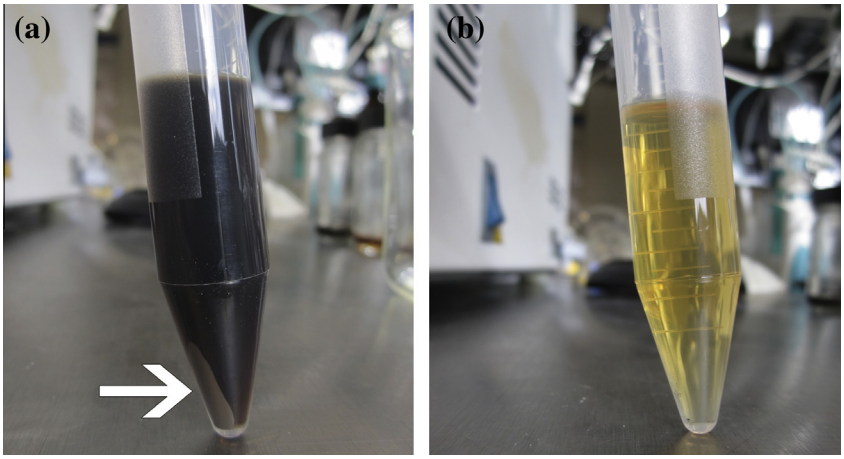

Fig. 3. Aqueous phase from experiments at 250 °C (a) and 375 °C (b) for the species *Scenedesmus obliquus*, after centrifugation at 10,414*g* for 10 min.

The solid residue obtained at 250 °C was exhibiting a dark green color, indicating that it was mainly composed of unconverted cells. In contrast, its aspect at 375 °C was similar to charcoal and the color was dark brown. This is in agreement with what has been reported elsewhere (García Alba et al., 2012), indicating that HTL at mild temperatures leads to a sort of "improved extraction" of certain algae compounds, while a harsher decomposition and thermal degradation of the algae cells occurs at 375 °C. The yields of solid residue are remarkably low at 375 °C (below 7 wt% for all the species). At 250 °C, the variation between solids yields is very wide, varying from 41.6 wt% for *S. obliquus* (with very resistant cell walls) to 17.8 wt% for *P. tricornutum* or 11.9 wt% for *D. tertiolecta*, the last two lacking a resistant cell wall.

The production of gas phase was low at 250 °C, and increased remarkably at 375 °C. It was composed at both temperatures almost entirely of CO_2, with very low amounts of CH_4 and C_2–C_3 hydrocarbons being promoted at 375 °C.

3.4. The role of water soluble inorganic matter

The behavior of salts in microalgae HTL was only briefly mentioned previously in a paper by Elliott et al. (2012), where they reported the formation of mineral precipitates from alkali metals and alkaline earths during the hydrothermal process. Other studies available in the literature about microalgae HTL overlook the influence of salts in the process, which can be attributed to the fact that most of the authors have calculated the yield of organics in the aqueous phase by difference, and not gravimetrically. This can lead to an overestimation of the yields of organics dissolved in the aqueous phase, because all the errors attributable to salts are transferred to this phase yield calculation and remain undetected. This would eventually explain why in other literature studies the yield of organics in the aqueous phase is so much higher (>60 wt%) (Biller and Ross, 2011) than the ones reported in this paper, despite using similar experimental conditions.

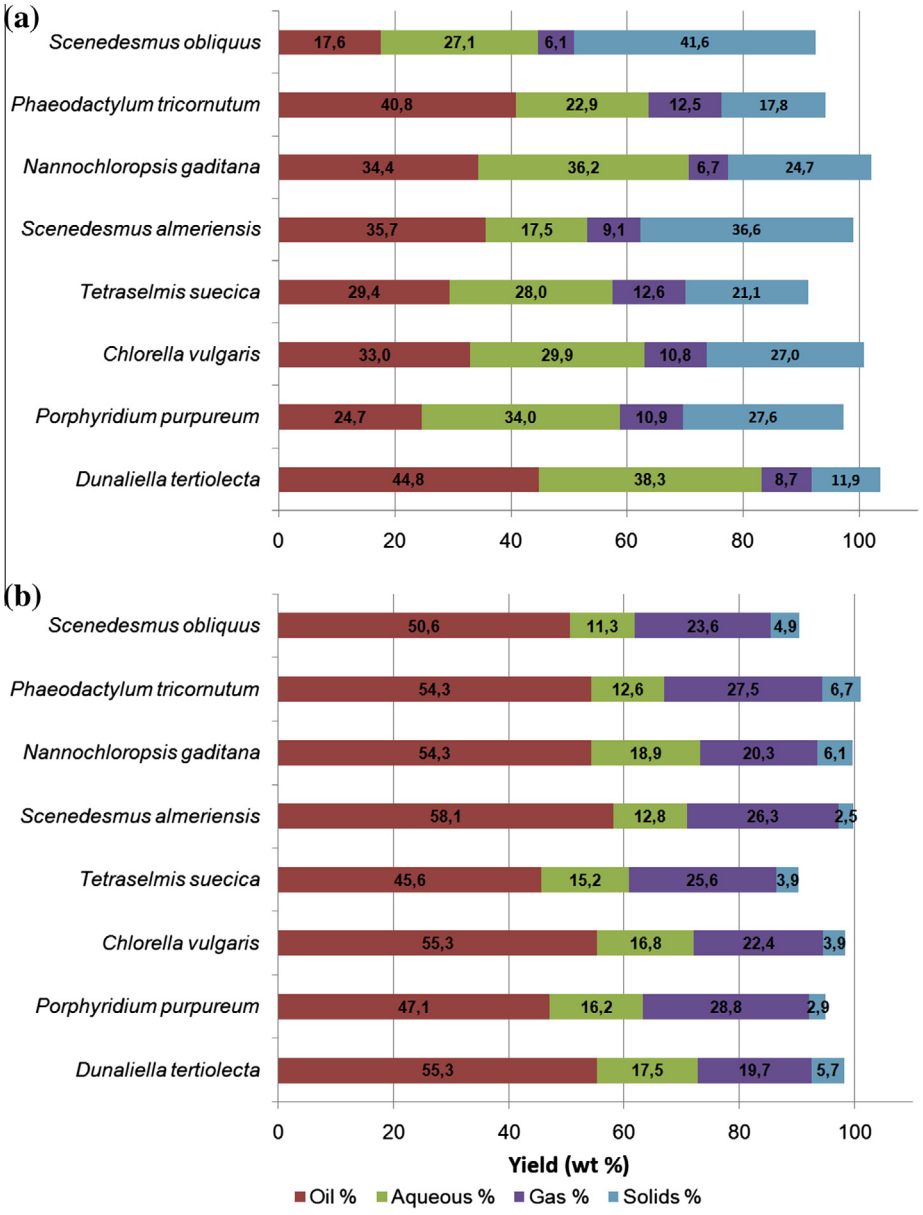

Fig. 4. Mass balances at 250 °C (a) and 375 °C (b).

The understanding of the behavior of salts under hydrothermal conditions appears to be limited due to the significant number of parameters and complex phenomena involved (i.e., multicomponent multi-phase thermodynamics, crystal growth kinetics from highly supersaturated solutions, heat and mass transfer) (Schubert, 2010; Armellini et al., 1994). The change in the water properties at near critical conditions (Peterson et al., 2008) is the cause of the changes in the state and structure of the salts accompanying the algae slurries. At these conditions, water is significantly less polar than at standard conditions, leading to a dramatic change of the dielectric constant (Leusbrock et al., 2008) and thus the solubility of inorganic ions is highly reduced (Schubert, 2010; Zhang et al., 2010). This decrease in the polarity results in a "shock-precipitation" and crystallization (Armellini et al., 1994), which could explain why the concentration of inorganic matter in the water and biocrude oil phases is higher at 250 °C (milder conditions) than at 375 °C (harsher conditions, less polar behavior of the water). These crystals seem to be thermally more stable, which is also consistent with the higher amount of ash recovered in the experiments at 375 °C, compared to those at 250 °C (despite using the same feedstock at both temperatures). In fact, when searching in the literature in view of the results obtained, it was found that hydrothermal processing has already been studied for the formation of more thermal stable crystals in the synthesis of ceramics (Byrappa and Yoshimura, 2013) or as a technique for solidification of thermal decomposable carbonate powders (Yamasaki and Weiping, 1993). Armellini et al. (1994) reported the formation of NaCl and Na_2SO_4 crystals by nucleation and agglomeration under hydrothermal conditions, leading to crystals with a particle size higher than 1 μm. Crystals with such a size would be part of the solid residue, given the separation procedure followed in our experiments.

FE-SEM analyses were carried out to assess the presence of crystals in different materials from the HTL process (see Supplementary material). No crystals were found in the samples of the dry algae cells. Even by analyzing separately the precipitated salts of the feedstock after centrifugation, the presence of crystals is not detected. This was happening as well for the solid residue after HTL experiments at 250 °C. In contrast, clearly crystals can be distinguished in the solid residue of experiments at 375 °C. The presence of crystals was also observed after subjecting the solid residue to 550 °C for 5 h, which proved their higher thermal stability. In all the cases, the main inorganic compounds detected by X-ray were Ca, P and Mg, indicating the possibility of recovering nutrients from the solid residue. This is especially interesting for P (a key nutrient in algae cultivation), as the depletion of its sources is a global concern (Cordell et al., 2009).

The information reported in this paper is new in terms of practical application of this technology for different microalgae as feedstock. The intention of this research was to test this technology in view of realistic, practical conditions for large-scale operation. Culturing algae in running water under non-axenic conditions has shown that the culture pH and strength of water directly influence the effect and appearance of salts and thus affect the overall process. The harvesting step required to concentrate the algae cultures can result in an accumulation of the salts in the algae slurries, depending on the culturing and harvesting technologies used. In order to obtain an economic and technical feasible operation, salt management becomes a critical issue, as salts can lead to precipitation, affecting the heat transfer and causing plugging of the equipment used at hydrothermal conditions. Moreover, they can enhance its corrosion. Eventually, if catalysts were used in the process, salts could also deactivate them due to precipitation, poisoning or fouling of the active sites; or they could even act as catalysts themselves. If microalgae HTL aims to take more steps towards its fully industrial development, this salts issue needs to be properly addressed and therefore research in this area should be continued.

3.5. GPC analysis

GPC analyses were carried out for the oils produced at both temperatures. Fig. 5 shows the molecular weight distribution for the oils obtained at 250 and 375 °C. The presence of three intense peaks at 250 °C in most of the species indicates that these oils have a limited range of molecules constituting the oil, and that these molecules fall into three major regions of molecular weights (400–500, 900–1000 and 1000–1100 g mol^{-1}, although these values should be taken as an approximation, rather than as absolute, as discussed in Section 2.6). In contrast, oils show a broader molecular weight distribution at 375 °C, with only a major peak in the range of 400–500 g mol^{-1}, although with a significantly lower intensity than at 250 °C. This indicates a higher degradation of the molecules from the oil at near critical conditions, with a higher presence of molecules with a lower molecular weight, regardless of the species used.

The peak at 400–500 g mol^{-1} is present for all the species tested in this study. Its intensity varies much more for the oils at 250 °C (log[M] of 2.5–5.0) than at 375 °C (log[M] of 1.0–1.5). At mild conditions, the strain used seems to have an effect in the relative abundance of the different types of molecules. High temperatures (375 °C) and pressures (250–270 bar) seem to be severe enough to degrade the microalgae molecules in such a way that they lead to similar types of oil molecular weight distributions. GPC results indicate again that harsh conditions make the process less sensitive to strain-specific parameters.

Heilmann et al. (2011) and Levine et al. (2010) reported that at mild hydrothermal conditions (200–250 °C), the lipids naturally present in microalgae undergo hydrolysis and form fatty acids that are adsorbed on the surface of the solid residue. This also agrees with the idea that HTL at mild temperatures results in an "improved" extraction, rather than a proper thermochemical conversion. This would explain why the GPC diagrams from this study show that the biocrude oil at 250 °C consists only of compounds with certain molecular masses. By adding the dichloromethane during the product separation, we could be recovering those extracted and hydrolyzed fractions, thus obtaining a biocrude with a limited type of molecules, rather than the complex mixture of organic compounds that appear to be present in the biocrude oil at 375 °C.

3.6. Suitability of the various microalgae strains for HTL

Recommending algae species for HTL appeared to be quite challenging. The results provided in this paper enable to propose some strains, although this selection is just based on gravimetrical yields and elemental composition of the product phases. It lacks a full perspective of the process, which should also consider aspects like recyclability of nutrients to the culture medium, composition of the organics dissolved in the aqueous phase or detailed characterization of the biocrude oil. These issues will be addressed in coming studies. From the results reported here, most of the species tested seem to be interesting as feedstock for microalgae HTL at 375 °C, according to the oil yield. Only *T. suecica* and *P. purpureum* lead to biocrude oil conversions lower than 50 wt%.

The reactivity of HTL is very complex, and any attempt to correlate the biochemical composition of the feedstock to the biocrude oil conversion was in vain. According to the results obtained, it seems that a gross composition of the algae (lipids, proteins and carbohydrates) is not sufficient to predict the product yields after HTL. A more detailed molecular characterization of the biocrude oil is needed to establish direct links between the biochemical

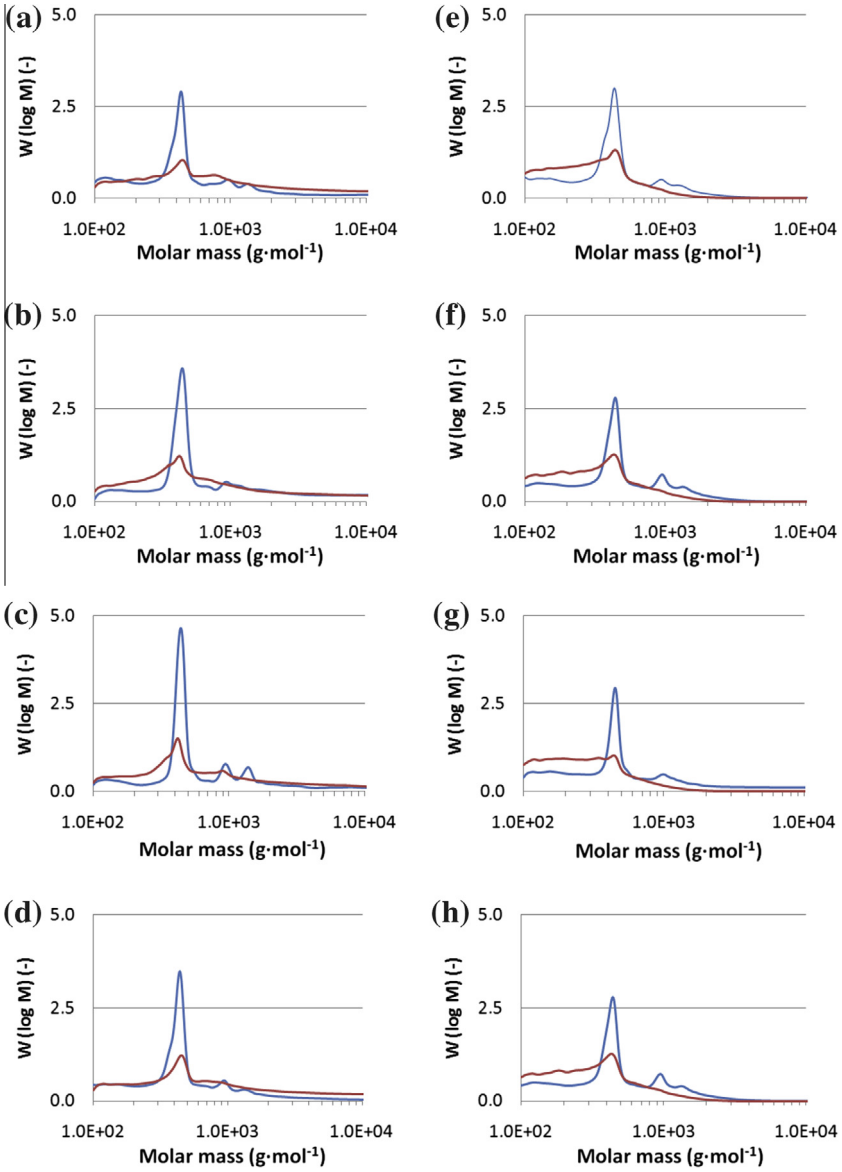

Fig. 5. Molecular weight distribution obtained by GPC analysis of the biocrude oil obtained at 250 °C (—) and 375 °C (—) for *Scenedesmus obliquus* (a), *Phaeodactylum tricornutum* (b), *Nannochloropsis gaditana* (c), *Scenedesmus almeriensis* (d), *Tetraselsmis suecica* (e), *Chlorella vulgaris* (f), *Porphyridium purpureum* (g) and *Dunaliella tertiolecta* (h).

composition of the feedstock and the amount of biocrude oil produced.

Considering the results provided in this paper, a high content of lipids is not a must for HTL (at least not in terms of biocrude oil yield) when sufficiently high temperatures are used (375 °C). However, the question about their effect in the quality of the biocrude remains uncertain. For all the species tested, the content (dry weight) of non-lipidic material is at least 75 wt% and still oil conversions in the range of 50–60 wt% were obtained, indicating that for this conversion technique, fast biomass-producing organisms are more interesting. Considering that usually the high-lipidic strains are slow growing organisms, this finding is an advantage that clearly encourages further research in microalgal HTL. Fast growing species with high biomass productivity appear to be a more suitable feedstock for this technology in order to maximize the quantity of biocrude oil produced.

It was not possible to find a direct relation between the nitrogen content of the initial algae slurry and the one in the biocrude oil. Torri et al. (2012) stated that the different microalgae fractions were not being converted independently, but by means of several cross-linked reactions. The results here presented are in accordance with that. It can be concluded that the absolute content of proteins cannot be directly linked with the concentration of nitrogen in the biocrude oil. Instead, the relative content of the different fractions might have a stronger influence. However, the mechanisms of the complex cross-linked reactions involved in microalgae HTL are yet to be understood.

In any case, from a process development point of view, it seems that near critical temperatures are required to obtain high biocrude oil yields. These conditions appear also to lead to biocrudes with a lower content of inorganic material, separating the salts present in the feedstock by precipitation. In this regard, *S. almeriensis*, *C. vulgaris, N. gaditana*, *D. tertiolecta* and *P. tricornutum* appear to be a suitable feedstock for HTL in terms of biocrude oil yield and elemental composition. This is also supported by their robust growth, commonly highlighted in the literature (Rodolfi et al., 2009). From each growth environment, the lowest nitrogen content in the bio-

crude oil was obtained with *N. gaditana* (marine) and *S. almeriensis* (freshwater). The biocrude oils from these species were also exhibiting the lowest content of inorganic material, regardless of the temperature used.

N. gaditana is a marine strain, while *S. almeriensis* is freshwater, but they both lead to similar conversions and elemental compositions. Hence, the growth environment does not seem to affect the process. Both *Nannochloropsis* and *Scenedesmus* genera are said to have resistant cell walls, and still they lead to high biocrude oil yields. This indicates that the severe conditions concurring at 375 °C take apart those structures.

Interestingly, remarkably high biocrude oil yields were obtained already at 250 °C for species lacking a cell wall. This information encourages further research towards the development of an algae biorefinery. In such a biorefinery the production of biofuels could be coupled with the extraction of other non-fuel valuable products from the microalgae. To extract those valuable compounds, cell-disrupting fraction-disclosing methods could be used, that would also harm the cell wall structure, making it weaker and thus facilitating the conversion of the inner compounds at lower temperatures. This would significantly decrease the process costs, due to a reduction in the temperature and pressure required for the conversion.

4. Conclusions

Short reaction times and near critical temperatures converted 50–60 wt% of the microalgae (daf) into biocrude, with similar yields and elemental composition for all the species tested, both marine and freshwater. At mild temperatures, strain-specific parameters seemed to directly affect the process. A correct characterization of the salts in the algae pastes was critical for interpreting the results. The fast growing species *N. gaditana* (marine) and *S. almeriensis* (freshwater) led to high biocrude oil yields and with low nitrogen content, thus appearing as interesting strains for HTL.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.07.123.

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