Hydrothermal liquefaction of a wastewater native Chlorella sp. bacteria consortium: biocrude production and characterization

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ABSTRACT
Along the recent developments of biofuel production, the hydrothermal liquefaction (HTL) of microalgae appears as a major process step which could take advantage of converting the whole microalgae content instead of only extracting its lipid content. This study presents the HTL conversion of a microalgae Chlorella sp. bacteria consortium using a batch type reactor. The experiments were carried out using a full factorial experimental design approach, where the main chosen factors were the temperature, the residence time at maximum temperature and the dry-matter-to-water ratio. The biocrude oil is the targeted product characterized along this work; its production and its composition were studied. The yields of biocrudes approach other microalgae studies using pure strains of microalgae and batch-type reactors. Maximum yields (37%daf) and lowest O/C ratio (0.167) were obtained at the highest severity conditions (310 °C for 55 min and 0.15 dry-matter-to-water ratio). The energy return on energy invested (EROEI) of the process has been determined. The simulated distillation by thermogravimetric analysis (Sim-Dis-TGA) was conducted on the different obtained biocrude and no significant effect was observed with respect to five evaporation ranges among the studied conditions. GC-MS characterization of biocrude presents the 10 relatively most abundant components for each studied condition.

Introduction
Nowadays, the liquid fuels demand has become a major environmental and economic concern. One of the considered alternatives has been the production of bioethanol from crops, but this path cannot satisfy an increasing and global demand for energy. Moreover, this alternative directly competes with arable lands and food production. Another alternative may be the use of algal biomass as biofuel feedstock. Indeed, microalgae are interesting bioproducers as they provide both cellular biomass and oil with high productivity. [1] they do not compete with arable lands or freshwater and they can even be used for CO2 sequestration [2] and/or water remediation. [3,4] Recently, increasing research works have been focusing on the use of municipal and industrial wastewaters as water and nutrients sources for the microalgae cultivation. [5–8] This way appears to be efficient for nutrients and carbon removal from wastewaters [9,10] and can provide inexpensive algal biomass for the production of biofuels. [11]

In the meantime, the hydrothermal liquefaction (HTL) process appeared to be an efficient way to convert algal biomass into biocrude oil. [12] By applying subcritical conditions to a wet biomass (280–371.14 °C and 6.4–22.1 MPa at saturation conditions), the HTL reaction mainly produces biocrude oil (BC), insoluble solid residues (SR), gases and volatiles (G) and dissolved aqueous solids (DAS). The appeal for this thermochemical conversion comes from many advantages: (1) the wet biomass, usually between 10 and 20% dry content, can be directly converted without prior drying step; (2) HTL reaction can convert many of the main microalgae cell components into biocrude oil, the yield doesn’t rely only on lipid concentration of the cell; (3) the use of catalysts is not mandatory for the conversion; and (4) the post-HTL aqueous phase could be used for nutrient recycling. [13] Moreover, the produced biocrude oil could not only be used for further biofuel production, but also for the production of higher value compounds. [14,15]

During a previous project, our team developed the mixotrophic cultivation of a native consortium of Chlorella sp. and bacteria, which can be grown in wastewaters issued from an aluminum smelter plant. [16,17] Nonetheless some studies have pointed out that grown-in-wastewaters algae have high ash contents, between 30 and 50% dry basis, [18] and this high ash content could have negative effects on the biocrude yield during the HTL processing. [19,20]
In a first attempt of exploring the HTL of our ‘in-wastewater’ developed microalgae bacteria consortium, these HTL experiments were conducted using the same consortium, grown in tap water. This paper reports the use of HTL to convert the harvested biomass into biocrude oil, with a special emphasis on its composition and its properties. A full factorial design of experiments was used to explore three main factors: the maximum operation temperature (T), the dry-matter-to-water ratio (DMW) and the holding time at maximum temperature (t). Various responses, such as the products yields and the biocrude physico-chemical characteristics, are investigated in function of these factors. Factorial relations and trends between the three main operational parameters and the biocrude characteristics are also discussed.

Materials and methods

Feedstock preparation

The RTA *Chlorella* sp. bacteria consortium used for this study is a native strain, dominated by *Chlorella* sp. It was isolated in previous works from aluminium smelter wastewaters. The mean size of the *Chlorella* sp. cells is 5 μm. The bacteria species present are unknown. The RTA wastewater native *Chlorella* sp. bacteria consortium was cultivated in the Alga Fuel™ tank, a pilot scale open tank of 10 m³, in phototrophic conditions. The inoculum was prepared by successively increasing the work volume, starting from 500 mL in flask, followed by 10 L, 150 L and 400 L open tanks. The 400 L volume was transferred in the Alga Fuel™ tank during its exponential growth. The culture medium was composed of tap water enriched with Bold’s Basal standard medium nutrients concentration which was modified as follows: KNO₃ 0.500 g/L, KH₂PO₄ 0.234 g/L, MgSO₄·7H₂O 0.150 g/L and sodium ferric EDTA 0.011 g/L.

The biomass was harvested for the HTL tests during the exponential growth phase. For harvesting, a flocculant, 1%w/w Organopol® 6485 (BASF), was introduced in the culture medium 10 minutes before the separation. Then, the algal biomass was concentrated using a vibrating sieve (400 mesh) Sweco Vibro-Energy® separator (Sweden) and was dried at 60 °C for 24 h, for storage conservation. The water obtained from the harvesting step was kept at 4 °C and used for HTL experiments.

Hydrothermal liquefaction

The HTL was performed in a Parr 4063 reactor (600 mL, Parr, USA). The dried biomass (10.17, 22.17 and 32.17 g) was introduced with 240.7 mL of the aqueous solution issued from the harvesting step (containing about 7.62 g DAS/L of solution, density solution: 1.00456 g/mL) in order to reach 0.05, 0.10 and 0.15 biomass-to-water ratios in the reactor (g_{dry biomass}/g_{water}). The characteristics of the initial biomass and the dissolved aqueous solids are presented in Table 1. The air was initially purged from the reactor using vacuum and replaced by nitrogen (99.99%, purchased from Praxair; after the air was purged, the reactor gas valve was quickly opened in order to bring the reactor initial pressure at 1 atm). The mixture was stirred continuously during the HTL experiment. The heating rate of reactor was at an average of 7.0 °C/min from the initial conditions up 275 °C for all tests, after that the temperature was increased by 5 °C steps until reaching the maximum operation temperature. The reactor was operated at three maximum temperature levels of 280, 295 and 310 °C, corresponding to pressures of about 64, 8.0 and 9.9 MPa (saturation conditions, respectively). The holding time of 5, 30 and 55 min was kept from the moment the reactor reached maximum desired operation temperature. The reactor heating was stopped after completing the desired holding time and the heating mantle was removed in order to cool the reactor and stop reactions; the cooling rates corresponded to about -3.2 °C/min (estimated from T_{max} to 195 °C). From 195 °C, the cooling was enhanced further by adding forced air (T_{air} ~ 25 °C) and plunging the reactor by three times into tap water (6–8 °C), replaced every five minutes.

Sample work-up

After the reactor was cooled off, 100 mL of distilled water and 200 mL of dichloromethane (DCM) (99%, Fisher Scientific, Canada) were introduced. The final mixture was stirred again during 1h30. The two-phases mixture was then filtered, introduced in a separatory funnel and the two phases were separated. The aqueous phase was extracted again using 200 mL of DCM. The filtration residue was extracted using 200 mL of DCM. The mixture was filtered again. The three organic phases were gathered and the solvent was removed under vacuum to finally get the biocrude (BC). The biocrude yields are expressed on a dry basis (\%_{db} \text{g biocrude/} \text{g dry biomass}) and dry ash-free basis (\%_{daf} \text{g biocrude/} \text{g dry ash-free biomass}).

Analysis and quantified parameters

Quantification of ash

Due to the limited amount of sample available for analysis, two methods for the ash quantification were used with respect to various fractions of reactants and co-products. The ash content was determined according

| Initial solid types | C  | H  | O  | N  | S  | Ash | HHV MJ/kg |
|--------------------|----|----|----|----|----|-----|-----------|
| Biomass powder     | 33.77 | 5.16 | 24.71 | 5.05 | 0.36 | 30.95 | 16.0       |
| DAS         | 0.37 | 0.30 | 2.26 | 3.35 | 2.04 | 91.68 | n.d.      |

*Expressed on a dry basis of each specific solid matter; HHV = higher heating value.*
to ASTM E1755-01 for initial added feedstock. The ash content of the biocrude and the dissolved aqueous solids (in HTL reactants) was evaluated using TGA analysis. About 10 mg of dried sample was introduced in a Diamond TG/DTA (Perkin Elmer, USA). The sample was heated from 105 °C to 1000 °C, at a heating rate of 10 °C/min, under an air flow of 200 mL/min. The mass at 575 °C was taken to evaluate the ash contents in the samples.

**Calorimetry**

The initial algal biomass and the biocrudes were analyzed for their higher heating value (HHV) using a Parr bomb calorimeter, model 6400 (Automatic Isoperibol Calorimeter).

**Elementary analysis**

The C, H, N, S organic elemental content was determined by dynamic flash combustion method using EA 1108, Fisons apparatus. The organic oxygen content (O) was determined by difference with respect to the C, H, N, S, the ash content and the total sample mass.

**Energy returned on energy invested**

In the study, the energy returned on energy invested (EROEI) was used to determine the energy efficiency of the process. The EROEI was determined using Equation 1. The Energy delivered corresponds to HHVbiocrude; Energy required to deliver that energy corresponds to $E_{\text{electrical}}$, which corresponds to the electrical energy used over each experiment by the Parr reactor heating mantle and for the stirring motor. The variables units (O) was determined by difference with respect to the C, H, N, S, the ash content and the total sample mass.

$$EROEI = \frac{\text{Energy delivered}}{\text{Energy required to deliver that energy}} = \frac{\text{HHVbiocrude} \times m_{\text{biocrude}}}{E_{\text{electrical}} \times M_{\text{biocrude}}}$$

**Simulated distillation thermogravimetric analysis (Sim-Dis-TGA)**

The simulated distillations of the biocrude were performed using TGA under nitrogen atmosphere. About 10 mg of samples was introduced in a Diamond TG/DTA (Perkin Elmer, USA). The sample was heated from 50 °C to 1000 °C, at a heating rate of 10 °C/min, under a nitrogen flow of 50 mL/min. The fractions are distributed by boiling points: heavy naphtha (<193 °C); kerosene (193–271 °C); gas oil (271–343 °C); vacuum gas oil (343–538 °C); and vacuum residue (>538 °C).

**FAME analysis and quantification**

The composition and the quantification of fatty acids have been made by GC-MS analysis, according to Li et al.[21] Hexane (>99.5%), boron trifluoride methanol solution (14%, v/v), hexane (99.9%), sodium chloride (99.9%) and Supelco 37 Component FAME Mix were purchased from Sigma-Aldrich. GC-MS analyses were performed using a 7820A Agilent gas chromatograph equipped with a DB-WAX capillary column (30 m, 0.25 mm id, 0.25 mm film thickness), connected to a 5977E Agilent quadrupole mass spectrometer.

**GC-MS compounds identification**

The final biocrudes were dissolved in DCM and analyzed for their chemical compounds using GC-MS, according to Biller et al.[22] using an HPS-MS capillary column (30 m, 0.25 mm id, 0.25 mm film thickness).

**Statistical analysis**

Using the SAS JMP® Pro 11 software (SAS Institute inc., Cary, North Carolina, USA) a 2³ factorial design with three central points was employed to study the effect of maximum operation temperature (T), dry-matter-to-water ratio (DMW) and holding time at maximum temperature (t) factors (see operating conditions in Table 2 and Supplemental data Table S1). The data of nine response variables were measured on biocrude (i.e. yield on dry basis (db) and on dry ash free basis (daf), O/C ratio, carbon recovery, HHV, N content and recovery, EROEI and fatty acid methyl esters profiles) and were analyzed by multiple linear regressions using the same software. The details of the statistical data are shown in Supplemental data.

Along the data presentations, in Tables and Figures, the order of presented conditions are always the same (see Table 2 and Figure 1): the experimental central point (295 °C–30 min–0.10 DMW) is presented at first, followed by all tested conditions regrouped by two, with respect to the temperature increase (280 vs. 310 °C), with the highest severity condition (highest temperature and residence times) presented at last. In Figures where error bars are presented, the bars correspond to standard deviation.

**Results and discussion**

**Biocrude yields and elemental composition**

Among all the conducted tests, the highest biocrude yield was reached at 310 °C–55 min–0.15 DMW (24.6%db and 37.4%daf, Table 2). According to the 2³
The lowest O/C atomic ratio is desired in the final biocrude since it generally corresponds to higher caloric value, approaching other petroleum products. According to the ANOVA on the fitted model, there was no significant linear regression among the factors to explain the O/C ratio variation. Nevertheless, the biocrude O/C and H/C ratios obtained along these experiments are consistent with other results reported in the literature (Figure 3).

The biocrude carbon recovery reached the highest level among the studied conditions at 310 °C–55 min–0.15 DMW; corresponding to 0.533 Cbiocrude/Cbiomass (Figure 4). According to the 2^4 factorial design, a linear models were fitted for the biocrude carbon recovery response. However, it was poorly significant ($p = .06$; Supplemental data Table S2) as well as the coefficient estimations, except for the DMW ratio. In the perspective of bioenergy applications, an optimal carbon recovery is desired as this value indicates an efficient thermochemical conversion of the organic carbon of the biomass into biocrude oil. In terms of biocrude carbon content (Table 2), no significant linear model was found among the factors to explain the variability according to the results obtained along this experimental design.

In terms of nitrogen content, which may represent an environmental issue related to the NOx formation during the biofuel combustion, the average N content in the biocrude was 5.4 ± 0.3 g N/100 gbiocrude while the N recovery was 0.22 ± 0.03 Nbiocrude/Nbiomass (Table 2). In the range of studied conditions, the biocrude’s N content and the N recovery would depend significantly on the DMW ratio. The linear model generated for these results showed an increase of the two responses (N content and N recovery) with respect to an increase in DMW factor only ($p = .008$ and .0007; but for low R^2 of 0.57 and 0.74, respectively; Supplemental data Table S2). Among all the results, 310 °C–55 min–0.05DMW is the condition which led to the lower N content and recovery (4.75 g N/100 gbiocrude and 0.18 Nbiocrude/Nbiomass) and 310 °C–55 min–0.15DMW was the condition which led to the highest N content and recovery levels (5.82 g N/100 gbiocrude and 0.34 Nbiocrude/Nbiomass).

### Table 2. Biocrude yields, organic elemental composition, ash content and HHV.

| Operating conditions | Yields | C (%) | H (%) | O (%) | N (%) | S (%) | Ash (%) | C_{rec} (%) | N_{rec} (%) | HHV (MJ/kg) |
|----------------------|--------|-------|-------|-------|-------|-------|---------|-------------|-------------|------------|
| 295°C–5min–0.10DMW   | 20.1±3.1 | 31.7±3.9 | 66.8±1.0 | 8.4±0.2 | 18.7±1.0 | 5.5±0.2 | 0.7±0.1 | 45.1±3.0 | 23.1±1.3 | 30.9±0.2 |
| 280°C–5min–0.05DMW   | 17.4±2.1 | 29.1±2.9 | 67.6±0.7 | 8.7±0.2 | 17.9±2.1 | 5.0±0.2 | 0.6±0.0 | 41.1±1.9 | 19.0±1.9 | 31.7±1.9 |
| 310°C–5min–0.05DMW   | 16.3±2.0 | 27.2±2.8 | 69.2±0.8 | 8.8±0.1 | 16.2±2.0 | 5.6±0.0 | 0.3±0.0 | 39.2±1.3 | 18.3±1.3 | 34.6±1.3 |
| 280°C–5min–0.15DMW   | 20.8±3.1 | 31.5±3.9 | 66.1±0.8 | 8.5±0.2 | 19.1±3.0 | 5.8±0.1 | 0.5±0.0 | 42.9±2.3 | 24.3±2.3 | 30.7±2.3 |
| 310°C–5min–0.15DMW   | 17.4±2.9 | 27.9±2.6 | 65.0±0.8 | 8.0±0.2 | 20.9±2.0 | 5.6±0.0 | 0.6±0.0 | 41.6±2.2 | 22.5±2.2 | 30.1±2.2 |
| 280°C–5min–0.05DMW   | 16.3±2.3 | 27.2±2.7 | 68.8±0.7 | 8.7±0.1 | 16.3±2.3 | 5.4±0.0 | 0.8±0.0 | 39.0±1.8 | 18.4±1.8 | 33.8±1.8 |
| 310°C–5min–0.05DMW   | 18.3±3.0 | 30.7±2.5 | 67.5±0.8 | 8.5±0.2 | 18.6±4.0 | 4.8±0.0 | 0.6±0.0 | 43.2±2.2 | 18.2±2.2 | 28.7±2.2 |
| 280°C–5min–0.15DMW   | 21.6±3.0 | 32.8±2.9 | 66.4±0.6 | 8.6±0.2 | 19.0±5.0 | 5.6±0.0 | 0.3±0.0 | 44.7±2.5 | 23.4±2.5 | 30.9±2.5 |
| 310°C–5min–0.15DMW   | 24.6±3.7 | 34.9±2.4 | 64.9±0.8 | 8.8±0.2 | 15.5±3.0 | 5.8±0.0 | 0.5±0.0 | 53.3±2.9 | 28.9±2.9 | 32.5±2.9 |

* per initial solids, before HTL; ** by difference; *** Means of the 11 trials of the factorial design of experiments; rec.: recovery; %1 with respect to all biocrude components; %2 with respect to the same respective element composition present in the initial solid content, before HTL.
100 \text{ g}_{\text{biocrude}} \text{ and } 0.29 \text{ N}_{\text{biocrude}}/\text{N}_{\text{biomass}} \text{ respectively). The highest levels of N were found for the condition reaching the highest biocrude yield, which is in accordance with other studies reported by Tian et al.\cite{12} 

The operating condition which led to a biocrude with the highest calorific value (HHV) was 310 °C—5 min—0.05 DMW (34.6 MJ/kg; Table 2). The standard deviation is fairly low for the central point (295 °C—30 min—0.10 DMW: 30.9 ± 0.2 MJ/kg; Table 2), which suggests a high reproducibility of the results. No significant linear model on the studied factors was found to explain the HHV variability, neither with respect to these operating conditions and the HHV results.

Energy return on energy invested

In terms of energy efficiency, energy return on energy invested (EROEI) has been evaluated to estimate the energy demand of the process. In this work, the higher

Figure 2. Surface representations of biocrude O/C atomic ratio based on experimental data. (a) Biocrude O/C in function of t and T for DMW values averaged; (b) biocrude O/C in function of t and DMW for temperature values averaged.

1Venderbosch et al. \cite{23} 2Tian et al. \cite{12}
the DMW, the higher the EROEI \((p < .0001)\). The fitted model \((p < .0001)\) for this response also presented the temperature to be a significant factor \((p = .05)\), as an increase of the maximum temperature led to a slight decrease of EROEI (Supplemental data Table S2). This is consistent with previous results as higher temperature necessitates higher electrical energy inputs, but higher temperature does not lead to higher yield in every case. As a result, the highest EROEI in biocrude appeared to be at a level of 0.111 MJ\text{biocrude}/MJ\text{HTL process}, at conditions of 280°C, 5 min, 0.15 DMW (Figure 5). Although some wide variations can be observed (particularly for heavy naphtha and gas oil fractions), no trend or linear relation between the operational parameters and the Sim-Dis profiles was observed. It is probable that the overall composition of the biocrude oils is more dependent on the initial biomass than on the operational parameters (in this range of operation). Other works on HTL algae biocrude \[25,26\] have also demonstrated that varying the temperature and the holding time have a slight impact on the Sim-Dis profiles. Nonetheless, the obtained profiles are quite similar to those observed in other works, operating in similar conditions with algal biomasses.\[14,23,27\] The major fraction is vac gas oil (33.2%) corresponding to high molecular compounds, however the lighter fractions (heavy naphtha, 13.2% and kerosene, 26.1%) are more abundant than in biocrudes analyzed in the aforementioned works. Based on Biller et al. conclusions,\[27\] this overall Sim-Dis profile seemed to indicate that, after catalytic hydro-treating in proper conditions, this biocrude could provide good yields in gasoline and diesel fractions (boiling points lower than 343°C).

**Simulated distillation by TGA**

The Sim-Dis-TGA analyses were performed on the 11 obtained biocrudes, indicating the distribution of the five fractions (heavy naphtha, kerosene, gas oil and vac gas oil and vac residue) by boiling points (Table 3). Although some wide variations can be observed (particularly for heavy naphtha and gas oil fractions), no trend or linear relation between the operational parameters and the Sim-Dis profiles was observed. It is probable that the overall composition of the biocrude oils is more dependent on the initial biomass than on the operational parameters (in this range of operation). Other works on HTL algae biocrude \[25,26\] have also demonstrated that varying the temperature and the holding time have a slight impact on the Sim-Dis profiles. Nonetheless, the obtained profiles are quite similar to those observed in other works, operating in similar conditions with algal biomasses.\[14,23,27\] The major fraction is vac gas oil (33.2%) corresponding to high molecular compounds, however the lighter fractions (heavy naphtha, 13.2% and kerosene, 26.1%) are more abundant than in biocrudes analyzed in the aforementioned works. Based on Biller et al. conclusions,\[27\] this overall Sim-Dis profile seemed to indicate that, after catalytic hydro-treating in proper conditions, this biocrude could provide good yields in gasoline and diesel fractions (boiling points lower than 343°C).

**Biocrude GCMS analysis (FAME and identified compounds)**

The methyl esterified fatty acids (FAME) and their compositions have been quantified in the biocrude after HTL (Figure 6). The proportion of FAME in the biocrude \((\text{mg FAME}/\text{mg biocrude})\) and the proportions of saturated, monounsaturated and polyunsaturated FAME \((\text{mg FAME}/\text{mg total FAME})\) are expressed in percentages on Figure 6. The proportion of FAME inside the biocrude varied between 2.6% and 8.2%, however no linear relation between the parameters and this proportion has been confirmed statistically. Two significant multiple linear regressions indicated that increasing the holding temperature and the time tended to increase the proportion of saturated FAME and to decrease the proportion of polyunsaturated FAME in the final biocrude. The DMW ratio and a linear interaction between the temperature and the DMW ratio seemed to have a similar effect on a minor scale (Figure 6). This effect could rely
on the specific stability of the polyunsaturated fatty acids and their derivatives. Indeed, this kind of structures is more sensitive to addition or oxidation reactions and increasing the time and the temperature may favor this type of reactions.

Other organic compounds present in the biocrude have been characterized by using GC-MS. For each obtained biocrudes, the 10 major compounds specific to the GC-MS analytical conditions have been retained and are shown in Table 4. In each biocrudes, phytol (17) has been identified as an abundant compound. The 2-methyl-2-Cyclopenten-1-one (4) was also present in each sample, indicating the hydrothermal conversion of polysaccharides, particularly starch and cellulose, as shown by Biller et al. [28] This conversion was also confirmed by the presence of phenol (7) in conditions 295°C–30min–0.10DMW, 310°C–55min–0.05DMW and 310°C–55min–0.15DMW. The presence of indole (9) and other N-heterocycles (1, 2, 3, 5, 6) indicated the thermal conversion of proteins and other N-compounds of the initial biomass. More surprisingly, every biocrude contained at least one of these cyclodipeptides: cyclo (valine-valine) (10), cyclo(leucine-leucine) (18) and cyclo (proline-leucine) (19). These three compounds were also observed by Gai et al. [29] and Cheng et al.[30] However, their thermochemical formation under hydrothermal conditions has been demonstrated by Faisal et al. [31] and the thermal degradation of proteins during HTL could have led to the formation of these compounds, as proposed by Yuan et al.[32] Finally, other observed long chain compounds (C > 10) could find their origins from the conversion of acylglycerides and other high molecular weight constituents of the cell (algaeenan).

### Table 3. Sim-Dis-TGA profiles of the obtained biocrudes.

| Operating conditions | Heavy naphtha (-<193 °C) | Kerosene (193–271 °C) | Gas oil (271–343 °C) | Vac gas oil (343–538 °C) | Vac residue (>538 °C) |
|----------------------|---------------------------|-----------------------|----------------------|--------------------------|----------------------|
| 295°C–30min–0.10DMW * | 15.5 (±6.6)%              | 29.9 (±1.0)%           | 10.0 (±1.8)%          | 30.4 (±6.2)%              | 14.3 (±1.2)%          |
| 280°C–5min–0.05DMW   | 5.8%                      | 22.3%                  | 18.9%                 | 34.3%                    | 18.7%                |
| 310°C–5min–0.05DMW   | 16.4%                     | 27.3%                  | 6.3%                  | 35.3%                    | 14.6%                |
| 280°C–5min–0.15DMW   | 19.8%                     | 25.7%                  | 5.5%                  | 33.7%                    | 15.4%                |
| 310°C–5min–0.15DMW   | 9.3%                      | 19.9%                  | 17.1%                 | 37.1%                    | 16.5%                |
| 280°C–55min–0.05DMW  | 14.6%                     | 30.5%                  | 7.3%                  | 31.3%                    | 16.3%                |
| 310°C–55min–0.05DMW  | 13.7%                     | 26.2%                  | 14.0%                 | 31.9%                    | 14.1%                |
| 280°C–55min–0.15DMW  | 7.6%                      | 18.4%                  | 16.4%                 | 39.5%                    | 18.1%                |
| 310°C–55min–0.15DMW  | 11.8%                     | 26.8%                  | 11.2%                 | 31.1%                    | 19.2%                |
| Average *            | 13.2 (±5.1)%              | 26.1 (±4.2)%           | 11.5 (±4.6)%          | 33.2 (±4.1)%              | 16.0 (±2.)%          |

*pMean of the central point replicated three times. *bMean of the 11 trials of the factorial design of experiments.

Figure 6. FAME proportion and composition in final biocrude.
Conclusion

The HTL of a *Chlorella sp.* bacteria consortium using a batch type reactor led to biocrudes maximum yields, maximum carbon recovery and lowest O/C ratio at the highest severity conditions (310°C / 55min / 0.15DMW). By using a 2³ factorial design of experiments, several multiple linear regression models were obtained. These models pointed out the direct effect of the DMW ratio on five responses (db yield, *C* recovery, *N* recovery, *N* content and *EROEI*). While this factor has a minor impact on dry-basis yields and *C* recovery, the models showed a stronger relation between DMW and *EROEI*, *N* content and *N* recovery. The DMW-*EROEI* direct positive relation could incite to work at higher dry matter concentration in the starting material as *EROEI* levels drive the viability of the HTL process. However, this would directly increase the final *N* content in the biocrude which may be a major issue for biofuel applications.

Although the effect residence time and maximum temperature could be modeled about the final lipids composition, the factorial design of experiments could not lead to satisfying models concerning the other biocrude characteristics, even if some trends can be observed. Finally, no clear trends or linear regression models could explain the variability of more ‘complex’ responses, such as the HHV, the Sim-Dis profiles or biocrude molecular composition. Further studies, implying design of experiments to assess non-linear relations between variables and responses, should be necessary to understand the mechanisms behind the formation of biocrude.

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