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Energy and nutrients recovery from lipid-extracted *Nannochloropsis* via anaerobic digestion and hydrothermal liquefaction

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Abstract

The biomass, generated after the lipid extraction from *Nannochloropsis* microalgae (LEM) for biodiesel production, demonstrated their suitability for both energy and nutrients recovery. The anaerobic digestion of LEM produced a minimum of 268 mL_{CH4}/g_{VS} in different experiments. The co-digestion of LEM and sewage sludge revealed that both waste can be co-digested without inhibition, although no synergy was

observed. The methane yields barely increased 10% after pre-treatments (ultrasound and ultrasound combined with alkali addition).

Regarding bio-oil production from hydrothermal liquefaction process, more than 28% of the LEM was converted into bio-oil. Moreover, the aqueous phase generated during the bio-oil production was successfully utilised as nitrogen source for microalgae cultivation.

Keywords:

Anaerobic co-digestion, Anaerobic digestion, Cultivation, Hydrothermal liquefaction, Nutrients recovery, Pre-treatments.

INTRODUCTION

Over the past years, researchers turned their attention to find renewable and cost-effective feedstocks for biofuels, as a consequence of the predicted fossil-fuels reserves depletion. Microalgae utilisation as feedstock was also fostered by the "food-versus-fuel competition" derived from the biofuels production from crops [1]. Amongst the different biofuels, biodiesel still gathers the attention of researchers. The process scale-up is not economically viable nowadays [2], but increasing efforts are being made to overcome the drawbacks. For example, the valorisation of the lipid-extracted microalgae (LEM) may be beneficial for the biodiesel process. Approximately 65% of the biomass remains as waste after lipid extraction, containing some valuable compounds [3].

The anaerobic digestion (AD) is a well-known technology used for the stabilisation of the sewage sludge generated in wastewater treatment plants (WWTPs). The versatility of this process allows the treatment of a wide range of feedstocks, including microalgae.

In recent years, the LEM have been also considered as promising substrates for AD [3]. The low C to N ratio in microalgae, which can lead to AD inhibition, is even lower in LEM. The anaerobic co-digestion (Aco-D) with sewage sludge is a viable alternative to balance the C to N ratio. Additionally of being widely available, sewage sludge is the second substrate utilised in co-digestion due to the over-sized digesters in WWTP [4]. The AD also offers the advantage of recycling nutrient. The aqueous phase generated during AD, rich in ammonia and phosphorous, may be used for microalgae cultivation [5,6]. On the other hand, the hydrothermal liquefaction (HTL) is a completely different technology which converts microalgae and several microalgae waste into bio-oil [7-11]. The bio-oil, a liquid fuel, can be further combusted or upgraded. Similar to AD, the HTL process generates a nutrients-rich aqueous phase (AP-HTL) which can be recycled for microalgae cultivation [12-14].

This paper is the first attempt to evaluate several processing routes for the biofuels production from LEM; the routes can be observed in the scheme presented in Figure 1. Several options to avoid AD inhibition and to improve the methane production were evaluated. The increased protein content after the lipid extraction and the presence of sea salt in the LEM are potential causes of inhibition [3,15]. The effects of high amounts of proteins were evaluated by setting several substrate to inoculum ratios (SIR) in the experiments. Regarding sea salt, the influence of washing biomass was evaluated. The LEM were also co-digested with sewage sludge, based on the advantages of combining both substrates as previously mentioned. The last option consisted of applying two pre-treatment methods to improve the methane production. The ultrasonic pre-treatment was chosen based on the suitability to increase the biodegradability of microalgae [16]. The combination of alkali addition and ultrasonic pre-treatment was chosen based on the benefits when applied prior to the AD of some waste [17,18].

In a completely different processing route, the LEM was used as for HTL. Although the process optimisation is not addressed in this report, the valorisation of the AP-HTL generated was evaluated as nutrients source for microalgae cultivation. The bio-oil production together with the nutrient recycling has not been reported yet.

MATERIALS AND METHODS

Materials

Lipid-extracted *Nannochloropsis oculata*, provided by AlgoSource's (Alpha Biotech, Asserac, France), was generated from a lipid-extraction process with supercritical carbon dioxide ($SCCO_2$). The solid LEM was stored in a desiccator until required. The LEM was the substrate used for biofuels production via AD and HTL.

For the experiments where microalgae were cultivated in the aqueous phase recovered from the HTL, the species was *Nannochloropsis gaditana* Lubián, strain CCMP 1775, obtained from NCMA (National Center for Marine Algae), formerly CCMP.

The inoculum for the AD experiments consisted of digested sludge from a pilot-plant under mesophilic conditions. The reactors were operated under semi-continuous conditions and fed with a sewage sludge blend (primary and secondary sludge in ratio 65:35 v/v); the same sludge blend was utilised as substrate in the co-digestion experiments. The sewage sludge blend was collected from the municipal WWTPs in Reus (Tarragona, Spain).

Experimental procedure

Analytical methods

Total solids (TS), volatile solids (VS), protein and carbohydrate were analysed according to the methods described in Caporgno et al. [19]. The residual lipid in the

LEM was determined by extraction of 3 g of LEM using a Soxhlet apparatus with a reflux period of 7 hours and hexane as solvent; lipids were then recovered by solvent evaporation and weighed. The TS content in the LEM was 928.8±0.7 g/kg, with VS/TS of 0.75±0.01. The organic fraction contained 61.7±4.1 % proteins, 7.6±0.1 % lipids and 25.3±2.0 % carbohydrates. The TS content and the VS/TS were 27.8±0.7 g/L and 0.76±0.01 in sewage sludge, and 17.2±1.0 g/L and 0.64±0.02 in the inoculum.

In the AD experiments, biogas production and composition, volatile fatty acid concentration (VFA), alkalinity, were determined as described in Caporgno et al. [19].

In the HTL experiments, the bio-oil was analysed by chromatography-mass spectroscopy (GC-MS). The samples were subjected to GS-MS analysis (G1099A/MSD5973) using a HP-5MS column (19091S-433) and helium as carrier gas with flow rate of 1.4 mL/min. A volume of 1 μL dichloromethane (DCM) extract was injected at 280 °C with a split ratio of 2:1. The initial temperature in the oven was 80 °C, and after 1 min, it was increased at 15 °C/min until 200 °C. Subsequently, the temperature was increased at 5 °C/min until 310 °C and held constant for 10 min. The higher heating value (HHV) in bio-oil was determined in a bomb calorimeter (Gallenkamp Ballistic Bomb Calorimeters), using benzoic acid as a standard substance. On the other hand, the HHV of the LEM was calculated based on the elemental composition of the biomass using the Dulong's formula as described in Vardon et al. [9] due to the high content of ashes in the LEW. The ammonia nitrogen (NH_3-N), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), phosphate phosphorus (PO_4-P), and chemical oxygen demand (COD) were measured in the aqueous phase prior to the microalgae cultivation, using a HI-83099-02 bench photometer and following the procedures described in the user's manual (Hanna Instruments, 2014). The total nitrogen (TN), total carbon (TC) and total inorganic carbon (TIC) were determined in

Multi NC 3100 (Analytic Jena) analyser. The gas-phase composition was analysed using the method for the biogas analysis described in Caporgno et al. [19].

Carbon, hydrogen and nitrogen content in the LEM, the bio-oil and the solid waste recovered after HTL have been analysed in an LECO TruSpec Elemental Analyser. Oxygen content was determined by difference.

In the cultivation experiments, cell growth was monitored by measuring the absorbance at 750 nm with a microplate reader (Infinite® M200 PRO, Tecan).

Anaerobic digestion

The batch reactors were set up following the procedure described in Caporgno et al. [19]. The substrate depended on the experiment. The effects of washing the LEM and the SIR variation were simultaneously evaluated. In the washing step, 20 g of LEM were mixed with 200 mL deionised water at room temperature, stirred 20 min and then centrifuged to recover the solid fraction; the procedure was repeated three times. Reactors with SIRs of 1:4, 1:2 and 1:1 $VS_{Substrate}$:$VS_{Inoculum}$ were prepared with different amounts of LEM, either washed or unwashed.

In the co-digestion experiments, the SIR was of 1:2 $VS_{Substrate}$:$VS_{Inoculum}$. Different mixtures of sewage sludge and unwashed LEM were fed, replacing 25%, 50% and 75% of the VS from LEM by VS from sewage sludge.

Two different pre-treatment methods were applied to unwashed LEM. The first one, ultrasonic pre-treatment, consisted in applying three different energy levels: 28, 48 and 55 MJ/kg_{TS} at 24 kHz working frequency and 93 W ultrasonic power (UP200S Hielscher Ultrasonics GmbH, Germany). The LEM were suspended in deionised water for the pre-treatment. The pre-treatment was carried out at room temperature and using a water bath to avoid the sample heating. The energy supplied was calculated according

to the ultrasonic power, the concentration of solids in the sample and the treatment duration. The second pre-treatment consisted in combining addition of alkali and ultrasound application at low-energy level. The energy applied during ultrasonic disintegration was 17 MJ/kg_{TS}. The alkali pre-treatment consisted in adjusting the pH at 9, 11 and 13 with NaOH, and keeping the samples at room temperature for 24 h before digestion. For the pre-treatment combination, the pH in the samples was first adjusted at 9, 11 and 13, and then the ultrasonic disintegration was applied. The pH in the pre-treated samples was neutralised to pH 7 with HCl.

Hydrothermal liquefaction

The experiments were performed at 300 ºC, ≈10 MPa, 30 min retention time in a 1L reactor (1 Liter EZE-Seal Bolted Closure, Autoclave Engineers). The reactor was loaded with 300 g of slurry, containing 20% LEM in water (w/w), and then purged with nitrogen to remove the oxygen before heating. The different product fractions were recovered (Figure 2) and analysed. After the reactor was cooled down to room temperature, the gases were vented off and collected in gas sampling bags for further analysis. The major part of AP-HTL was removed by pouring it into a beaker. Dichloromethane (DCM) was used as solvent to recover the bio-oil, which was adhered to the reactor walls. The DCM containing the bio-oil was separated from the remaining aqueous fraction using a separatory funnel. Both AP-HTL and DCM were vacuum-filtered through pre-weighted filters for the quantification of the amount of suspended solids. DCM was dried under anhydrous sodium sulphate and evaporated in a rotary evaporator to determine the amount of bio-oil. The AP-HTL was recovered and used for microalgae cultivation.

For the mass balance, the products yields were defined as weight percentages relative to the raw material. The bio-oil yield accounted the DCM-soluble fraction, the AP-HTL accounted dissolved constituents remaining after the DCM extraction, the solid fraction accounted the mass of dried particulates retained after filtration, and finally, the gas-phase yield was calculated by difference.

Nutrients recycling

A growth experiment was performed to evaluate the feasibility of replacing the nitrogen source in the algal culture medium by the aqueous phase from HTL. *N gaditana* was cultivated in culture media containing different proportions of AP-HTL. Blank assays were performed with seawater enriched with a modification of Guillard's f/2 formulation [20]. Since f/2 is a rather diluted medium for biomass production purposes, nitrogen and phosphorous concentrations in this modified medium ($f/2^+$) were increased up to 60 mg $N\text{-}NO_3$/L and to reach a molar N:P ratio equal to 16:1. Based on the total nitrogen in AP-HTL, five different mixtures of AP-HTL and $f/2^+$ were prepared by the replacement of 7, 15, 30, 45 and 60 mg N/L in the $f/2^+$ medium by the AP-HTL addition. Media were filter sterilized through 0.2 μm.

Microalgae were cultured in sterile 6-well microplates, with a working volume of 3 mL per well and an initial microalgae density of $3 \cdot 10^5$ cells/mL. Triplicate cultures were grown in continuous agitation at 25±1°C, under an irradiance of ca. 115 μmol photons/$m^2 \cdot s$ at the surface of the microplates, provided by daylight fluorescent lamps in a 16:8 light:dark cycle.

Microalgae growth was daily monitored by light absorbance measurements, at 750 nm, with a M200Pro Infinite Tecan microplate reader. Division rates (k) were obtained by

calculating the slope of the linear least-squares regression for the $\log_2$-transformed values of the sample absorbance at 750 nm during the exponential growth phase [21].

RESULTS AND DISCUSSION

Anaerobic digestion

Table 1 summarises the ultimate methane productions obtained in all the digestion experiments, the kind of substrate fed and the SIR used in the reactors. It is worth mentioning that the experiments were not performed simultaneously, therefore the methane productions from LEM slightly differ in some of the experiments.

LEM *washing and SIR variation*

The ultimate methane productions from washed and unwashed LEM indicated that the washing influenced the AD negatively. The methane production decreased between 29% and 34% after washing. The salt concentration in marine microalgae can strongly affect the methane production [15]; however, the washing removed not only salts from the biomass. The analysis of the washing water revealed that 67±1% of the solids fraction removed was constituted by organic compounds. The loss of these organics caused the significant reduction in the methane production [22]. Moreover, not only the organic fraction content was affected, but also the characteristics of the organic fraction. As mentioned 2.2.2., the same amount of substrate on VS basis was fed in all the reactors. For this reason, the lower methane production can not be attributed to the organic fraction content in the digesters. Easily-biodegradable compounds were removed by washing, thus decreasing the methane production from washed substrates.

Regarding the SIR, it was clearly demonstrated that the ratios evaluated cause no influence on the digestion of washed and unwashed LEM (Table 1). The differences in

the ultimate methane production from washed LEM were less than 7%, and from unwashed LEM, less than 2%. In the literature, 1:1 is commonly considered a SIR threshold for inhibition; however, it depends on the substrate characteristics. Long chain fatty acid, hydrolysis products of lipids, lead to inhibition during the AD of microalgae with high lipid content. For this reason, the SIR can be increased in the digesters with LEM [23]. Similarly, substrates characterised by high protein content cause inhibition by ammonia during AD [3]. Ammonia is the most probable cause of inhibition due to the high protein content in the LEM. The ammonia concentration, VFA concentration and pH amongst other parameters indicated stable operation.

In reactors with unwashed LEM, the methane production throughout the first days of the experiment was affected (Data not shown). These effects, observed at 1:1, were also reported during the AD of other LEM [23,24]. The VFA were not simultaneously converted into methane, leading to significant accumulation of VFA and methane production decrease. The VFA accumulation can lead to inhibition; however, the VFA accumulated but they were converted into methane soon afterwards. The ratio 1:1 was close to the SIR threshold for inhibition. Regarding the washed LEM ~~LEW~~, the phenomenon did not occur due to the low degradability of the organic fraction.

LEM *co-digestion with sewage sludge*

The ultimate methane production from LEM and sewage sludge co-digestion indicated no synergy by mixing both substrates. The sewage sludge and the LEM produced the highest and the lowest methane yields, 360±12 mL_{CH4}/g_{VS} and 274±4 mL_{CH4}/g_{VS} respectively. The methane productions from the mixtures were proportional to the percentage of each substrate in the mixture. Similar results were reported by Neumann et al., who highlighted that the absence of inhibition allows mixing both substrates

without affecting the process operation [25]. The co-digestion with sewage sludge is even more advantageous when considering the non-used capacity of the digesters in WWTP, approximately 30% [4]. Recently, Astals et al. emphasised the benefits of integrating pig manure processing and microalgae cultivation, in spite of the absence of synergy in Aco-D [6].

LEM *pre-treatment*

The effects of the ultrasonic pre-treatment on the AD of LEM can be observed in Table 1. The lowest energy input (28 MJ/kg_{TS}) seemed to be inefficient to increase the LEM degradation, since the methane production was similar to the obtained with untreated LEM. Higher energy inputs (48 and 65 MJ/kg_{TS}) only resulted in a 7-9% increase in methane production. Similar results were reported after ultrasonic pre-treatment of *Nannochloropsis sp.* LEM [24]. The results suggest that this pre-treatment was probably unsuitable for microalgae wastes from the genus *Nannochloropsis*, probably due to the cell wall characteristics. The cell wall in the genus *Nannochloropsis* has a cellulosic inner-wall protected by an outer hydrophobic algaenan layer [26], which hampers AD degradation [27]. For this reason, powerful pre-treatment methods have been suggested for *Nannochloropsis* species [27].

In order to improve the limited degradation of the LEM, an ultrasonic pre-treatment with low-energy input was combined with alkali addition. For comparison, the effects of alkali and ultrasound were also separately tested. The first visible effects of the pre-treatment were noticed in the LEM composition. Alkali treatment caused organic matter destruction in the LEM, so that after the pre-treatment with alkali addition up to pH 13 the VS/TS ratio decreased from 0.75 to 0.62. Likewise, the combined pre-treatment decreased the VS/TS ratio down to 0.66. Similar VS/TS decreases reported during the

alkali and ultrasonic pre-treatment of other substrates were attributed to the mineralization of some components [28]. Pre-treatment with alkali also increased the methane production up to 8% (Table 1), the highest methane production being obtained with the lowest NaOH dosage. The pre-treatment of undamaged microalgae with similar NaOH dosages also gave higher methane productions with lower dosages [29]. Furthermore, the highest methane production was obtained from the non pre-treated microalgae [29]. Regarding the ultrasonic pre-treatment (17 MJ/kg_{TS}), the methane production remained unchanged, as expected considering the inefficiency of a stronger input (28 MJ/kg_{TS}) discussed at the beginning of this section. Surprisingly, the pre-treatment combination did not increase the methane production (Table 1). References regarding the AD of LEM are currently scarce, especially those focused on the effects of pre-treatments. Only Suresh et al. reported an alkali pre-treatment of LEM and its combination with ultrasonication. The authors suggested inhibition due to VFA accumulation, being the methane production negatively affected [30]. However, in the present work, the absence of inhibition was corroborated in all the reactors at the end of experiments. On the other hand, the combined pre-treatment applied to other lignocellulosic substrates did not affect the total methane production [28]. The alkaline pre-treatment affects the intermolecular linkages and functional groups of lignin, cellulose, and hemicellulose [31]. The cell walls structures are more vulnerable to the shear forces in the ultrasonic pre-treatment when the pre-treatments are combined. However, the effectiveness of the alkaline pre-treatment is considered dependent on the content of lignin in the biomass and the content of lignin in microalgae is considered low [31].

Hydrothermal liquefaction

The mass balance allows establishing the product distribution after the HTL process. The results, presented in Table 2, indicated that similar amounts of the biomass were distributed between the bio-oil, the AP-HTL, the gas and the solid fraction.

The bio-oil showed the highest yield, 28±2 g/100g_{LEM}. This yield is comparable to the obtained by processing *Scenedesmus sp.* LEM, with high-protein and low-lipid content, under the same experimental conditions [9]. Higher bio-oil yields have been obtained from *N. salina* LEM [10], but with a high amount of residual lipids (≈20 g/100g_{sample}). Bio-oil yields close to 45 g/100g_{sample} have been recently reported from protein-extracted *Scenedesmus sp.* [11]; once again, the lipid fraction which remains after extracting the proteins ((≈18.5 g/100g_{sample}) may contribute to the high bio-oil yield. Lipids present the highest bio-oil yield, followed by proteins and carbohydrates [12]. On the other hand, the bio-oil obtained by processing *D. tertiolecta* from β-carotene production yielded approximately 5 g/100g_{sample} under operating conditions similar to the present experiments; quite severe operating conditions were required to increase the bio-oil yield [7]. The bio-oil yields obtained in the experiments here reported are also comparable with the reported in the literature, after processing from the whole *Nannochloropsis oculata*, in spite of the differences in the lipid content [12]. It worth mentioning that bio-oil yield was calculated on the dry weight basis of the LEW; the yield increases when is calculated on the dry ash-free basis due to the high content of ashes in the LEM.

Regarding the characteristics of the bio-oil, the analysis of the HHV resulted in 37.2±0.9 MJ/kg, which is in agreement with the values reported for other LEM under the same experimental conditions [9] and other microalgae species under different HTL conditions [8,12]. The HHV was considerably low for the LEM, 17.7±0.1 MJ/kg, mainly as a consequence of the high content of ashes in the LEW. The qualitative GC-

MS analysis of the bio-oil evidenced its complexity. The high protein content in the LEM led to the presence of nitrogenous compounds in the bio-oil. The main compounds can be included in the category of aromatic compounds such as indole derivatives or in the category of highly aliphatic compounds, including long chain alkanes and alkenes, free fatty acids and amide derivatives. Similar compounds have been also identified by other authors [7,9,11]. The analysis elemental of the bio-oil determined C, H, N and O percentages of 70%, 9%, 5% and 17% respectively. According to these results, the bio-oil quality is better than the reported by Vardon et al. [9] and comparable to the bio-oil quality reported by Zhu et al. [10] in terms of N and O contents. The analysis elemental of the LEM determined C, H, N and O percentages of 40%, 6%, 5% and 24% respectively. Based on these results, 48±4% of the C from the LEM was converted into bio-oil, similar to value reported by Vardon et al. [9].

The gas yields indicated that a large portion of the initial biomass was converted into gaseous products. Although the gas composition was not analysed in detail, the chromatogram revealed that the gas fraction was mainly CO_2 and small amounts of simple hydrocarbons as it was expected based on the literature [8,11]. The high content of CO_2 suggests the possibility of using the exhausted gas in the microalgae cultivation unit [32].

The yield of the solid fraction was considerably higher than the yields obtained after processing LEM under similar experimental conditions [9,11]. Comparable yields of the solid fraction were reported during the HTL of *Dunaliella tertiolecta* waste, but in this case, the bio-oil yield indicates low biomass conversion [7]. As mentioned before, the LEM here used was characterised by high ashes content, and most of the ashes contributed to increase the yield of the solid fraction. The analysis of the solid fraction revealed the presence of the major part of the inorganic fraction from the LEM, 78±3%.

The analysis elemental of the solid determined C, H, N and O percentages of 78%, 12%, 4% and 6% respectively. The high percentage of C and the appearance of the solids, suggest the formation of bio-char as a consequence of the re-polymerisation and carbonisation of water-soluble compounds derived mainly from carbohydrates, due to the high temperature in the process [33]. Based on the percentages of ashes and C in the solid fraction, it can be stated that a considerable high biomass conversion was achieved during the process.

The mass balance revealed that the AP-HTL contained a high percentage of the inorganic fraction originally present in the LEM, but also organic compounds. The COD and the TOC analysis resulted in 75500 mg O_2/L and 21450 mg C/L respectively, in agreement with the concentrations reported in the literature [13,34]. The COD and TOC values indicate the presence of dissolved organic compound. Although the compounds were not identified, they may be formic acid, acetic acid, lactic acid, glycerol amongst other are some polar organic compounds which remained solubilised in water [35]. As can be seen in Table 2, 26±1% of the C from the LEM remained solubilised in the AP-HTL. Based on the high protein content, its decomposition can generate nitrogen compounds such as pyroles, indoles and phenols [12]. The nitrogen content was approximately 7000 mg N/L, and around 68% of the TN was in the form of NH_4^+. The major part of the N from the LEM remained solubilised in the AP-HTL (Table 2). The C to N ratio resulted ≈3.4 in the aqueous phase, as reported during the HTL of several microalgae species under similar experimental conditions [8,12,14]. The PO_4^{-3} was 90 mg P/L, considerably low compared with the N and C concentrations. The phosphorous distribution in the HTL products can be affected by reaction temperature and retention time, and by the reaction between the amino acids with reducing sugar [13].

As mentioned before, the optimisation of the HTL process was not studied in this manuscript. However, the production of bio-oil from LEM and the subsequent upgrading process was already reported by Zhu et al. [10]. The similarities in the composition of the biomass and the yields and characteristics of the bio-oil suggest that the HTL of lipid-extracted *N. oculata* may be also a promising alternative to produce liquid fuels which can compete with conventional fossil fuels. Moreover, the nutrient recycling can significantly contribute to improve economic aspects.

Nutrients recycling

The suitability of the AP-HTL for microalgae cultivation was evaluated. According to the nutrient analysis performed, the N:P ratio of the AP-HTL was 78:1. Such a high N:P ratio is far from the optimal N:P ratios for microalgae development [36]; this was confirmed by the low *Nannochloropsis gaditana* growth on dilutions of AP-HTL in a previous culture experiment (data not shown). Therefore, it was decided to check the suitability of the AP-HTL only as nitrogen source. With this aim, part or the entire N-NO_3 source of the standard medium was substituted by a volume of AP-HTL that provided with an equal amount of nitrogen. Several AP-HTL loads were tested considering that a high AP-HTL concentration could have an inhibitory effect on the culture due to toxic compounds such as amides or heterocyclic compounds [13].

Figure 3 shows *N. gaditana* growth curves for different loads of AP-HTL; the curves are plotted in semi-logarithmic scale for better visualisation of the exponential-growth phase. The amount of AP-HTL that accounted for the total substitution of N-NO_3 (HTL60) inhibited growth from the beginning, as evidenced by the reduced division rate (Table 3) and the final biomass which was 25% of the obtained in the standard medium. On the contrary, AP-HTL loads contributing up to 30 mg N (HTL30) supported growths

only slightly smaller than in the standard medium. Division rates were similar or even slightly higher than in the standard medium, but at the end, final biomass (in terms of Abs_{750nm}) was 90-77% of that obtained in standard medium, the higher the final biomass the lower the AP-HTL load.

The applicability of an AP-HTL as microalgae culture medium depends on the concentration and form of macro- (nitrogen, phosphorus, N:P ratio) and micro- nutrients [37], the absence of harmful compounds [12,34,38], the pH [14] and the microalgae species used [12,14]. In the present experiments, the growth inhibition at HTL60 concentrations may reflect a compound at harmful concentration rather than the form of inorganic N supplied. Although high amounts of NH_4^+ are known to be toxic [39], *N. gaditana* has demonstrated to thrive at concentrations up to 190 mg $N\text{-}NH_4^+$/L when the pH of the culture is controlled at 8.0 [40]. Therefore, the 60 mg·$N\text{-}NH_4^+$/L in HTL60 at the initial pH of 8.3 (Table 3) should not be harmful.

The AP-HTL contributing up to 30 mg N (HTL30) supports growth close to the maximum growth obtained with standard medium. Further experiments are needed to ascertain which other nutrients, such as iron and other micronutrients, can be substituted from standard media by AP-HTL.

CONCLUSIONS

This study demonstrated the valorisation of the (lipid-extracted *Nannochloropsis* can be carried out producing energy and a nitrogen-source for biomass cultivation. These ways of valorisation can be beneficial for the biodiesel production process, and also for other process which extract valuable compounds from microalgae. Additionally to the possibility of converting the LEM into methane, the co-digestion with sewage sludge demonstrated the viability of the co-digestion process. The co-digestion of LEM and

sewage sludge allows taking advantage of the over-sized digesters in some WWTPs. The pre-treatment of LEM before digestion resulted unsuitable for microalgae wastes, probably due to the cell wall characteristics which hamper degradation. For this reason, powerful pre-treatment methods should be evaluated to increase the biodegradability of the biomass.

Another alternative for LEM valorisation is the conversion into bio-oil. The results revealed the possibility to obtain high bio-oil yields. Moreover, recycling the aqueous phase generated during HTL to the microalgae cultivation unit, the amount of fertilisers for cultivation is reduced. The high nutrient content in the aqueous phase is problematic for water discharge, thus the microalgae cultivation can contribute to accomplish the regulation for water discharge.

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FIGURE CAPTIONS

Figure 1. Scheme of the processing routes evaluated for the valorisation of the LEM. **1-column fitting image.**

Figure 2. Procedure for the separation of the different products fraction from HTL. **1-column fitting image.**

Figure 3. Growth curves in semi-logarithmic scale for *N. gaditana* in several culture media. **1-column fitting image.**

Table 1. Ultimate methane production from LEM ~~LEW~~. Batch reactors at 33ºC and 29 days digestion. **Note:** These experiments were not done simultaneously, thus the methane production from LEM ~~LEW~~ samples differs in some of the experiments.

Experiment	Substrate	SIR	CH_4 mL_{CH4}/g_{VS}
Effect of washing	Unwashed LEM	1:4	269±6
		1:2	268±17
		1:1	273±6
	Washed LEM	1:4	192±16
		1:2	185±9
		1:1	180±11
Aco-D	LEM	1:2	274±4
	25% Sludge	1:2	293±3
	50% Sludge	1:2	319±4
	75% Sludge	1:2	345±10
	100% Sludge	1:2	360±12
Ultrasonic pre-treatment	LEM	1:2	274±4
	28 MJ/kg_{TS}	1:2	269±7
	48 MJ/kg_{TS}	1:2	299±6
	55 MJ/kg_{TS}	1:2	294±4
Combined pre-treatment: US and NaOH	LEM	1:2	286±8
	17 MJ/kg_{TS}	1:2	289±3
	pH 9	1:2	309±6
	pH 11	1:2	300±9
	pH 13	1:2	290±5
	US + pH 9	1:2	290±3
	US + pH 11 [a]	1:2	244±4
	US + pH 13	1:2	296±3

[a] Experimental error confirmed by analysis at the end of the experiment.

Table 2. Mass balance of HTL, and C and N recovery from the LEW.

	Bio-oil	AP-HTL	Gas	Solid fraction
Mass balance ($g/100g_{LEM}$)	28±2	24±2	27±3	21±2
C recovery (% of the C in LEW)	48±4	26±1	14±1 [a]	9±2
N recovery (% of the N in LEW)	24±2	56±1	- [b]	3±1

[a] Only the amount of CO_2 determined in the gas was considered.

[b] The N content in gas was not analysed.

Table 3. N source in the culture media and summarised results obtained during cultivation.

		Media					
		f/2^{+}	HTL7	HTL15	HTL30	HTL45	HTL60
Source of N (mg N/L)	*NO_3^- from f/2^{+}*	60	53	45	30	15	0
	NH_4^+ from AP-HTL [a]	0	7	15	30	45	60
	Total Inorganic N	60	60	60	60	60	60
k (duplications/day)		0.95±0.03	0.97 ±0.11	1.04±0.02	0.97 ±0.07	0.79±0.02	0.54±0.02
Initial pH		8.1	7.4	7.8	8.3	8.2	8.3
Final pH		9.8±0.1	9.7±0.1	9.9±0.0	9.6±0.0	9.1±0.0	7.8±0.1

[a] the amount of NO_3^- was negligible in the AP-HTL

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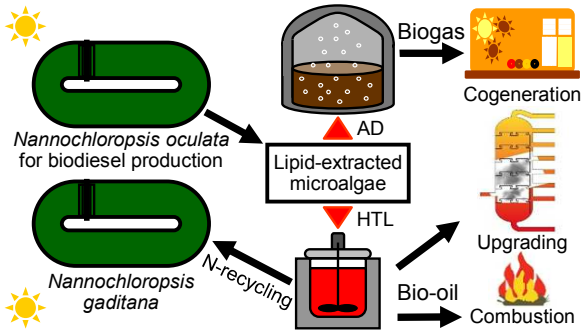
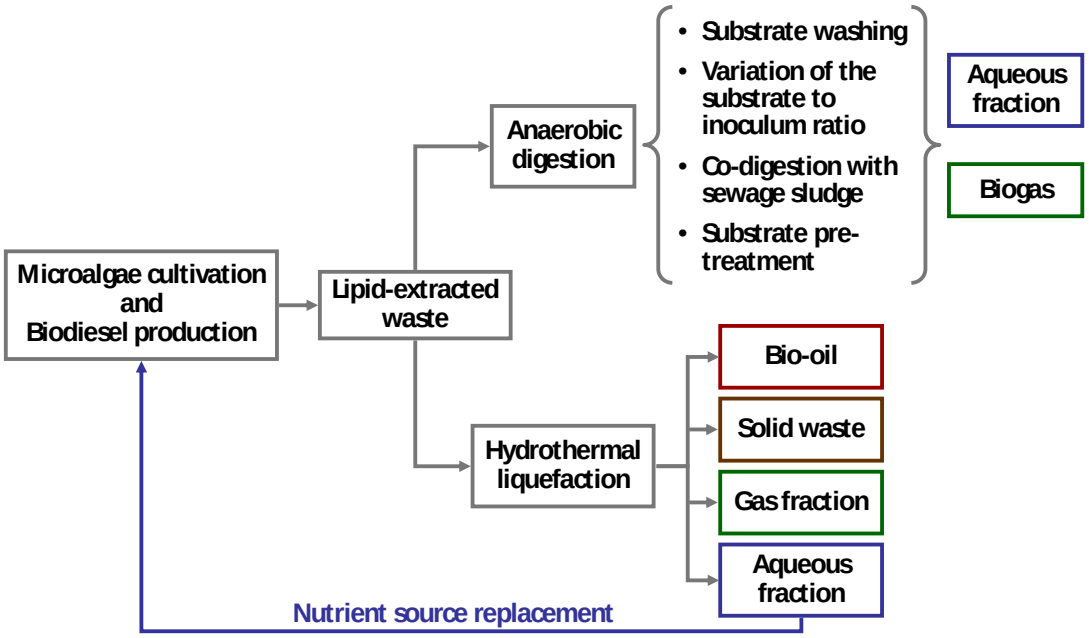
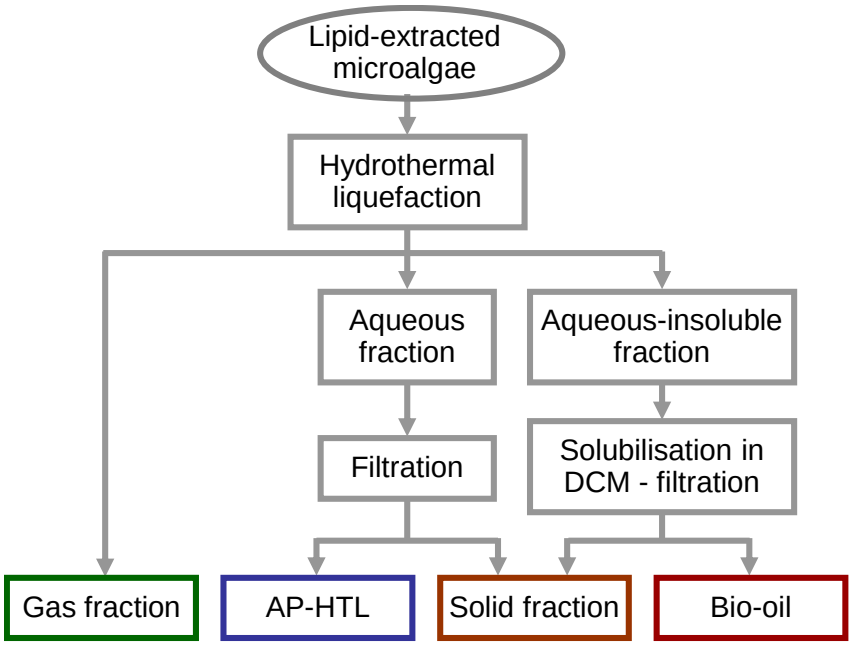


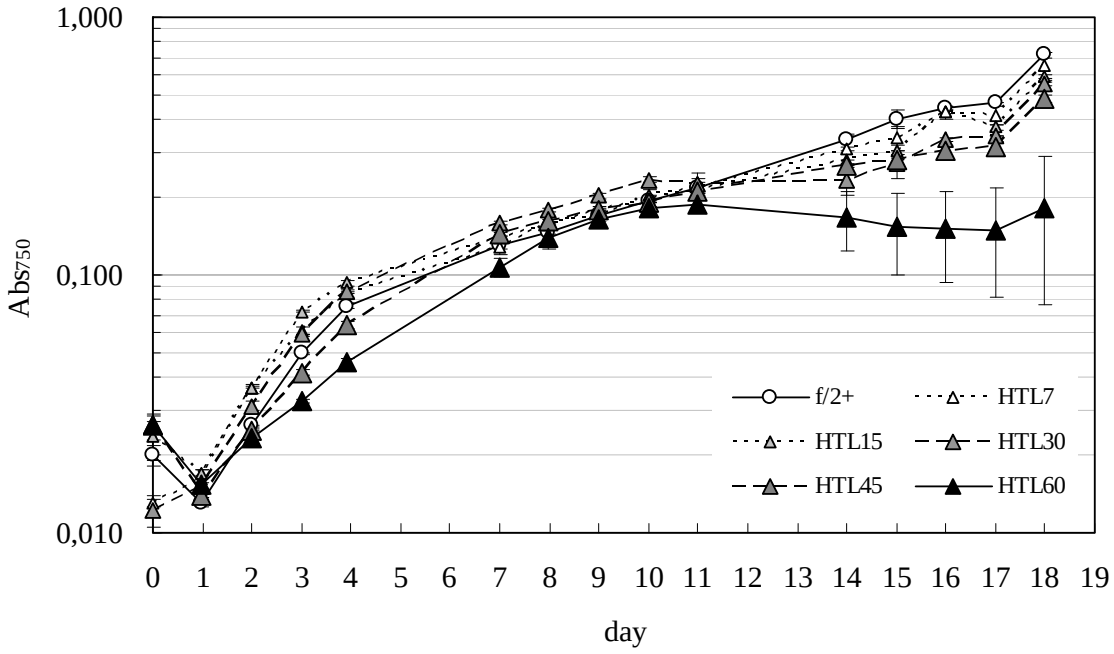
Energy and nutrients recovery from lipid-extracted *Nannochloropsis* via anaerobic digestion and hydrothermal liquefaction

M.P. Caporgno, E. Clavero, C. Torras, J. Salvadó, O. Lepine, J. Pruvost, J. Legrand, J. Giralt, C. Bengoa

Energy and nutrients from microalgae cultivation were recovered from lipid-extracted *Nannochloropsis oculata*


Microalgae cultivation and Biodiesel production
Lipid-extracted waste
Anaerobic digestion
Substrate washing
Variation of the substrate to inoculum ratio
Co-digestion with sewage sludge
Substrate pre-treatment
Aqueous fraction
Biogas
Hydrothermal liquefaction
Bio-oil
Solid waste
Gas fraction
Aqueous fraction
Nutrient source replacement


Lipid-extracted microalgae
Hydrothermal liquefaction
Aqueous fraction
Aqueous-insoluble fraction
Filtration
Solubilisation in DCM - filtration
Gas fraction
AP-HTL
Solid fraction
Bio-oil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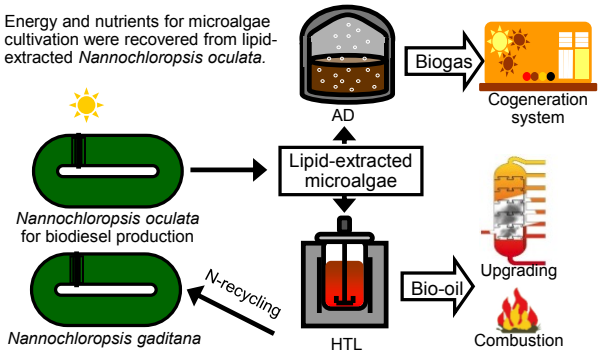
Energy and nutrients for microalgae cultivation were recovered from lipid-extracted Nannochloropsis oculata.
AD
Biogas
Cogeneration system
Lipid-extracted microalgae
Nannochloropsis oculata for biodiesel production
Nannochloropsis gaditana
N-recycling
HTL
Bio-oil
Upgrading
Combustion