Biochemical methane potential of microalgae biomass after lipid extraction

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HIGHLIGHTS

- The CH4 production of oil-extracted microalgae was higher than the non-extracted one.
- Lipid-extraction process can be considered as a pretreatment.
- Thermal hydrolysis resulted in the maximum increase on CH4 productivity.
- Process economics were related to CH4 productivity and microalgae concentration.

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ABSTRACT

The anaerobic digestion of lipid-extracted Nannochloropsis at different substrate to inoculum ratios (SIR), biomass concentrations and after thermal hydrolysis pre-treatments exhibited higher CH4 production rates than its non-extracted counterpart. Thermal pretreatment supported a CH4 productivity enhancement of 40% for the non-extracted Nannochloropsis and 15% for the lipid-extracted Nannochloropsis. The higher initial rates of CH4 production for the extracted microalgae, together with this lower extent of enhancement by thermal hydrolysis, suggested that lipid-extraction constituted itself a pretreatment to increase the biochemical CH4 potential of microalgae. From an energy balance viewpoint, the minimum microalgae concentration necessary to achieve an energy-sufficient thermal hydrolysis process depends directly on the CH4 productivity of the pre-treated microalgae.

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1. Introduction

The imminent exhaustion of fossil fuel reserves has promoted intensive research on the potential of microalgae as feedstock for the production of biofuels. Despite the fact that research on microalgae-based biofuels started 50 years ago, the number of successful lab and pilot-scale studies on biodiesel, bioethanol, biogas and biohydrogen production from microalgae has rapidly increased over the last 5 years [1–3]. Interestingly, biodiesel production has received most of the attention worldwide, especially from oil-companies and public administrations (e.g. US Department of Energy) concerned about securing renewable oil replacement automotive fuels sources for the future. In this context, the use of microalgae as a source for biofuel production on an industrial scale would generate huge amounts of residual biomass. This biomass can be used as a feedstock in the production of animal feed or as a slow-release biofertilizer. Nevertheless, the chemical processing of the microalgal biomass to obtain biofuels might negatively impact on the perception of farmers to employ it as fertilizer or as a supplement in animal nutrition. An alternative use to this residual microalgal biomass is anaerobic digestion, which allows the recovery of a significant fraction of the energy and nutrients provided during microalgae cultivation. Obviously, the biomethane potential (BMP) of microalgae depends mainly on its composition (carbohydrate, lipid and protein content, which itself depends on the growth conditions [4–6]) and is species-specific [7,8]. For instance, the higher the lipid content of microalgae, the higher the potential methane yield is, but at expenses of lower kinetics rate [4]. However, although the BMP of microalgae has been extensively investigated over the last 5 years [7–10], the number of studies assessing the effect of lipid extraction (or other technics to obtain biofuels from microalgae) on the microalgae BMP is limited [11–13]. In this context, the recovery of CH4 from post-transesterified microalgae and the simultaneous H2/CH4 potential from lipid-extracted microalgae in a two stage anaerobic digester have been preliminarily evaluated [12,14].

In addition, the conversion of microalgae to CH4 is often limited by the high resistance of the microalgae cell wall to microbial...
attack [15,16]. Therefore, the application of pretreatments to disrupt the microalgae cell wall and release their intracellular content is crucial to enhance microalgae digestibility. Among the different existing pretreatments, those used to hydrolyze the cellulose in microalgae, to access to their lipid material or other specific-product may be useful to increase the BMP of microalgae [4]. Indeed, the few experimental works available on this topic have highlighted the beneficial effects of thermal hydrolysis and ultrasound pretreatment on microalgae BMP [7,10]. However, the impact of conventional pretreatments (thermal hydrolysis, ultrasound or biological pretreatment) on the BMP of lipid-extracted microalgae has not been yet assessed.

This study aimed at comparatively determining the influence of lipid extraction on the CH₄ productivity of the microalgae Nannochloropsis at different concentrations and substrate to inoculum ratios (SIR). In addition, the potential of 3 pretreatment technologies, namely thermal hydrolysis, ultrasound and biological treatment, to enhance the methane productivity of both untreated and lipid-extracted microalgae was assessed for the first time to elucidate whether lipid extraction constitutes itself a pretreatment. Finally, a global energy balance of thermal hydrolysis as a model pretreatment was conducted to determine the economic viability of this pretreatment to increase the methane productivity of oil-extracted microalgae.

2. Materials and methods

2.1. Microalgae and Inoculum

Dried Nannochloropsis gaditana was used as a model microalgae in the present study based on its high neutral lipid content and ease of large-scale cultivation. This microalgae was provided as an unwashed powder, spray-dried at 80–90 °C, frozen and vacuum-packed before lipid extraction. The spray-dried microalgae without oil extraction, namely Nannochloropsis A, possessed a lipid content of 16.3% and a concentration of total microalgae solids and volatile microalgae solids of 946 gTS/kg spray-dried sample and 684 gVS/kg spray-dried sample, respectively (VS/TS = 0.72). The dried microalgae after lipid extraction using ethanol as a solvent, namely Nannochloropsis B, contained a final solvent and lipid content of 1.5% and 6.5%, respectively. The concentration of total microalgae solids and volatile microalgae solids in the Nannochloropsis B spray-dried sample as received was 889 gTS/kg spray-dried sample and 625 gVS/kg spray-dried sample (VS/TS = 0.70). Nannochloropsis was grown in a medium with a high salt content, which resulted in the low VS/TS ratios here reported. Both microalgae were kindly supplied by Feyecon Carbon Dioxide Technologies (The Netherlands).

The anaerobic inoculum was collected from a pilot anaerobic digester treating activated sludge at 35 °C operated at the Department of Chemical Engineering and Environmental Technology at the University of Valladolid (Spain).

2.2. Anaerobic digestion batch tests

The Biochemical Methane Potential (BMP) assay was used to determine the methane productivity of Nannochloropsis A and B. Three series of tests were performed to evaluate the influence of the Substrate to Inoculum Ratio (SIR), microalgae concentration and microalgae pretreatment. The tests were conducted in serum bottles of 160 ml filled with 80 ml of a mixture of anaerobic inoculum and microalgae, provided at the concentrations below described depending on the test series. The anaerobic inoculum was supplemented with 5 g NaHCO₃/L to provide enough buffer capacity for anaerobic digestion. The bottles were closed with butyl septa, sealed with aluminum caps, purged with helium for 5 min and incubated in a thermostated room at 35 °C in a rotary shaker at 120 rpm. Reference tests containing 80 ml of inoculum were carried out in order to determine the CH₄ production potential of the inoculum. The methane production on the three series of tests was monitored by periodic measurements of the pressure of the headspace and biogas composition and it was expressed at a standard temperature and pressure (STP) of 0 °C and 1 atm, respectively. The production of CH₄ from the reference tests was subtracted from the total CH₄ production to obtain the microalgae CH₄ production. The CH₄ productivity was calculated as mlCH₄/gVS added. All tests were carried out in duplicate.

Microalgae biodegradability was calculated as the ratio of the empirical to the theoretical CH₄ production, the latter estimated assuming a theoretical production of 350 ml CH₄/g COD degraded.

2.2.1. Influence of the SIR

The concentration of microalgae was fixed at 10 gTS/kg and the SIR was set at 0.5, 1.0 and 3.0 (VSalgae:VS inoculum). The SIR ratios here tested ranged among typical values reported in literature for organic substrates BMP assays, and corresponded to Food to Microorganisms ratios that guarantee both a sufficient anaerobic population to conduct the biodegradation process (0.5) and sufficient substrate to activate the anaerobic inoculum and avoid interferences from the endogenous organic matter present in the inoculum (3).

2.2.2. Influence of microalgae concentration

The SIR was maintained constant at 1.0 (VSalgae:VS inoculum) and the microalgae concentrations were 3 gTS/kg, 10 gTS/kg and 20 gTS/kg, which correspond to concentrations typically found in conventional settlers and were selected to evaluate the anaerobic biodegradability of microalgae without further pre-concentration steps.

2.2.3. Influence of microalgae pretreatment

Nannochloropsis A and B at 10 gTS/kg were subjected to 3 different pretreatments:

2.3. Ultrasound pretreatment

This pretreatment was performed in a plastic beaker (no T control) containing 200 mL of microalgae using an ultrasound system (UP400S Ultrasonic Processor Hielscher Ultrasonomics with the ultrasonic probe immersed in the middle of the microalgae sample. Four energy inputs, calculated according to Alzate et al. [7], were tested: U₁ = 10,000 kJ/kgTS, U₂ = 27,000 kJ/kgTS, U₃ = 40,000 kJ/kgTS and U₄ = 57,000 kJ/kgTS.

2.4. Thermal hydrolysis

Samples of 200 mL of microalgae were maintained for 15 min in a stainless steel vessel heated at T₁ = 110 ± 5 °C (1.4 ± 0.2 bar), T₂ = 140 ± 4 °C (4 ± 0.2 bar) and T₃ = 170 ± 3 °C (6.4 ± 1 bar). Due to the dilution of the microalgae sample as a result of steam condensation, the final volume of the pretreated samples was measured at the end of the experiment and used for further calculations.

2.5. Biological pretreatment

A microaerobic biological pretreatment was conducted in 2 L bottles containing 500 mL of microalgae culture in the absence of any other microorganisms in order to assess the hydrolytic potential (release of extracellular enzymes) of Nannochloropsis under micro-aerobic conditions [7]. The bottles were closed with
butyl septa and aluminum caps but the air was not removed from the headspace of the bottles. The bottles were incubated in the dark in a roller shaker at 55 °C for 12 h (B1) and 24 h (B2) according to previous studies conducted with activated sludge [17]. Preliminary tests showed that the systems remained aerobic after 24 h of incubation.

The anaerobic digestion of the pretreated microalgae was conducted at a SIR of 1.0 as above described for 53 days. The solubilization degree (SD) of chemical oxygen demand (COD) was calculated according to Eq. (1) in order to evaluate the efficiency of the pretreatments:

\[
SD = \frac{COD_S - COD_{S0}}{COD_T - COD_{S0}} \times 100
\]

where \(COD_S\) is the soluble COD after pretreatment, \(COD_{S0}\) is the soluble COD in the raw microalgae and \(COD_T\) the total COD of the microalgae [19].

The concentrations of total nitrogen (TN), ammonium (\(N - NH_4^+\)), total solid (TS), volatile solid (VS), \(COD_T\) and \(COD_S\) were determined for the raw (initial), pretreated and digested microalgae, in order to assess their impact on the BMP.

2.6. Analytical procedures

\(TS, VS, COD_T\) and \(COD_S\) concentrations were determined according to APHA Standard Methods [18]. To obtain the soluble phase for the TN, \(NH_4^+\) and \(COD_S\) analysis, samples were centrifuged at 5000 rpm for 5 min in a Kubota 5100 centrifuge (Kubota Corporation-Japan). The soluble TN was measured in a TNM-1 analyzer (Shimadzu, Japan). Ammonium was determined colorimetrically according to the Nessler method using a Hitachi U-2000 Spectrophotometer (Hitachi Corporation-Japan). For the batch BMP tests, the pressure in the headspace of the bottles was maintained with a 0–1000 mbar pressure sensor PN 5007 (IFM, Germany), while the biogas composition was analyzed periodically using a gas chromatograph coupled with a thermal conductivity detector (Varian CP-3800, USA) according to Donoso-Bravo et al. [19].

The ethanol content remaining in \(Nannochloropsis\) B after lipid-extraction was determined by extracting 1 g of microalgae with 5 ml of distilled water for 2 days in a thermostated room at 35 °C. The extracted microalgal suspension was filtered through a 0.2 μm filter and the ethanol concentration quantified by HPLC-IR according to Bellido et al. [20].

3. Results and discussion

3.1. Influence of the SIR

The \(CH_4\) productivity rates for \(Nannochloropsis\) A at a SIR of 0.5 and 1 were comparable except for the two first days (Fig. 1). Hence, at day 1 and 2 the \(CH_4\) productivity rates were 90 ± 1 and 73 ± 1 mllCH4/gVS\(_{algae}\) d, respectively, for a SIR of 0.5, and 56 ± 1 and 75 ± 1 mllCH4/gVS\(_{algae}\) d for a SIR of 1, respectively. At a SIR of 3, \(Nannochloropsis\) A exhibited significantly lower \(CH_4\) production rates. For instance, the \(CH_4\) productivity rate at day 3 was 14 ± 1 mllCH4/gVS\(_{algae}\) d compared to the average 55 ± 2 mllCH4/ gVS\(_{algae}\) d at a SIR of 0.5 and 1. However, despite the initial differences in the anaerobic digestion performance at the 3 SIR tested, similar \(CH_4\) productivities were recorded at the end of the tests (303 ± 5 mllCH4/gVS\(_{algae}\) d).

For \(Nannochloropsis\) B, similar \(CH_4\) productivities rates were recorded at a SIR of 0.5 and 1, except for the 2 first days of incubation. At day 1 and 2, the \(CH_4\) productivity rates accounted for 97 ± 3 and 102 ± 1 mllCH4/gVS\(_{algae}\) d, respectively for a SIR of 0.5 and 49 ± 2 and 72 ± 2 mllCH4/gVS\(_{algae}\) d for a SIR of 1. Similarly to \(Nannochloropsis\) A, \(Nannochloropsis\) B digestion at a SIR of 3 exhibited lower initial \(CH_4\) productivity rates (17 ± 1 mllCH4/gVS\(_{algae}\) d at day 3 compared to 71 ± 3 mllCH4/gVS\(_{algae}\) d at a SIR of 0.5 and 1), and its \(CH_4\) productivity was almost the same for the three SIR tested by the end of the test (327 ± 2 mllCH4/gVS\(_{algae}\) d).

At this point it must be highlighted that despite the \(CH_4\) productivity rates here recorded after 42–52 days of experimentation do not corresponded strictly to the final BMP values of \(Nannochloropsis\) A and B (since the tests were not run to completion), the values are of practical relevance since most anaerobic digesters operate at residence times of 20–40 days.

3.2. Influence of microalgae concentration

Fig. 2 presents the BMP test performed at the three microalgae concentrations tested. The anaerobic biodegradability of \(Nannochloropsis\) A until day 6 supported similar initial \(CH_4\) production rates at biomass concentrations of 10 and 20 gTS/kg. However, the \(CH_4\) production rate at a biomass concentration of 3 gTS/kg was slightly lower than for the other two concentrations. From days 6–13, comparable \(CH_4\) production rates and productivities (205 ± 6 mllCH4/gVS\(_{algae}\) d by day 13) were recorded regardless of the microalgae concentration tested (Fig. 2a). From this day on, a significantly higher methane productivity was recorded at a concentration of 3 gTS/kg, which supported a \(CH_4\) productivity at the end of the tests of 356 ± 9 mllCH4/gVS\(_{algae}\) compared to 301 ± 4 and 282 ± 2 mllCH4/gVS\(_{algae}\) for 10 and 20 gTS/kg, respectively.

During the first 8 days, \(Nannochloropsis\) B supported similar initial \(CH_4\) production rates at biomass concentrations of 10 and 20 gTS/kg and an average methane productivity of 244 ± 4 mllCH4/ gVS\(_{algae}\). This \(CH_4\) productivity was slightly higher than that recorded at 3 gTS/kg at day 8 (203 ± 1 mllCH4/gVS\(_{algae}\) d). At the end of the test, the methane productivity at a concentration of 10 gTS/kg (326 ± 0 mllCH4/gVS\(_{algae}\) d) was 6% higher than that recorded at 20 gTS/kg (308 ± 5 mllCH4/gVS\(_{algae}\) d) but comparable to that achieved at 3 gTS/kg (326 ± 5 mllCH4/gVS\(_{algae}\) d). Once again, despite not corresponding to the final BMPs, these final productivities
The difference recorded in the overall production rate and productivity of methane at the SIRs and concentration tested, together with the duration of the tests (53 and 41 days for Nannochloropsis A and B, respectively), suggest a higher biodegradability of the oil-extracted Nannochloropsis B. The disruption of the microalgae cell wall as a result of the solvent-based oil extraction might have contributed to the biodegradability increase and the higher CH₄ productivity rates in Nannochloropsis B, although further research would be needed to elucidate the mechanisms underlying this solvent-mediated cell degradation. In addition, those differences might be also explained by the lower carbohydrate and protein content (lipids 16.3%, carbohydrates 20.6%, proteins 41.9%) of Nannochloropsis A compared to Nannochloropsis B (lipids 6.5%, carbohydrates 23.0%, proteins 46.8%), as lipid hydrolysis is slower than protein and carbohydrate hydrolysis [4]. In addition, a theoretical estimation of the CH₄ production potential based on the macromolecular composition of Nannochloropsis A and B predicts a comparable BMP for both microalgae, which agrees well with the empirical methane yields here obtained at the end of the tests. No significant differences were also recorded in the NH₄⁺ released during anaerobic digestion (Table 1). The presence of 1.5% of residual ethanol (extraction solvent) in Nannochloropsis B could have also caused the difference in the initial CH₄ production rate since alcohols often present a high anaerobic biodegradability. However, based on the equivalent COD of ethanol (1043 gO₂/gEthanol) and the ethanol content in Nannochloropsis B, the increase in CH₄ productivity associated to the presence of ethanol would be negligible (4 mL CH₄/gVSalgae). The results here obtained are in agreement with those obtained from Ehimen et al. [12], who evaluated the anaerobic biodegradability of the Chlorella residue originated from different lipid extraction processes and concluded that microalgae without lipid extraction presented the highest CH₄ potential (420 mLCH₄/gTSadded). Likewise, the anaerobic biodegradability of the extracted Chlorella was influenced by the solvent used during the lipid extraction process, with final productivities of 268 and 222 mLCH₄/gTS for the microalgae extracted with 1-butanol and the acid-catalyzed in situ transesterficated microalgae, respectively. Kinnunen and Rintala [13] also determined the CH₄ productivities of oil extracted Nannochloropsis sp. with and without a previous air-spray-drying. Wet extracted microalgae exhibited the highest final CH₄ productivities (482 mLCH₄/gVSadded) compared to the spray-dried microalgae (192 mLCH₄/gVSadded), but unfortunately those values were not compared with the microalgae without lipid extraction.

### Table 1

<table>
<thead>
<tr>
<th>Concentration (gTS/kg)</th>
<th>SIR (gVSalgae/gVSmax)</th>
<th>Ammonium (mgN-NH₄⁺/gVSalgae added)</th>
<th>Digested Nannochloropsis A</th>
<th>Digested Nannochloropsis B</th>
</tr>
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<tbody>
<tr>
<td>3</td>
<td>1.0</td>
<td>63</td>
<td>44</td>
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<tr>
<td>10</td>
<td>0.5</td>
<td>50</td>
<td>36</td>
<td></td>
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<tr>
<td>10</td>
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<td>46</td>
<td>32</td>
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<tr>
<td>10</td>
<td>3.0</td>
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<tr>
<td>20</td>
<td>1.0</td>
<td>45</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

The results obtained in this study are presented in Tables 2 and 3. Control tests refer to non-treated Nannochloropsis A or B. Ultrasonication at 40,000 and 57,000 kJ/kgTS (U3 and U4) were the most effective pretreatment to solubilize the particulate COD of Nannochloropsis A (S₀ of 19% and 21%, respectively), followed by thermal hydrolysis at 170 °C (T3) (S₀ of 18%). The lowest COD solubilizations were recorded after thermal hydrolysis at 110 °C (T1), 140 °C (T2) and after biological pretreatment B2. The concentrations of TN were much higher than the NH₄⁺ values (also in the control test), which suggests that most of the nitrogen was present as organic nitrogen. The increase in NH₄⁺ concentration after pretreatment did not correlate with the S₀ or the increase in TN concentrations, which also suggest that organic N was mainly released during biomas pre-treatment.

In terms of CH₄ productivity, thermal hydrolysis mediated the largest enhancements, the results being similar for the three temperatures tested (33–39% increase in the productivity recorded at the end of the tests compared to the control). Sonication at 10,000 kJ/kgTS (U1), 30,000 kJ/kgTS (U2) and biological pretreatments B1 and B2 resulted in a reduction in CH₄ productivity and biodegradability despite the positive S₀ values. This reduction in methane productivity after biological pretreatment might have been due to the aerobic oxidation of the solubilized organic matter through endogenous respiration during microalgae incubation [7]. In this context, despite significant BMP enhancements have been recorded during the autohydrolysis of activated sludge due to the high hydrolytic potential of bacteria [17], the reduction in methane productivity recorded agrees well with previous studies conducted in our lab during the biological pretreatment of mixed microalgae consortia [7].

For Nannochloropsis B, the results are qualitatively rather similar to those obtained for Nannochloropsis A. However, the best results in terms of solubilization were obtained for thermal hydrolysis at 140 °C and 170 °C, reaching S₀ values of 20% and 56%, respectively. Biological pretreatment resulted in negligible S₀ values. The N released was mainly present as organic N regardless of the pretreatment, and the increase in N concentration in the final medium did not correspond with the S₀. The maximum increase in methane productivity was 15% for T1 and U4, followed by 7% for T2 and U3, without any correlation with the empirical S₀ observed for these particular pretreatments. Similarly to the anaerobic digestion of Nannochloropsis A subjected to biological
productivity decreased by 3% and 13% in B1 and B2. The biodegradability decrease in the microalgae subjected to microaerobic autohydrolysis was higher, the higher the time of pretreatment applied compared to the control tests (300 ± 1 vs 331 ± 1 mL/gVS_{algae}, for Nannochloropsis A and B, respectively). This suggests that the lipid extraction process might be considered as a pretreatment step and therefore the application of a second pretreatment would have not a significant effect on the final biodegradability of the microalgae.

Overall, it can be stated that thermal hydrolysis showed the best pretreatment performance.

### 3.4. Energy balance

From the different pretreatments applied to Nannochloropsis A and B, thermal hydrolysis was the one that gave the highest increase in CH₄ productivity. Fig. 3 shows the flowchart of the thermal hydrolysis process according to Perez-Elvira et al. [21], where energy integration was considered in order to minimize operational costs. In this optimized process, the steam recovered from the flash tank is used to pre-heat the microalgae feeding. Mass and energy balances were applied to the thermal hydrolysis of Nannochloropsis A and B at 110 °C to determine the minimum microalgae concentration (X gTS/kg_{solution}) to achieve a self-sufficient process in terms of energy usage. The global mass and energy balances to the control volume selected (dashed lines in Fig. 3) can be written as follows:

\[ I + S = H \]  
\[ I(h_{L \rightarrow TL}) + S(h_{V \rightarrow TS}) = H(h_{L \rightarrow TF}) \]

where \( I \) and \( H \) represent the mass flow rate (kg/h) of the influent (at a concentration \( "x" \)) steam and hydrolyzed microalgae, respectively. \( h_{L \rightarrow TL} \) and \( h_{V \rightarrow TS} \) represent the liquid and vapor enthalpy at a temperature \( T \) (°C), respectively, while \( TL \), \( TS \), and \( TF \) correspond to the influent, steam and flash temperatures (°C), respectively.

\( X \) stands for the total solids (TS) concentration of the influent (gTS/kg).

The total flow rate of methane produced from the anaerobic digestion of the microalgae (\( M \) (m³CH₄/h)) can be calculated according to following equation:

\[ M = I(X) \times Y \frac{m^3CH_4}{kgST_{algae}} \]

where \( Y \) is the methane productivity (m³CH₄/kgTS_{algae}) of Nannochloropsis A or B after thermal hydrolysis at the end of the BMP. A conservative CH₄ productivity of 0.31 and 0.27 m³CH₄/kgTS_{algae} was considered in the energy balance conducted for Nannochloropsis A and B, respectively, which corresponds to conservative values of 300 ± 1 mL/gVS_{algae}.

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### Tables

**Table 2**

<table>
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<th>Pretreatment</th>
<th>Pre-treated Nannochloropsis A</th>
<th>Digested Nannochloropsis A</th>
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<td>S₀ (%)</td>
<td>N – NH₄⁺ (mg/L)</td>
<td>TN (mg/L)</td>
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</tr>
<tr>
<td>U1</td>
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**Table 3**

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<td>TN (mg/L)</td>
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</tr>
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<td>T3</td>
<td>56 48</td>
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Fig. 3. Scheme of the hydrolysis unit with energy recovery from flash steam to pre-heat the microalgae feed.
achieved with pretreated microalgae in all tests here carried out after 53 days.

A Combined Heat and Power (CHP) engine with a conventional efficiency of 33% in the generation of electricity was considered (former lines 305–306). Likewise, an overall efficiency of 60% was considered for the conversion of the remaining thermal energy into 9 bar steam. Methane heat combustion is 35,800 [kJ]/m^3 CH₄. So, knowing the experimental methane production Y (Table 4), the steam production is calculated by:

\[ S(hv_{TS}) = M(35800)(1 - 0.33)(0.60) \] (5)

Therefore, 27% of the methane combustion heat is not converted into electricity or steam due to heat losses in the boiler and to technical-thermodynamic constraints in the CHP. For an inlet \( I = 1 \) kg/h, the results of the balance and the values of each parameter are shown in Table 4.

Hence, the minimum microalga concentration to achieve an energy self-sufficient process should be 84 gTS/kg and 97 gTS/kg for Nannochloropsis A and B, respectively, due to the difference in their specific methane productivity. For fresh Nannochloropsis A, the typical harvested concentration is likely to be lower than 10 gTS/l, so it will be necessary to apply a strong concentration process to achieve the recommended thermal hydrolysis concentration. This pre-concentration process involves an extra cost of their specific methane productivity. For fresh Nannochloropsis A and B, respectively, due to the difference in

<table>
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<th>Parameter</th>
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<td>( I )</td>
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<td>1</td>
</tr>
<tr>
<td>( S )</td>
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<td>0.142</td>
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<tr>
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<td>1.142</td>
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<td>( TS/VS )</td>
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<td>0.71</td>
</tr>
<tr>
<td>( Y )</td>
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<td>0.311</td>
</tr>
<tr>
<td>( M )</td>
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<td>0.270X</td>
</tr>
<tr>
<td>( X )</td>
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</table>

3.5. Conclusions

The oil-extracted Nannochloropsis B exhibited higher initial methane productivities than its non-extracted counterpart, likely due to the pre-treatment effect of the solvent-based lipid extraction process, which allows the release and increased bioavailability of the intracellular microalgae content. The optimum anaerobic digestion of both the oil-extracted and non-extracted Nannochloropsis biomass was conducted at a SIR of 0.5–1, with no significant influence of biomass concentration within the tested range (0.5–2%). The methane productivity increase of 15% after thermal hydrolysis of Nannochloropsis B compared to 40% reached by Nannochloropsis A, supports the hypothesis that the lipid extraction process serves as a pretreatment for microalgae. Thermal hydrolysis of Nannochloropsis A and B could be an energy self-sufficient process at minimum microalgae concentrations of 84 and 97 gTS/kg, respectively. This minimum microalgae concentration depends on the specific methane production of microalgae, lower concentrations being needed at higher methane productivities.

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References