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Research article

Effect of pre-treatments on the production of biofuels from *Phaeodactylum tricornutum*

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ABSTRACT

Several characteristics make *Phaeodactylum tricornutum* potential candidate for biofuels production such as methane and biodiesel. For this reason, some alternatives are evaluated in this manuscript to improve the conversion of this microalgae into methane.

One of these alternatives is the addition of sewage sludge to *Phaeodactylum tricornutum* for anaerobic co-digestion. Although the co-digestion resulted in lack of synergy, the absence of inhibition indicated that both substrates could be co-digested under certain circumstances, for example if microalgae are cultivated for wastewater treatment purposes.

The extraction of lipids using organic solvents has been evaluated for biodiesel production but also as a pre-treatment for anaerobic digestion. The results revealed that the type of solvent influences lipid and biodiesel yields. The high polarity of the mixture methanol/hexane increased the lipid and the biodiesel yields from 10 ± 1 to 53 ± 2 $g_{Lipids}/100\ g_{VS}$ and from 7 ± 1 to 11 ± 1 $g_{Biodiesel}/100\ g_{VS}$ compared with hexane. However, none of these solvents affected the composition of biodiesel. Regarding the methane production after the extraction, it yielded 257 ± 8 and 180 ± 6 mL_{CH4}/g_{VS} from lipid-extracted *P. tricornutum* using hexane and methanol/hexane respectively. The methane production from the raw microalga was 258 ± 5 mL_{CH4}/g_{VS} in the same experiment. The difference in methane production, mainly after the extraction with methanol/hexane, was a consequence of the changes in the composition of the microalgae after extraction. The extraction did not influence the biodegradability.

The ultrasonic pre-treatment prior anaerobic digestion completely disrupted the microalgae cells, but the solubilisation of the organic fraction was scarce (<9.5%). The methane production from pre-treated samples was barely 10–11% higher than the obtained from non pre-treated samples, indicating that the refractory nature of the organic fraction in *P. tricornutum* is the main obstacle for the methane production.

1. Introduction

In a wide variety of microalgae species considered as promising feedstocks for renewable biofuels, the seawater *Phaeodactylum tricornutum* presents several advantages: cultivation at commercial scale, high biomass productivities and accumulation of lipids (Silva Benavides, 2013; Bellou et al., 2014; Song et al., 2014; Davis et al., 2015; Vinayak et al., 2015). Unfortunately, the scarce literature about methane production from *Phaeodactylum tricornutum* reports that the species present low degradability during anaerobic digestion (AD) (Zamalloa et al., 2012; Frigon et al., 2014; Zhao et al., 2014).

The low degradability of microalgae is usually attributed to several causes, one of which is the low C:N. The low ratio lead AD to fail due to the high concentration of ammonia generated, but it can be overcome by mixing microalgae and carbon-reach substrates. Several substrates have been co-digested with microalgae (Schwede et al., 2013; Wang et al., 2013; Olsson et al., 2014; Prajapati et al., 2014; Caporgno et al., 2016). Sewage sludge has shown synergy when co-digested with some microalgae species (Wang et al., 2013; Olsson et al., 2014; Mahdy et al., 2015). The oversized digesters in wastewater treatment plants (WWTP) (Mata-

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Alvarez et al., 2014) and the possibility of recycling nutrients by growing *P. tricornutum* in wastewaters (Davis et al., 2015) are additional reasons to investigate the co-digestion of this species and sewage sludge.

The low degradability can be also consequence of strong cell walls, which hamper the microorganisms attack. Pre-treatments, such as the ultrasonic, can break the cell walls, release internal compounds and increase the methane production (González-Fernández et al., 2012; Alzate et al., 2014). However, the effects of pre-treatment on the degradability of *P. tricornutum* have not been evaluated yet. Furthermore, organic solvents used for the extraction of lipids in the biodiesel production process can act as pre-treatment increasing the degradability of the microalgae waste (Alzate et al., 2014; Ramos-Suárez and Carreras, 2014; Caporgno et al., 2016). Although some authors reported the AD *P. tricornutum* after lipid-extraction (Frigon et al., 2014; Zhao et al., 2014), the extraction was not always performed using organic solvents. More information about the influence of the solvents is necessary.

This communication attempts to show some preliminary results about the possibility of coupling the AD of *P. tricornutum* with other processes like the sewage sludge treatment in WWTP. For this reason, the first option considers the possibility of co-digest microalgae and sewage sludge. The influence of the substrate to inoculum ratio (SIR) was evaluated for both substrates, and then, different mixtures of substrates were co-digested. Furthermore, the possibility of increasing the degradability of microalgae was evaluated by applying an ultrasonic pre-treatment at varying intensities.

A second option considers the lipid-extraction for biodiesel production and as pre-treatment for AD. The effects of different solvents on biodiesel yields and composition, and methane productions from lipid-extracted microalgae have been evaluated in these experiments.

2. Materials and methods

2.1. Materials

2.1.1. Microalgae, inoculum and sewage sludge

The marine microalgae *Phaeodactylum tricornutum* Bohlin (strain CCAP 1055/5) were obtained from the Culture Collection of Algae and Protozoa (CCAP). The cultivation was started in 200 mL culture and scaled up through 4 L cultures, using seawater (37 g/L salinity) filtered through 0.22 μm, enriched with Walne's medium (Walne, 1970) and autoclaved. Cultures were kept at 22 ± 2 °C, illuminated (16:8 light: dark cycle) with cool daylight fluorescents (Osram L30W/865) to give an irradiance of 100–140 $\mu E/m^2s$, and aerated with air. For 300 L culture, a vertical bag was used as photobioreactor. Seawater was filtered through four filter cartridges with 25, 10, 5 and 1 μm pore sizes and treated with UV light to eliminate biological contamination, and then enriched with 0.3 mL/L of the commercial fertilizer Codafol 14-6-5 and 107 μM Na_2SiO_3. Cultures were kept at 22 ± 2 °C, illuminated (16:8 light: dark cycle) with cool daylight fluorescents (Philips TLD 58W/865) to give an irradiance of ca. 200 $\mu E/m^2s$ at the culture surface, and aerated with air. Microalgae were then concentrated to approximate 70 g_{TS}/L in a continuous centrifuge. Microalgae were stored in a freezer at −20 °C until utilisation.

The sewage sludge consisted of a primary and secondary-sludge blend (65:35 v/v), collected from the municipal WWTPs in Reus (Tarragona, Spain). Regarding the inoculum, it consisted of digested sludge taken from an anaerobic semi-continuous plant as described in a previous work (Caporgno et al., 2015).

2.2. Experimental procedure

2.2.1. Biomass processing

The first experiments consisted in the co-digestion of microalgae and sewage sludge. For co-digestion, mixtures of both substrates containing 25%, 50% and 75% sewage sludge on a VS basis were fed into the reactors.

In the following experiments, the lipids from microalgae were first extracted and converted into biodiesel, and the remaining microalgae was converted into methane. The lipid extraction was performed using hexane and methanol/hexane in ratio (2:3 v:v), following the procedure detailed in (Caporgno et al., 2016). Since the microalgae were dried using freeze-drying equipment (FT33-A Freeze Drier, Armfield Inc.) prior extraction, the dried microalgae were digested to evaluate the effects of drying on AD.

The ultrasonic pre-treatment was carried out using an ultrasonic device (UP200S Hielscher Ultrasonics GmbH, Germany) at 24 kHz working frequency and 93 W ultrasonic power. The samples were disintegrated at room temperature in a water bath to avoid heating the sample. Three energy inputs were evaluated, 21 MJ/kg_{TS}, 36 MJ/kg_{TS} and 52 MJ/kg_{TS}. The Disintegration degree (Dd) was measured by the soluble COD increase:

$$Dd = \frac{(SCOD - SCOD_0) \cdot 100}{TCOD_0 - SCOD_0} \quad (1)$$

where SCOD is the soluble COD; $SCOD_0$ represents the values of soluble COD before the disintegration treatment; $TCOD_0$ represents the values of total COD before the disintegration treatment.

Microalgae samples were observed under a light microscope (ZEISS Axio Scope.A1, with ProgRes® SpeedXT core 3 camera) to evaluate the effects of ultrasonic pre-treatment on the microalgae cells.

2.2.2. Anaerobic digestion experiments

Batch reactors were set up at 33 °C following the procedure described in (Angelidaki et al., 2009). The effects of the SIR were evaluated using raw microalgae and sewage sludge as substrates; the SIRs were set at 1:4, 1:2 and 1:1 $VS_{Substrate}$:$VS_{Inoculum}$, where VS is the volatile solid content in substrates and inoculum. Based on the results, the SIR was decided at 1:2 $VS_{Substrate}$:$VS_{Inoculum}$ for the experiments using lipid-extracted and ultrasonic pre-treated microalgae.

The first order hydrolysis model was used to determine the hydrolysis constant, k_h ($days^{-1}$) (Caporgno et al., 2016). The theoretical methane potential was calculated based on the biochemical composition of the substrates, and assuming the specific methane yields of 1014 mL_{CH4}/g_{VS}, 496 mL_{CH4}/g_{VS}, 415 mL_{CH4}/g_{VS} for lipid, protein and carbohydrate respectively (Caporgno et al., 2016). The biodegradability was defined by the following equation:

$$\text{Biodegradability}(\%) = \frac{\text{measured methane production}}{\text{theoretical methane potential}} \times 100 \quad (2)$$

2.2.3. Analytical techniques

Total solids (TS), volatile solids (VS) and chemical oxygen demand (COD) were analysed according to standard methods 2540B, 2540E and 5220D respectively (Rice et al., 2012). The soluble COD (SCOD) was measured following the same procedure that for COD, but the sample consisted of the supernatant after centrifugation. The biochemical composition of microalgae was determined according to the Lowry method for protein determination (Lowry