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Evaluation of different strategies to produce biofuels from *Nannochloropsis oculata* and *Chlorella vulgaris*

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ABSTRACT

The lipid extraction using hexane and methanol:hexane increased the biodegradability of *Nannochloropsis oculata* by 36% and 24% respectively. Moreover, hexane increased the methane production from raw microalgae, from 253 ± 11 to 313 ± 9 mL_{CH4}/g_{VS}. Methanol:hexane did not affect the methane production, which yielded 254 ± 10 mL_{CH4}/g_{VS}, mainly due to the significant changes in the biomass composition.
On the other hand, the lipid extraction failed to increase the biodegradability of *Chlorella vulgaris*, which resulted around 44% for raw and lipid-extracted microalgae. The methane productions were 219 ± 6, 202 ± 1 and 200 ± 4 mL_{CH4}/g_{VS} from raw and pre-treated microalgae using hexane and methanol:hexane respectively.
Regarding the lipid extraction yields, using methanol:hexane the yields were 4.7 and 3.7 times higher for *N. oculata* and *C. vulgaris* than using hexane. The biodiesel yields were also higher using methanol:hexane, 2.4 and 1.9 times than using hexane. However, the biodiesel composition was unaffected by the solvent.
The substrate to inoculum ratio influenced raw *N. oculata* digestion. At 1:1 $VS_{Substrate}$:$VS_{Inoculum}$, the methane production throughout the first days decreased but not the ultimate methane production. *C. vulgaris* digestion was unaffected, probably due to the biomass characteristics.
Finally, the co-digestion of microalgae and sewage sludge showed no synergy, nor inhibition.

1. Introduction

Microalgae present extraordinary characteristics for producing renewable biofuels, such as high biomass productivity and accumulation of lipids amongst some others [1,2]. The genus *Nannochloropsis* and *Chlorella* are examples of promising microalgae for biodiesel and methane production [3–6]. Biodiesel has high biodegradability, low toxicity, low emission profile, and does not need major modification in engines and refuelling technology [7]; however, the biodiesel from microalgae presents economical and sustainable limitations. There are considerable efforts underway to achieve favourable energy balances and production costs, for example, trying to find cost-effective and efficient lipid extraction technologies [1]. Processes using ionic liquids [7] or wet extraction can reduce the high costs of harvesting and drying [8,9], but they are not large-scale processes yet. Methane is an alternative to biodiesel. The anaerobic digestion (AD) or methanisation is ideal for processing wet biomass, reducing the cost derived from microalgae drying. This well-known technology, widely used with manures, municipal organic solid waste and sewage sludge, has gained more attention for processing microalgae in recent years [2,6]. Another driving force behind AD is the possibility of recycling nutrients for microalgae cultivation; microalgae can uptake nutrients from the aqueous phase recovered after digestion [5,10,11].

In spite of the differences, biodiesel and methane production can be complementary. The organic solvents can break the cell walls during the lipid extraction from microalgae [12,13], acting as a pre-treatment before AD. The lipid extraction also mitigates the inhibitory effect of high concentration of lipid on AD [14]. Hexane is one of the solvents commonly used in the commercial extraction of edible lipid, and in the extraction of omega-3 LC-PUFA from microalgae [15]; it is inexpensive and offers high efficiency and suitability for industrial processes [1]. High extraction yields have been reported using polar solvents or mixtures, but most of the mixtures contain halogenated solvents considered carcinogenic [15]. Methanol:hexane (2:3 v:v) proved to be one of the best non-halogenated polar mixtures for the lipid extraction from of *Nannochloropsis sp.* [16]. After the extraction, the AD of the microalgae generates methane which can be use for electricity and/or heat in the biodiesel production [2]. An increasing number of publications recently appeared showing the benefits of coupling both processes [17].

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The anaerobic co-digestion (Aco-D) of microalgae is another favourable option to convert microalgae into methane, mainly due to the unbalanced C to N ratio (C:N) in microalgae [2,6,18,19]. Unlike AD, the co-digestion processes mixtures of substrates, resulting in a more favourable C:N. The sewage sludge is one of the most widely used co-substrates; it is generated in wastewater treatment plants (WWTP), available in large quantities and suitable for AD. The over-sized digesters in WWTP promote the utilisation of sewage sludge in co-digestion as well [20]. Sewage sludge has been co-digested with some microalgae species [21–23], but no results were reported using *Nannochloropsis*.

The present study considers various strategies to produce methane from *Nannochloropsis* and *Chlorella* species. Firstly, the influence of the substrate to inoculum ratio (SIR) on AD was evaluated for both microalgae species. The SIR markedly affects the performance of the digesters, depending on the species and characteristics of microalgae [12,14,24]. The next experiments were performed applying the lipid extraction as pre-treatment to improve AD, using hexane and methanol:hexane (2:3 v:v). The lipids recovered from the pre-treatment were converted into biodiesel, and the influence of solvents was evaluated. Finally, the last strategy consisted in the co-digestion of microalgae and sewage sludge.

2. Materials and methods

2.1. Materials

2.1.1. Microalgae, inoculum and sewage sludge

Nannochloropsis oculata and *Chlorella vulgaris* were provided by AlgoSource's (Alpha Biotech, Asserac, France). *N. oculata* was received as frozen slurry with 28% total solids, thus stored at −15 °C until required. *C. vulgaris* was received dried, thus stored in a dessicator. Both microalgae are hereinafter referred to as "raw" biomass.

The inoculum utilised in AD experiments consisted of digested sludge obtained from a mesophilic pilot-plant (33 °C) under semi-continuous operating conditions. Prior to setting the experiments, the inoculum was "degassed" as described in Caporgno et al. [23]. The sewage sludge utilised for feeding the pilot-plant and the batch digesters in co-digestion consisted of a primary and secondary-sludge blend (65:35 *v/v*), collected from the municipal WWTPs in Reus (Tarragona, Spain). This blend is representative of the sludge generated in the WWTPs.

2.2. Experimental procedure

2.2.1. Pre-treatment with organic solvents: lipid extraction

Before the pre-treatment, *N. oculata* was freeze-dried (FT33-A Freeze Drier, Armfield Inc.); *C. vulgaris* drying was unnecessary. For the pre-treatment, 2 g of dried microalgae were extracted in a Soxhlet apparatus with a reflux period of 7 h. Hexane and methanol:hexane (2:3 v:v) were utilised as solvents. The recovered lipids were converted into biodiesel, identified and quantified according to the procedure described by Olkiewicz et al. [25]. The pre-treated microalgae were anaerobically digested. Prior to AD, the biomass was left unstoppered in a hood for several hours until complete evaporation of the remaining solvent.

2.2.2. Anaerobic digestion experiments

Batch reactors were set up at 33 °C following the procedure described by Angelidaki and Sanders for the determination of methane potential [26]. The substrates differed in the experiments. The microalgae samples were re-suspended in deionised water before digestion, resulting in a solid concentration similar to sewage sludge.

The SIR experiments were performed using only raw microalgae 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.

The effects of the pre-treatment were evaluated using pre-treated and raw microalgae. Since *N. oculata* was freeze-dried before the pre-treatment, the possible effects of the drying process were evaluated using freeze-dried microalgae. The SIR was 1:2 $VS_{Substrate}$:$VS_{Inoculum}$ in all reactors.

Co-digestion was performed using mixtures of raw *N. oculata* and sewage sludge. The mixtures contained 25%, 50% and 75% sewage sludge on a VS basis. The SIR was 1:2 $VS_{Substrate}$:$VS_{Inoculum}$ in all reactors.

2.3. Analytical techniques

2.3.1. Substrate characterisation

Total solids (TS), volatile solids (VS) and chemical oxygen demand (COD) were analysed according to standard methods 2540B, 2540E and 5220D respectively [27]. Protein, carbohydrate and lipid content in raw and pre-treated biomass were quantified as described in [23]. The characteristics of the inoculum and the substrates are summarised in Table 1. The TS and COD values for raw microalgae correspond to the microalgae suspensions in deionised water used in the experiments.

Raw and pre-treated microalgae samples were analysed by Fourier Transform Infrared (FTIR) spectroscopy using a Fourier Jasco FT/IR-600 Plus spectrometer with a diamond golden gate ATR (GS10542, Specac Ltd) reflectance cell.

2.3.2. Products characterisation

The biogas production and its composition, and the volatile fatty acid concentration (VFA) were measured following the procedure described in [23]. The ammonia concentration was determined with an ion selective electrode (Ammonia Gas Sensing combination electrode, mod. 51927–00, HACH).

The first order hydrolysis model [26] was used for hydrolysis rate calculation, Eq. (1):

$$\ln \frac{B_0 - B}{B_0} = -k_h \cdot t \tag{1}$$

where B is the cumulative methane yield at the time t (units: mL_{CH4}/g_{VS}), k_h ($days^{-1}$) is the first order hydrolysis constant and B_0 (mL_{CH4}/g_{VS}) is the ultimate methane production. The values of k_h and B_0 were determined in MS Excel 2007® using the Solver tool, by minimising the residual sum of squared errors between the experimental data and the data predicted by the model.

The theoretical methane potential was calculated based on the relative fractions of lipid, protein and carbohydrate in 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 [26]. The biodegradability was then calculated considering the measured and the theoretical methane production, Eq. (2):

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

Microalgae cells before and recovered after AD were observed under light microscope to evaluate the integrity of the cells (ZEISS Axio Scope.A1, with ProgRes® SpeedXT core 3 camera).

3. Results and discussion

3.1. The influence of the SIR on anaerobic digestion

The influence of the SIR on AD was evaluated in order to determine the most suitable SIR for the experiments. The ultimate methane productions and the k_h for *N. ocualta* and *C. vulgaris* are listed in Table 2.

The SIR did not affect the ultimate methane production from *N. oculata* and *C. vulgaris*; the differences were smaller than 3% in the experiments using the same microalgae species. On the contrary, the k_h