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Short Communication

A novel pre-treatment for the methane production from microalgae by using N-methylmorpholine-N-oxide (NMMO)

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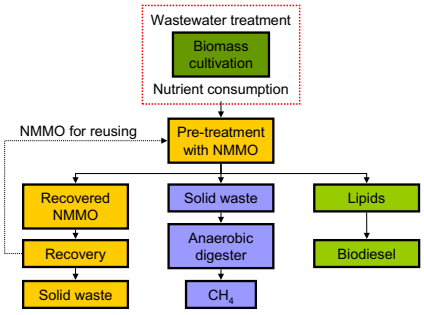
HIGHLIGHTS

- The NMMO damages the *N. oculata* cells, increasing the biodegradability.
- The lipid fraction remains inside the microalgae cells after the pre-treatment.
- The drying of *C. vulgaris* may have reduced the effectiveness of the pre-treatment.
- The NMMO could be fully recycled since does not decompose during the pre-treatment.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of this work was to study the effect of the solvent N-methylmorpholine-N-oxide (NMMO) to pre-treat *Nannochloropsis oculata* before the anaerobic digestion process. The results indicated that the pre-treatment affects the characteristics of the cell wall, which consequently becomes more susceptible to the microorganisms attack during anaerobic digestion. The methane production was increased by 43% after the pre-treatment, from 238 ± 6 mL_{CH4}/g_{VS} until 339 ± 4 mL_{CH4}/g_{VS}. On the contrary, the methane production from *Chlorella vulgaris* decreased after the pre-treatment from 251 ± 4 mL_{CH4}/g_{VS} to 231 ± 3 mL_{CH4}/g_{VS}. The failure on the pre-treatment was attributed to the particular characteristics of the substrate in consequence of a previous drying step.

1. Introduction

Microalgae have the ability accumulate high amount of lipids, being promising feedstocks for biofuels (Rawat et al., 2013). Over the past years, researchers turned their attention to produce biodiesel from microalgae. Unfortunately, some challenges still required still need to be faced to scale up the biodiesel production, mainly derived from the high water content in microalgae cultures (Rawat et al., 2013). The anaerobic digestion (AD) is an alternative to produce energy from microalgae, in this case, biogas. The AD does not require the biomass drying, thus reducing the cost associated with harvesting (Kwietniewska and Tys, 2014). Moreover, AD results favourable when the biomass has low lipid content for the biodiesel production (Pragya et al., 2013). Some microalgae species such as *Chlorella* sp. and *Nannochloropsis* sp. demonstrated their suitability to grow in different wastewaters consuming nutrients, reducing the demand for fertilizer during their cultivation (He et al., 2013; Caporgno et al., 2015b). This allows coupling the cultivation and the AD of microalgae in a wastewater treatment plant (WWTP), with environmental and economic benefits.

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The organic solvent N-methylmorpholine-N-oxide (NMMO) has facilitated the AD of carbohydrates in lignocellulosic material, which is characterised by high resistance to degradation (Teghammar et al., 2014; Kabir et al., 2014). Since the NMMO is usually used as an aqueous solution, is does not require the biomass drying. However, the amount of water affects the process (Jeihanipour et al., 2010a). This solvent is commercially used in the Lyocell fibre-production process due to the feasibility to act as solvent for cellulose at mild conditions (90–130 °C and ambient pressure) (Zheng et al., 2014). The NMMO shows low toxicity, high thermal stability, and the possibility of being recovered and reused, amongst other advantages (Jeihanipour et al., 2010a). The NMMO affects hydrogen bonds and weaken van der Waals forces between the cellulose chain molecules, thus changing the cellulose structure (Zheng et al., 2014). Some of these changes, for example a decreased crystallinity and an increased porosity make cellulose more susceptible to AD (Jeihanipour et al., 2010a).

This paper is the first attempt to pre-treat microalgae, *Nannochloropsis oculata* and *Chlorella vulgaris*, using NMMO in order to improve the methane production.

2. Methods

2.1. Materials

N. oculata and *C. vulgaris* species were provided by AlgoSource (Alpha Biotech, Asserac, France). They were cultivated in a raceway unit placed in a greenhouse with thermal control, and harvested via centrifugation. *N. oculata* microalgae was received as frozen slurry with 28% solids and stored at −15 °C. In order to facilitate the pre-treatment, the biomass was dried before the experiment. *C. vulgaris* was received dried, and the solid was stored in a dessicator.

NMMO 50% w/w in aqueous solution was concentrated to 85% as described by Jeihanipour et al. (2010a).

2.2. Microalgae pre-treatment

The pre-treatment process is described in Fig. 1. The microalgae sample, 6 g of dried biomass, was added into a round-bottom flask containing 94 g of 85% NMMO solution and placed in an oil bath at 120 °C for 3 h in atmospheric conditions. Agitation was provided by a magnetic stirrer. After the 3 h heating, 150 mL of boiling deionised water was added to stop the process. The pre-treated microalgae were recovered by centrifugation as a solid fraction and washed with deionised water to eliminate the NMMO prior to the anaerobic digestion. The supernatant, which is the recovered NMMO, and liquid fraction from washing were collected together for further analysis. The pre-treatment was evaluated in three different opportunities.

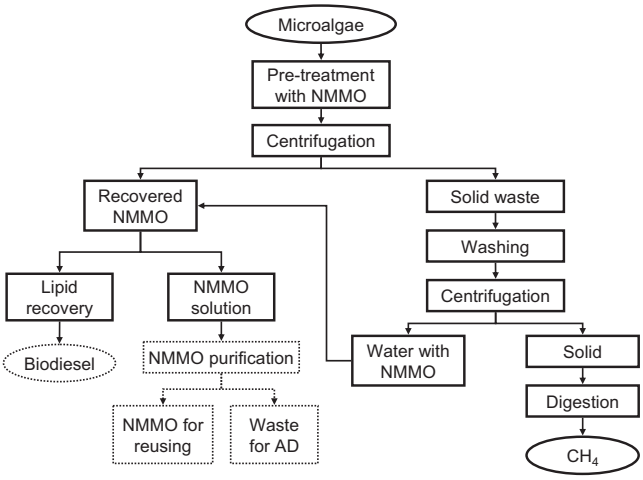

Fig. 1. Scheme of the pre-treatment process and the uses of the different products.

Microalgae samples, before and after the pre-treatment, were observed under a light microscope to evaluate their integrity The samples were also analysed by Fourier Transform Infrared (FTIR) spectroscopy to observe the changes in the protein, carbohydrate and lipid content in the biomass. Protein, carbohydrate and lipid content in raw and pre-treated samples were quantified as described by Caporgno et al. (2015a). In *C. vulgaris*, the carbohydrate content was calculated by the difference between total organic matter content and the contents of proteins and lipids.

2.3. Lipid extraction

The lipid content was analysed in the mixture containing the NMMO solutions collected after the microalgae pre-treatment and after washing, as indicated in Fig. 1. A sample of the mixture containing the NMMO was mixed with 10 ml of hexane; the hexane phase containing lipids was separated by centrifugation (3500 rpm, 10 min). This step was repeated three times. Hexane was dried under anhydrous sodium sulphate and evaporated in a rotary evaporator. For the lipid analysis in the pre-treated microalgae, a sample of microalgae was re-suspended in deionised water and acidified until pH 2 prior to the hexane addition. The results were expressed as gram of extractable lipids per gram of volatile solids in the dry microalgae used for the experiments, g/g_{VS}.

A thin-layer chromatography (TLC) was performed in order to evaluate the composition of the extracted-lipids. The lipids were dissolved in hexane and spotted on a TLC plate which was then developed in a solvent system of hexane/diethyl ether/acetic acid (60:40:1, v/v/v). Separated compounds were visualised under iodine vapour and identified by using authentic standards. The fatty acids in the extracted lipids were identified and quantified as described by Olkiewicz et al. (2014).

2.4. Anaerobic digestion

The AD experiments were performed in batch reactors at 33 °C in triplicate. The solid samples were re-suspended in deionised water for better handling. The inoculum consisted of digested sludge described in Caporgno et al. (2015a). The total solids (TS) and the volatile solids (VS) in all the substrates and in the inoculum were analysed according to standard methods as described by Caporgno et al. (2015a), and the substrate to inoculum ratio was adjusted to 1:2 $VS_{Substrate}$:$VS_{Inoculum}$ in all reactors.

The methodology for quantifying the biogas production and composition, the volatile fatty acid concentration (VFA), the ammonia concentration and the substrates biodegradability is fully described in Caporgno et al. (2015a).

3. Results and discussion

3.1. Lipid recovery after pre-treatment

Since the lipids are not solubilised in the NMMO solution due to the differences in the polarity of lipids and the NMMO solution, the released lipids can be recovered by hexane addition. The results indicated that 3.0 ± 0.2 and 0.9 ± 0.3 g/g_{VS} were recovered from the NMMO solution after *N. oculata* and *C. vulgaris* pre-treatment respectively. These were minor fractions considering the initial lipid content in both microalgae species (Table 1). The results indicated that the lipids were not released during the pre-treatment