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Biochemical methane potential of microalgae biomass after lipid extraction

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HIGHLIGHTS

- The CH_4 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 CH_4 productivity.
- Process economics were related to CH_4 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 CH_4 production rates than its non-extracted counterpart. Thermal pretreatment supported a CH_4 productivity enhancement of 40% for the non-extracted *Nannochloropsis* and 15% for the lipid-extracted *Nannochloropsis*. The higher initial rates of CH_4 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 CH_4 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 CH_4 productivity of the pre-treated microalgae.

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 CH_4 from post-transesterified microalgae and the simultaneous H_2/CH_4 potential from lipid-extracted microalgae in a two stage anaerobic digester have been preliminarily evaluated [12,14]

In addition, the conversion of microalgae to CH_4 is often limited by the high resistance of the microalgae cell wall to microbial

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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 CH_4 productivity of the microalga *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 microalga 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 $gTS/kg_{spray\text{-}driedsample}$ and 684 $gVS/kg_{spray\text{-}driedsample}$, 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 $gTS/kg_{spray\text{-}driedsample}$ and 625 $gVS/kg_{spray\text{-}driedsample}$ (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 $NaHCO_3$/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 CH_4 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 CH_4 from the reference tests was subtracted from the total CH_4 production to obtain the microalgae CH_4 production. The CH_4 productivity was calculated as $mlCH_4/gVS_{algae\text{-}added}$. All tests were carried out in duplicate.

Microalgae biodegradability was calculated as the ratio of the empirical to the theoretical CH_4 production, the latter estimated assuming a theoretical production of 350 ml $CH_4/g\ COD_{degraded}$.

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 (VS_{algae}:$VS_{inoculum}$). 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 (VS_{algae}:$VS_{inoculum}$) 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 Ultrasonics 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 °C ± 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 microalgal 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