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Biochemical methane potential of microalgae: Influence of substrate to inoculum ratio, biomass concentration and pretreatment

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HIGHLIGHTS

- Anaerobic digestion of microalgae have highest productivities at S/I ratios of 0.5.
- Methane productivity is depended of microalgae specie.
- The microalgae concentration for anaerobic digestion must be greater than 10 gTS/kg.
- COD solubilization no imply an improvement on methane productivity.
- Thermal hydrolysis substantially improves methane productivity of microalgae.

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ABSTRACT

The anaerobic digestion of three microalgae mixtures was evaluated at different substrate to inoculum (S/I) ratios (0.5, 1 and 3), biomass concentrations (3, 10 and 20 gTS/kg) and pretreatments (thermal hydrolysis, ultrasound and biological treatment). An S/I ratio of 0.5 and 10 gTS/kg resulted in the highest final methane productivities regardless of the microalgae tested (ranging from 188 to 395 mL CH_4/gVS_{added}). The biological pretreatment supported negligible enhancements on CH_4 productivity, while the highest increase (46–62%) was achieved for the thermal hydrolysis. The optimum temperature of this pretreatment depended on the microalgae species. The ultrasound pretreatment brought about increases in CH_4 productivity ranging from 6% to 24% at 10,000 kJ/kgTS, without further increases at higher energy inputs. The results here obtained confirmed the lack of correlation between the solubilization degree and the methane enhancement potential and pointed out that anaerobic digestion of algae after thermal pretreatment is a promising technology for renewable energy production.

1. Introduction

Microalgae as a feedstock for biogas production have been studied since the early fifties based on their large areal productivities compared to conventional crops (Chisti, 2007; Golueke et al., 1957). In the recent years, the need to seek for renewable energy sources to replace fossil fuels has triggered the research on microalgae anaerobic digestion. However, few systematic studies have been conducted to date to fully explore the maximum biodegradability of microalgae and to enhance their CH_4 productivity.

The biochemical methane potential (BMP) assay constitutes a useful tool to determine both the ultimate biodegradability and the methane conversion yield of organic substrates. In this context, the determination of the BMP of an organic residue can help in the design and economic evaluation of a biogas plant (Angelidaki et al., 2009). In this assay, the substrate to inoculum (S/I) ratio is a key parameter affecting the result. In addition, the determination of the optimum S/I ratio for a specific residue could also help to establish a start-up protocol for continuous anaerobic digesters in order to optimize this critical operational stage (Fernández et al., 2001).

The concentration of substrate in the BMP assay also impacts on the final biodegradability and methane productivity. Hence, an excessive concentration of solids makes proper mixing more difficult and could generate inhibition by accumulation of fatty acids. On the other hand, too low substrate concentrations significantly increase the process heating costs and require larger digester volumes in order to avoid the wash-out of anaerobic biomass. Additionally, some studies have shown that the initial substrate concentration can modify the methane content in the biogas and the methane productivity (Fernández et al., 2001, 2008). In this regard, microalgal cultures have low biomass concentrations (<0.5–27 g/L) (Molina et al., 2003; Suh and Lee, 2003) and the need to concentrate this biomass before anaerobic digestion could increase

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the production cost of microalgae, which could eventually jeopardize the economics of the entire biofuel production process. Hence, in order to avoid extra biomass harvesting costs the determination of the optimal concentration for anaerobic digestion of microalgae is crucial.

Preliminary studies on anaerobic digestion of microalgae have shown low methane productivities (0.18–0.39 LCH_4/g VS) compared to municipal solid waste or fruit and vegetables waste (0.39–0.53 LCH_4/g VS) (Gunnaseelan, 1997; Mussgnug et al., 2010). These experimental findings have been attributed to the strong cell walls of microalgae, which make them highly resistant to bacterial attack even when cells are not alive (Golueke et al., 1957). Recalcitrant compounds like polyaromatics, heteropolysaccharides, algaenan, sporopollenin, silica, uronic acid and lignine were found in the cell walls of microalgae that showed a low biodegradability (Gunnison and Alexander, 1975a,b,c; Sander and Murthy, 2009). In this context, a pretreatment step able to break up the cell wall of microalgae could increase their biodegradability and therefore their CH_4 productivity. Different pretreatments have been successfully applied to activated and primary sludge to enhance its CH_4 productivity. These pretreatments can be classified as mechanical (ultrasonic, lysis – centrifuge, liquid shear, collision plate, grinding, etc.), biological (aerobic, microaerophilic or anaerobic), thermal and chemical (oxidation, alkali treatments, etc.). For instance, CH_4 productivity enhancements of up to 100% have been recorded when using thermal hydrolysis and ultrasound in activated sludge (AS) (Carrère et al., 2010). Biological pretreatments based on increasing the bacterial hydrolytic activity have also enhanced methane productivity by 86% when applied to activated sludge (Carrère et al., 2010). Even though these pretreatments could also be used for microalgae, little information is available regarding the potential of microalgal pretreatments to enhance their anaerobic digestion of algal biomass.

In this study, the influence of different S/I ratios and biomass concentrations on the BMP was evaluated in three different microalgae. In addition, the potential of thermal hydrolysis, ultrasound and a biological treatment (hydrolytic activity) for increasing the biodegradability and CH_4 productivity in these microalgae was assessed.

2. Methods

2.1. Microalgae and inoculum

Microalgae A, a mixture of microalgae cultivated in a synthetic mineral salt medium in a tubular photobioreactor and free of bacterial contamination was kindly provided by Cajamar Foundation (Almeria, Spain). This microalgae mixture was composed of 40% *Chlamydomonas*, 20% *Scenedesmus* and 40% of an unknown microalgae tentatively characterized as *Nannocloropsis*. The concentration of total solids as received was ≈180 gTS/kg. These microalgae were refrigerated at 4 °C prior to use.

Microalgae B and C were cultivated in a 180-L open photobioreactor operated in a continuous culture mode at 36 days of hydraulic residence time and artificially illuminated at the Department of Chemical Engineering and Environmental Technology at the University of Valladolid (Spain). The photobioreactor was fed with anaerobic digestion effluent and synthetic biogas. Microalgae B were harvested from the bottom of a settler located at the photobioreactor outlet with a concentration of ≈10 gTS/kg. The composition of this microalgae mixture was 58% *Acutodesmus obliquus*, 36% *Oocystis* sp., 1% *Phormidium* and 5% *Nitzschia* sp. Microalgae mixture C, mainly composed of *Microspora,* was harvested from the surface of the photobioreactor (autoflotation) at ≈40 g TS/kg. Both microalgae mixtures were refrigerated at 4 °C prior to use.

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

2.2. Anaerobic digestion batch tests

Three series of tests (BMP assays) were conducted to determine the influence of the S/I ratio, microalgae concentration and microalgae pretreatment on the BMP and microalgae biodegradability. The tests were performed in serum bottles of 160 ml filled with 80 ml of a mixture of anaerobic inoculum and microalgae (untreated or pretreated). To provide enough buffer capacity for anaerobic digestion, the anaerobic inoculum was supplemented with 5 g $NaHCO_3$/L. The bottles were closed with butyl septa, sealed with aluminum caps, purged with helium for 15 min and incubated in a thermostated room at 35 °C in a rotary shaker at 120 rpm. Control tests containing 80 mL of inoculum were carried out in order to determine the CH_4 production potential of the inoculum. The production of methane from the inoculum (obtained from the control tests) was subtracted from the total methane production to obtain the microalgae methane production.

2.2.1. Ratio test

The microalgae concentration was kept constant at 10 gTS/kg and three S/I ratios were tested: 0.5, 1 and 3 ($VS_{microalgae}$:$VS_{inoculum}$).

2.2.2. Concentration test

The S/I ratio was keep constant at 1 ($VS_{microalgae}$:$VS_{inoculum}$) and three microalgae concentrations were tested: 3, 10 and 20 gTS/kg approximately. This concentration range was selected to evaluate the BMP of microalgae harvested using low cost technology such as sedimentation, where microalgae concentrations reach concentrations of ≈1,6% TSS (Mohn, 1980).

2.2.3. Pretreatment test

Tests with pretreated microalgae (as described below) were conducted at a S/I ratio of ≈1 and microalgae concentrations of 9–10 gTS/kg, except for the microalgae subjected to thermal hydrolysis, which experienced a dilution during this pretreatment. Tests carried out with non-pretreated biomass were used as controls.

The anaerobic digestion process in the three series of tests was monitored by periodic measurements of the pressure of the headspace and biogas composition. The BMP assays were stopped when the daily methane production was less than the 1% of the total accumulated methane. All tests were carried out in duplicate. The methane production was expressed at a standard temperature and pressure (STP) of 0 °C and 1 atm, respectively. 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 mLCH_4/g $COD_{degraded}$. The total nitrogen (TN), total ammonium (N–NH_4^+), total solid (TS), volatile solid (VS), total and soluble chemical oxygen demand (COD_T–COD_S) concentrations were determined for the raw (initial) and digested microalgae.

2.3. Microalgae pretreatments

Microalgae were diluted (when necessary) with distilled water to a concentration of 9–10 gTS/kg prior to pretreatment. All pretreatments were carried out in batch mode. TN, N-NH_4^+, TS, VS, COD_T and COD_S concentrations were determined before and after each pretreatment. Three different pretreatments were applied to the three target microalgae: