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Chlorella vulgaris vs cyanobacterial biomasses: Comparison in terms of biomass productivity and biogas yield

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ABSTRACT

The aim of the present study was to compare cyanobacteria strains (*Aphanizomenon ovalisporum*, *Anabaena planctonica*, *Borzia trilocularis* and *Synechocystis* sp.) and microalgae (*Chlorella vulgaris*) in terms of growth rate, biochemical profile and methane production. Cyanobacteria growth rate ranged 0.5–0.6 day^{-1} for *A. planctonica*, *A. ovalisporum* and *Synecochystis* sp. and 0.4 day^{-1} for *B. tricularis*. Opposite, *C. vulgaris* maximum growth rate was double (1.2 day^{-1}) than that of cyanobacteria. Regarding the methane yield, microalgae *C. vulgaris* averaged 120 mL CH_4 g COD in^{-1} due to the presence of a strong cell wall. On the other hand, anaerobic digestion of cyanobacteria supported higher methane yields. *B. trilocularis* and *A. planctonica* presented 1.42-fold higher methane yield than microalgae while this value was raised to approximately 1.85-fold for *A. ovalisporum* and *Synechochystis* sp. In the biogas production context, this study showed that the low growth rates of cyanobacteria can be overcome by their increased anaerobic digestibility when compared to their microalgae counterpartners, such is the case of *C. vulgaris*.

1. Introduction

Algae biofuels may provide a viable alternative to fossil fuels. Among biofuel production processes using microalgal biomass, biogas generation seems to be the least complex. Opposite to biodiesel and bioethanol production, where only small fractions of the cell (lipids or sugars) are used, methane might be produced using all three macromolecules. Additionally, biogas production through anaerobic digestion avoids energy intensive steps such as biomass drying and extraction. Photosynthetic microorganisms could potentially be integrated in a wastewater treatment plant and combine the benefits of nutrient removal, energy production, and CO_2 sequestration. Nevertheless, the methane production potential of the different photosynthetic microorganisms that could conduct this dual purpose (bioremediation and energy production) should be further investigated.

Microalgae cell wall and biochemical composition affects markedly their anaerobic digestion potential [1,2]. More specifically, microalgae cells are protected by a semi-rigid structure that hinders the hydrolysis of organic matter [3]. In order to increase the efficiency of the digestion process, microalgae cell walls should be disrupted prior anaerobic digestion. These pretreatment methods to enhance methane production are increasing the cost of production and thus compromising the feasibility of this technology [3,4]. Cyanobacteria may play a crucial role to circumvent this major drawback of microalgae. The resemblance is that these microorganisms are also photosynthetic but their cell walls characteristics are different. Cyanobacteria present a cell envelope typical of gram-negative bacteria (peptidoglycan outer-membrane [5]). The lack of cellulose and other complex polymers renders cyanobacteria biomass as an ideal substrate for anaerobic digestion. Up to now, cyanobacteria has not been extensively studied for biogas production. As a matter of fact, studies dealing with that topic mainly focused on *Arthrospira* (*Spirulina*) *maxima* and *Microcystis* spp. [6,7]. In those studies, methane production ranged 0.2 and 0.36 L g VS^{-1} for *Microcystis* and *Arthorspira maxima* when operated at 30 days hydraulic retention time of digestion, respectively.

It is commonly found in literature that cyanobacteria growth rate is much lower than that of many algal species [8,9]. Nevertheless, Lürling et al. [10] demonstrated that at optimum temperature of 29 °C, mean growth rates were similar for cyanobacteria (0.92 day^{-1}) and chlorophytes (0.96 day^{-1}), while when those photosynthetic microorganisms were cultivated at lower temperatures, the growth rates of cyanobacteria is 30% of that of microalgae. Opposite, under low light intensities, cyanobacteria growth rate is higher than that of microalgae [11]. For both types of photosynthetic microorganisms, it seems likely that growth rate

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is size-dependent when grown as unicells, while this does not stand when colonies are formed [12]. Cyanobacteria are unicellular and usually aggregate in colonies and therefore, this may explain the lower growth rates observed. However, their slow growth rates are compensated by the high prevalence of populations once they have been established [13].

The goal of the current study was to explore the biomethane production potential of different cyanobacteria strains (*Aphanizomenon ovalisporum*, *Anabaena planctonica*, *Borzia trilocularis* and *Synechocystis* sp.). Additionally, those cyanobacteria were compared to a common and robust microalgae strain (*Chlorella vulgaris*) in terms of biomass productivity, biochemical characterization and methane production.

2. Material and methods

2.1. Photosynthetic microorganisms and culturing conditions

Biological substrates used as substrates for anaerobic digestion included four cyanobacteria and a microalgae strain. *A. ovalisporum*, *A. planctonica* were kindly provided by Autónoma University of Madrid (Spain) while *B. trilocularis* and *Synechocystis* sp. were kindly supplied by Georg-August-Universität Göttingen (Germany). The cultures were prepared by inoculating in a 0.5 L erlenmeyer flask all the biomasses at 0.4 g VSS (volatile suspended solids) L^{-1}. *C. vulgaris* was collected in the wastewater treatment plant of Valladolid (Spain) and cultivated in Modified Basal medium [3]. On the other hand, cyanobacteria strains were grown in BG 11. Culture broth was maintained at 25 °C under continuous agitation supplied by air bubbling and continuous illumination 2460 lux. Microorganisms were periodically collected and analyzed for growth determination (VSS). All these biomasses were cultured in duplicates for a period of 13–15 days. The specific growth rate was determined by a first order dynamic Eq. (1):

$$dX/dt = \mu X \quad (1)$$

where X is the concentration of biomass (g L^{-1}) at time t(d), and μ is the specific growth rate (day^{-1}). The specific growth rates can be calculated using the following equation:

$$\mu = [\mathrm{Ln}(Xt/X_o)]/(t - t_o) \quad (2)$$

where X_o is the concentration of biomass (g L^{-1}) at initial time (t_o) and Xt is the concentration of biomass at specific time (t).

The volumetric productivity rate (Q, g L^{-1} day^{-1}) was estimated using the following equation:

$$Q = (Xt - X_o)/(t - t_o) \quad (3)$$

2.2. Biomethane potential assays

Anaerobic sludge employed was collected at the wastewater treatment plant of Valladolid (Spain). Anaerobic biomass presented total solids (TS) concentration of 17.2 g L^{-1} and volatile solids (VS)/TS of around 60%. Anaerobic digestion was conducted in batch mode for approximately one month. Fermenters were glass bottles with 0.120 L capacity incubated at 35 °C and agitated at 120 rpm. To keep anaerobic conditions, oxygen was removed from digesters purging the headspace with helium, and closed with butyl rubber seals and aluminum caps. Calculations were set to achieve a final volume of 0.070 L of liquid fraction for each bottle, and thus allowing 42% of the total volume for biogas production. Anaerobic sludge was mixed with the tested biomasses in order to obtain COD/VS ratio of 0.5 (g g^{-1}) [14]. Photosynthetic microorganisms were concentrated previous anaerobic digestion by centrifugation. Digesters were run in duplicates and inoculated with microalgae and cyanobacteria biomasses. The volume of biogas produced by the substrates was calculated by measuring the pressure of the bottle's headspace. In addition, bottles containing only anaerobic sludge were run as blanks for quantification of endogenous methane production, and controls using ethanol as substrate to check the correct performance of the anaerobic microorganisms.

2.3. Analytical methods

Total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS) and total Kjeldahl nitrogen (TKN) were measured according to Standard Methods [15]. Proteins were calculated by multiplying TKN results by 5.95 [16]. The carbohydrates content was analyzed by phenol–sulphuric acid method [17]. COD was analyzed by a colorimetric method using Hach vials. To obtain soluble fractions, the samples were centrifuged at 14,000 rpm for 10 min (Eppendorf 5424). Biogas composition was measured by gas chromatography (Agilent 7820A) equipped with HP-PLOT Q column and thermal conductivity detector.

3. Results and discussion

3.1. Growth rate and productivity

Since different nutrition modes, light–dark cycles, temperature and other parameters may affect markedly the results obtained in the different studies found in literature, the present investigation was designed to provide a fair comparison between cyanobacteria and microalgae. The microalgae strain, *C. vulgaris*, was selected in accordance to its predominance in wastewaters. Even though maximum growth rate would entail optimum growing conditions, this study was designed for comparison purposes between cyanobacteria and microalgae. In this context, from now on when referring to maximum growth rate, this parameter refers to the cultivation conditions used for comparison. As it can be seen in Fig. 1, cyanobacteria growth rates were quite lower than microalgae. As a matter of fact, the maximum growth rate ranged 0.5–0.6 day^{-1} for *A. planctonica*, *A. ovalisporum* and *Synecochystis* sp. while even lower results (0.4 day^{-1}) were recorded for *B. tricularis*. These values are in good agreement with Lürling et al. [10] who also reported an average growth rate of 0.6 day^{-1} for several cyanobacteria grown at 25 °C. With regard to the microalgae strain, the maximum growth rate achieved during the first days of cultivation of *C. vulgaris* was double than the observed for cyanobacteria. In this context, *C. vulgaris* exhibited a maximum growth rate of 1.2 day^{-1}. While the growth rates shown by this strain when grown in wastewater is slightly lower, the values can be raised up to 1.6 day^{-1} when grown in mineral medium [18].

Similar tendency was attained for the volumetric productivities (Qv). *C. vulgaris* exhibited an exponential growth since inoculation. In this manner, after 2 days of cultivation, the highest volumetric biomass productivity was 0.5 g DW L^{-1} d^{-1} and decreased onwards until day 10th, after which the Qv remained constant (Fig. 2A). Opposite to *C. vulgaris* that grew exponentially right after inoculation, cyanobacteria concomitantly increased biomass production along with cultivation time and therefore Qv remained constant along the cultivation time. This trend was observed for *A. planctonica* (0.12 g DW L^{-1} d^{-1}) and *B. tricularis* and *A. ovalisporum* (0.1 g DW L^{-1} d^{-1}, Fig. 2B). Opposite to the other cyanobacteria, *Synechocystis* sp. displayed an exponential growth during the first days of cultivation and then remained constant after 6 days of cultivation. No comparison can be made with literature dealing with this parameter since different reactor configuration and operational conditions reported different results. In this manner, Cea-Barcia