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Influence of growth kinetics of microalgal cultures on biogas production

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ABSTRACT

The microalgal cultures growth curve shows different phases involving changes in the physical-chemical characteristics of the microalgae. The aim of this study was to determine if the physico-chemical differences involve different biogas productions during the different stages of growth kinetics of *C. sorokiniana*. The microalgae growth kinetics was evaluated in batch cultures. Three phases were differentiated on the growth curve: P1, beginning of the exponential growth; P2, end of the exponential growth, and P3, steady state phase. The physico-chemical characterization, the biochemical methane potential, the biogas production rates by the Gompertz model, and the energy balance for each one of the three phases of the growth curve were evaluated. Significant differences were established in the four parameters evaluated. The highest biogas production and maximum production rate was obtained at the end of exponential growth P2, with 322 mL CH_4/g VS and 17.6 mL CH_4/g. VS/d, respectively. Regarding the energy balances, the methane potential from the biomass harvested in P2 and P3 phases contributed with 16% and 14% of the harvesting requirements, respectively.

1. Introduction

The microalgae anaerobic digestion is often proposed to overcome the high energy requirements of a microalgal biorefinery [1,2]. The biomethanization of microalgae for biogas production depends, among other parameters, on the physico-chemical composition of the microalgae [3]. Lipids have the greatest theoretical biomethane potential of the main microalgae components, corresponding to 1.014 L CH_4/g VS. However, they are the slowest to hydrolyse, followed by proteins with 0.851 L CH_4/g VS and carbohydrates with 0.415 L CH_4/g VS [3].

It has been described that microalgae presents growth kinetics in which it is possible to distinguish at least five phases: i) the lag phase, in which growth is delayed due to the presence of non-viable cells or physiological adjustments to adapt to the new medium conditions; ii) the exponential phase, where cells grow and divide as an exponential function of time, with non-limiting light intensity and nutrients; iii) the linear phase, in which the light becomes limited due to the increase in microalgae quantity, decreasing the growth rate; iv) the steady state phase, where there is nutrient limitation, determining when growth stops, and v) the death phase, where microalgae cell concentration declines rapidly due to a depletion of nutrients and pH disturbance [4,5]. In each of these phases, the physico-chemical composition of the microalgae changes. For example, in the steady state phase, in which the nutrients are exhausted, storage carbon products such as starch and lipids begin to accumulate [4,6]. Currently, there are no studies linking the biomethane potential with the different microalgal growth phases.

The aim of this study was to determine if there are significant differences in biogas production at different phases of the growth kinetics of the *Chlorella sorokiniana* microalgae in batch cultures. The methane produced was compared with the energy requirements of the microalgal harvesting process.

2. Material and methods

2.1. Batch cultivation of the C. sorokiniana microalgae

The *C. sorokiniana* microalgae was donated by the University of Huelva, Spain. This microalgae was previously isolated from a

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sludge anaerobic digester effluent belonging to a wastewater treatment plant in Spain, and was subsequently identified using molecular analysis.

For microalgal growth, a Sueoka culture medium was used, which is composed of 0.72 [g/L] KPO_4H_2, 1.44 [g/L] K_2PO_4H, 0.061 [g/L] $MgSO_4$*$7H_20$, 0.002 [g/L] CaCl2,* 2 H_20, 0.5 [g/L] NH_4Cl, 0.95 [g/L] KNO_3, 5[ml/L] hunter traces (2.28 [g] H_3BO_3, 4.4 [g] $ZnSO_4$* 4 H_20, 1.02 [g] $MnCl_2$ * $4H_20$ 1.0 [g] $FeSO_4$ * 7 H_20, 0.32 [g] $CaCl_2$ *$6H_20$, 0.32 [g] $CuSO_4$ * $5H_2O$, 0.22 [g] $Mo_7O_{24}(NH_4)_6$ *4 H_20). This culture medium was chosen because it contains the components required for non-limiting growth conditions. The culture conditions used were: temperature of 21 ± 2 °C, artificial lighting source F24-39W with I = 127.60 μmol of photons/(m^2x s)), a 24-h light photoperiod, and an atmospheric air aeration of 1.3–1.5 L/min.

The growth kinetics of the *C. sorokiniana* cultures was determined by measuring the biomass concentration over time by optical density at 600 nm. The dry weight estimation was determined based on the Volatile Solids (g VS/L) of the microalgal culture [7]. Subsequently, the dry weight concentration was related to the optical density at 600 nm (spectrophotometer Jenway 6715 UV/VIS), obtaining a linear relationship between both variables (Fig. 1). This linear relationship was evaluated and confirmed for each studied phase. The culture biomass concentration was expressed in g of VS per L of microalgal culture.

Once the growth kinetics was described, three growth phases were differentiated: P1, beginning of exponential growth; P2, end of exponential growth, and P3, steady state phase of the culture. After reaching these phases, the microalgal cultures were harvested by centrifugation with a Hermle Z 400 centrifuge (Labortechnik GmbH, P: 480 W).

2.2. Characterization of microalgal cultures

The characterization of cultures into the three different growth phases was based on the measurement of their physico-chemical composition, composed by protein content, lipids, carbohydrates, and recalcitrant material. For these analyses, samples from P1, P2, and P3 were lyophilized at a pressure of 10–15 mTorr and a temperature of −53 °C using the ILSHIN Mod. FD5518 freeze dryer.

Protein determination was performed using the Kjeldahl method through the measurement of total organic nitrogen [8,9]. Lipid identification was conducted using the Soxhlet method, [10]. Carbohydrate determination was performed using the Dubois method [11]. Finally, recalcitrant material determination, measured as the insoluble fiber content of the sample, was determined using acid digestion followed by alkaline digestion to remove proteins, sugars, and lipids until the residue was considered as recalcitrant material [10].

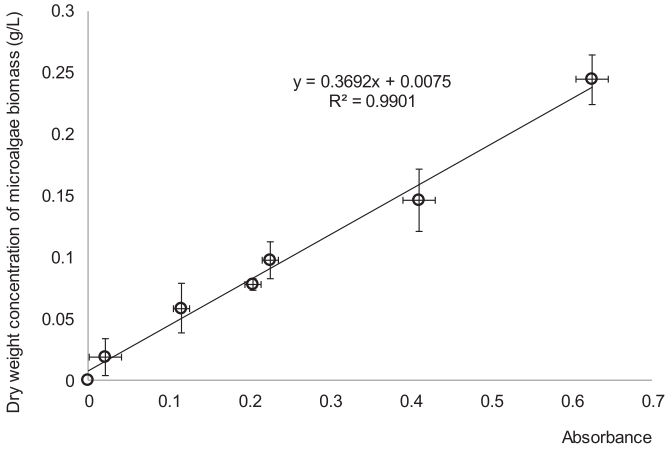

Fig. 1. Calibration curve for *Chlorella sorokiniana* culture concentration was carried out in triplicate. The dry weight concentration was related to optical density at 600 nm, obtaining a linear relationship between both variables. Mean values ± SD.

2.3. Biomethane potential and CH_4 productivity

Biogas production generated by cultures of *C. sorokiniana* in phases P1, P2, and P3 was evaluated using the biochemical methane potential test (BMP). BMP is a conventional test to evaluate the anaerobic biodegradability of any substrate [12].

The anaerobic inoculum used in the BMP tests was obtained from a lab-scale anaerobic reactor that treats sewage sludge. BMP tests were performed in 100 mL flasks under mesophilic conditions (37 °C). In all cases, the substrate to inoculum ratio was maintained at 0.5 g. $VS_{substrate}$/g $VS_{inoculum}$. The biogas produced was passed through a NaOH solution to capture CO_2; the remaining gas was assumed to be methane. The test ended once biogas production ceased. Each experiment was carried out in triplicate.

The quantification of accumulated methane production was measured in time (mL CH_4 accumulated/g. VS.) and normalized to mL CH_4/g VS.

Subsequently, biogas productivity was determined with the modified Gomperzt model [13], using the production values obtained from the BMP test, whose equation can be expressed as (Equation (1)):

$$B = P \times \exp\left(- \exp\left(\frac{Rm \times e}{P}(\lambda - t) + 1\right)\right) \quad (1)$$

where B represents the accumulated volume of biogas production at time t (d), P is the maximum biogas production potential (mL CH4/g VS), Rm is the maximum production rate (mL CH4/g VS/d), λ is the duration of the lag phase (h) and t is the incubation time (d).

2.4. Analytical methodologies

All analyses were performed in triplicate, estimating mean values and their standard deviations. Both the microalgal biomass as well as the inoculum were characterized, estimating the following parameters: total solid (TS) concentration, and volatile solids (VS) concentration, according to standard methods [10]. pH was analysed by means of a pH meter (HI/111 Hanna Instrument).

2.5. Energy balance

Energy balance was based on the difference between the energy obtained as CH_4 in each phase and the energy required for microalgae harvesting, since this is one of the steps that requires most of the energy [14]. Energy balance for each of the phases was calculated considering an energy input (*Ei*) that corresponded to the specific energy used for microalgae harvesting in each one of the three phases and an energy output (*Eo*), considering the biogas production in each of the three phases. *Ei* and *Eo* were calculated using Equations (2) and (3) [15,16].

$$Ei = \frac{\mathrm{P} \times \mathrm{t}}{\mathrm{V} \times \mathrm{TS} \times \left(\frac{\mathrm{VS}}{\mathrm{TS}}\right)} \quad (2)$$

where Ei is the input energy (KJ/g VS); P is the power applied in centrifugation (480 W); t is the centrifugation time needed to reach around 9 g TS/L after centrifugation (s); V is the volume of the culture media (L); TS is the total solid concentration before centrifugation (g TS/L) and VS is the total solid concentration before