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Effect of harvesting time in the methane production on the anaerobic digestion of microalgae

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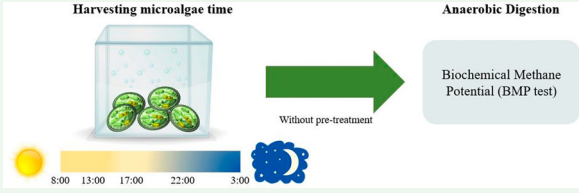
ABSTRACT

Microalgae are being proposed as excellent substrates for different biorefinery processes. Anaerobic digestion process of microalgae is one of these interesting processes but has some limitations in deleting cell walls. For this reason, many studies proposed different types of pre-treatments, entailing energy, operation, and investment costs. This work aims to optimize the anaerobic digestion of the microalgae *Chlorella sorokiniana* and *Chlorella sorokiniana* (strain S12/S13/S16) without any pre-treatment by selecting the optimal harvesting time. The greatest influence is seen at 5:00 PM in methane production for both microalgae. For *Chlorella sorokiniana,* it is the most optimal moment for anaerobic digestion, whereas *Chlorella sorokiniana* (strain S12/S13/S16) is the least optimal. In the other harvesting times, both microalgae present a similar methane production, i.e. 173 ± 12 mL CH_4/g of total volatile solids. The highest methane production rate values were obtained during peak sunlight, 1:00 PM and 8:00 AM, respectively, and lower overnight.



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KEYWORDS
Biochemical potential test; carbohydrate; *Chlorella sorokiniana*; kinetic model; protein

1. Introduction

Biorefinery based on microalgae cultures has gained a lot of attention in the last years due to the possibility of obtaining interesting compounds, such as amino acids, fine chemicals, or pigments, as well as the generation of fuels such as biogas, biodiesel, or biohydrogen [1, 2]. The yield of these microalgae-based biorefineries is related to the composition of the microalgae cells, which depends on the stimulus received from surroundings such as carbon dioxide, water, nutrients deficit, light intensity, pH, and temperature [3, 4]. For example, lipid content, fatty acid, and lipid class compositions of microalgae are strongly influenced by the culture age [5], light intensity, and photoperiod [6]. Other compounds such as photosynthetic pigments, proteins, and carbohydrates have been reported to be also influenced by light [7].

Microalgae have been widely proposed as a substrate for anaerobic digestion processes, allowing the conversion of the microalgae into biomethane for energy production. Species from the genus *Chlorella* and *Scenedesmus* have been the most widely studied as a substrate for anaerobic digestion processes [8]. Generally, the specific methane yield described for these processes ranged between 90 and 440 mL CH_4/g VS (VS, total volatile solids) [8,9]. However, the biomethanization of microalgae can be limited due to the difficulty of degrading the cell walls, whose hydrolysis has been widely reported as one of the main challenges in the anaerobic digestion of microalgae [8,10]. Different pre-treatments have been reported as a possible solution to overcome this challenge, such as thermal treatments, mechanical or the application of microwaves or ultrasounds [4,10]. These methods aim to break down the microalgae cell wall, allowing the solubilisation of the organic matter and, thus, facilitating the biological

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access of the anaerobic microorganisms to the organic matter to be converted into methane [8]. However, the energy consumption during the pre-treatment stage is not always compensated during the subsequent anaerobic digestion, especially for high-intensive pre-treatments [11]. For the pre-treatment of microalgae, the implementation of mechanical treatments is partly limited by the low solid concentration of the microalgae biomass, which results in negative energy balances [10, 12]. Likewise, the energy balances of the thermal treatments would be energetically unfeasible due to the high cost associated with heating the high water content in the microalgae biomass. Most of the studies about microalgae pre-treatments reviewed by Passos et al. [10] showed a ratio between the energy output from the anaerobic digestion and the energy input from the pre-treatments lower than one. On the contrary, Schwede et al. [13] reported a positive energetic balance in the anaerobic digestion of *Nannochloropsis salina* after a thermal pre-treatment of 100–120 °C for 2 h, relating their positive energy balance with the high concentration of the used microalgae feedstock.

Another factor that would influence the methane yield of microalga is the organic matter composition, i.e. proteins, carbohydrates, or lipids content [10]. The composition of the microalgae can be related to the specific microalga species. For example, of the capability of accumulating polyunsaturated fatty acids, microalgae from the genus *Bacillariophceae or Chlorophyceae* tend to accumulate eicosapentaenoic acid. In contrast, other microalgae such as *Crypthecodinium cohnii* or species from the genus *Schizochytrium* have been reported to accrue docosahexaenoic acid [4]. Other authors have described that the anaerobic digestion of microalgae can also be influenced by different parameters such as cultivation conditions, the growth phase of the microalgae culture, or storage conditions due to variations in the microalgae composition [14]. The variation in the time of harvesting during the day would vary the microalgae composition due to variations in different interrelated factors, thus, affecting the anaerobic digestion of the microalgae biomass.

This work aimed to determine the optimal moment of the one natural day to harvest the microalgae culture for enhancing methane production, through biochemical methane potential tests, as an alternative to usually proposed pre-treatments methods for enhancing the methane production. The microalgae *Chlorella sorokiniana* and *Chlorella sorokiniana* (strain S12/S13/S16) were cultured as model microalgae. The knowledge offered by this research relates the time of harvesting of the selected microalgae with the production of methane that leads to their biodegradation. Also, it was evaluated the substrate composition at each harvesting time as possible explanation of the differences in the methane production. These results could be applied not only to these microalgae, but also to other similar biomasses.

2. Materials and methods

2.1. Microalgae culture and harvesting conditions

An initial culture of *C. sorokiniana* and *C. sorokiniana* (strain S12/S13/S16) were obtained from Algal Biotechnology group from CIDERTA-University of Huelva (Spain). The identification and description of *C. sorokiniana* (strain S12/S13/S16) can be found at Tapia et al. [15]. Both microalgae were cultured in a modification *Chlamydomonas reinhardtii* medium which contained [16]: 0.72 g KH_2PO_4; 1.44 g K_2HPO_4; 1 mL $MgSO_4 \cdot 7H_2O$ (61% w/v); 1 mL $CaCl_2 \cdot 2H_2O$ (20% w/v); 0.5 g NH_4Cl; 0.95 g KNO_3; 0.0114 g H_3BO_3; 0.0637 g Na_2·EDTA; 0.022 g $ZnSO_4 \cdot H_2O$; 0.005 g $MnCl_2 \cdot 4H_2O$; 0.005 g $FeSO_4 \cdot 7H_2O$; 0.0016 g $CoCl_2 \cdot 6H_2O$; 0.0016 g $CuSO_4 \cdot 5H_2O$ and 0.0011 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ per liter. Both microalgae were cultivated in outdoor batch photobioreactors at natural light, temperature, and light/dark period conditions. Each photobioreactor contained approximately 8-L of microalgae culture and was aerated and mixed with air to provide the needed CO_2 at 100 L/h.

Stationary phase culture was harvested every 5 h in a 24-hour cycle, i.e. 8:00 AM, 1:00 PM, 5:00 PM, 10:00 PM, and 3:00 AM, by centrifuging at 5000 rpm for 5 min. The concentrate was immediately frozen in liquid nitrogen at −195.8°C for preservation by lyophilization in a lyophilizer Telstar LyoQuest (Spain). Lyophilized microalgae were stored at −20 °C for subsequent analysis.

2.2. Biomethane potential tests

The potential of each microalgae sample taken at different times was evaluated in triplicate through biochemical methane potential (BMP) tests in reactors with a working volume of 120 mL at mesophilic conditions (35–37 °C) ,more details about the BMP system can be found in Serrano et al. [17]. The reactors were mechanically continuously stirred through magnetic bars at 300 rpm. The inoculum to substrate ratio was fixed in 2, based on VS. Initially, 120 µL of a micronutrient solution was added to supply all the required micronutrients to the microorganisms with the following composition: 2.28 g/L H_3BO_3, 2.747 g/L $ZnSO_4$, 1.02 g/L $MnCl_2 \cdot 4H_2O$, $FeSO_4 \cdot$ 1.0 g/L $7H_2O$, 0.32 g/L $CoCl_2 \cdot 6H_2O$, 0.32 g/L $CuSO_4 \cdot 5H_2O$, and 0.22 g/L $(NH_4)_6Mo_7O_{24}$

· $4H_2O$ [18]. 5 mL of a 65 g/L $NaHCO_3$ solution as an alkalinity source was added to each reactor to ensure the correct pH. Blanks were also used, in triplicate, to subtract the endogenous inoculum activity of the methane produced from the substrates. Methane production was measured daily by liquid displacement after CO_2 removal by bubbling in 2N NaOH. The methane volumes were expressed at standard temperature and pressure conditions for all measurements.

2.3. Chemical analyses

The VS and the chemical oxygen demand (COD) determinations were carried out according to the protocols defined by the American Public Health et al. [19]. The determination of the concentration of ammonia was carried out by the colorimetric Nessler Method by Hannah kits. The Lowry method [20] was used to measure the protein content of the microalgae samples. Casein was used as a standard for the calibration curve, expressing the results of proteins in the samples as mg of casein equivalent. Total carbohydrates were estimated by the Dubois colorimetric method [21]. A calibration curve using glucose standards was used as a reference, expressing the results as mg of glucose equivalents.

2.4. Kinetic model

A first-order kinetic model was used for evaluating the methane production for the calculation of the maximum methane yield coefficient (Equation 1) and the methane production rate (Equation 2), according to the following expressions [22]:

$$G = G_{\max}\,[1 - \exp(-k \times t)] \quad (1)$$

where G (mL CH_4/g VS) is the cumulative specific methane production, $G_{\max}$ (mL CH_4/g VS) is the ultimate specific methane production, k (d^{-1}) is the specific rate constant or apparent kinetic constant, and t (d) is the time.

$$R_m = G_{\max} \times k \quad (2)$$

where R_m (mL CH_4/(g VS·d)) is the methane production rate.

3. Results and discussion

3.1. Relation between the harvesting time and microalgae composition

The chemical characterization of the samples of both microalgae taken at different times is presented in Table 1. As it can be seen, both *C. sorokiniana* and *C. sorokiniana (strain S12/S13/S16)* showed similar COD values, ranging from 655 ± 20 mg O_2/L to 759 ± 11 mg O_2/L (Table 1). Therefore, it was impossible to establish any relation between the harvesting time and the COD for both microalgae. Also, the almost constant organic matter concentration would indicate that the microalgae cultures were at a stationary growth phase at the different sampling times [23], ensuring that the obtained differences would not be a consequence of the growing process. The concentration of ammonia in the microalgae samples at each harvesting time is also shown in Table 1. As can be seen, the concentration remained almost constant at the different harvesting times, with mean values of 13.1 ± 0.4 mg/L and 9.2 ± 0.1 mg/L for *C. sorokiniana* and *C. sorokiniana (strain S12/S13/S16)*, respectively, concentrations similar to those described by Rossi et al. [24]. The ammonium concentration is much lower than those shown to the anaerobic digestion process inhibition, i.e. higher than 1000–1500 mg / L [25].

The concentration of proteins in both *C. sorokiniana* and *C. sorokiniana (strain S12/S13/S16)* presented a similar trend, reaching a maximum concentration in the sample harvested at 10:00 PM. However, the proteins concentration in *C. sorokiniana (strain S12/S13/S16)* was markedly higher than in *C. sorokiniana* at all the harvesting times (Table 1). The maximum concentration of proteins was 96.6 ± 12.4 mg casein eq/g VS and 129.2 ± 5.8 mg casein eq/g VS for *C. sorokiniana* and *C. sorokiniana (strain S12/S13/S16)*, respectively. These values mean an increment of around 20% to the lowest concentration of protein for each microalga (Table 1). These protein values are lower than the percentages described for Chlorella sp. by other authors, which varied between 35 and 60% in dry weight [26, 27]. Similarly, the concentration of carbohydrates was also higher in *C. sorokiniana (strain S12/S13/S16)* than in *C. sorokiniana* (Table 1). Both microalgae presented a reasonable constant carbohydrate concentration regardless of the harvesting time, except for abnormal data for each microalga (8:00 AM for *C. sorokiniana* and 10:00 PM for *C. sorokiniana (strain S12/S13/S16)*). Concretely, the mean values for the concentration of carbohydrates were 12.2 ± 2.8 mg glucose eq./g VS and 15.2 ± 2.4 mg glucose eq./g VS for *C. sorokiniana* and *C. sorokiniana (strain S12/S13/S16)*, respectively (Table 1). The increase in the protein concentration in the samples harvested at 10:00 PM would be intriguing due to these compounds' high theoretical methane potential, i.e. 0.851 L CH_4/g VS, compared to the carbohydrates, whose theoretical methane yield is 0.415 L/g VS [28].

Table 1. Characterization of the microalgae samples harvested at each different time, where COD, chemical oxygen demand, VS, total volatile solids.

	Chlorella sorokiniana			
Harvesting time	COD (mg O_2/L)	Proteins (mg casein eq./g VS)	Carbohydrates (mg glucose eq./g VS)	Ammonia (mg/L)
3:00 AM	707 ± 8	87.9 ± 2.5	12.8 ± 0.6	13.7 ± 0.4
8:00 AM	658 ± 8	86.0 ± 3.5	7.2 ± 0.6	12.9 ± 0.5
1:00 PM	681 ± 8	80.9 ± 5.5	13.6 ± 0.6	13.0 ± 0.3
5:00 PM	655 ± 20	89.6 ± 6.2	13.5 ± 0.5	13.1 ± 0.3
10:00 PM	759 ± 11	96.6 ± 12.4	13.9 ± 0.4	12.8 ± 0.1
Chlorella sorokiniana (strain S12/S13/S16)				
Harvesting time	COD (mg O_2/L)	Proteins (mg casein eq./g VS)	Carbohydrates (mg glucose eq./g VS)	Ammonia (mg/L)
3:00 AM	682 ± 7	113.9 ± 2.7	15.5 ± 0.2	9.2 ± 0.3
8:00 AM	733 ± 66	109.6 ± 0.9	12.7 ± 0.2	9.0 ± 0.5
1:00 PM	693 ± 35	120.7 ± 2.0	14.1 ± 0.2	9.2 ± 0.4
5:00 PM	682 ± 22	108.3 ± 1.1	15.1 ± 0.4	9.3 ± 04
10:00 PM	665 ± 7	129.2 ± 5.8	19.1 ± 0.5	9.2 ± 0.3

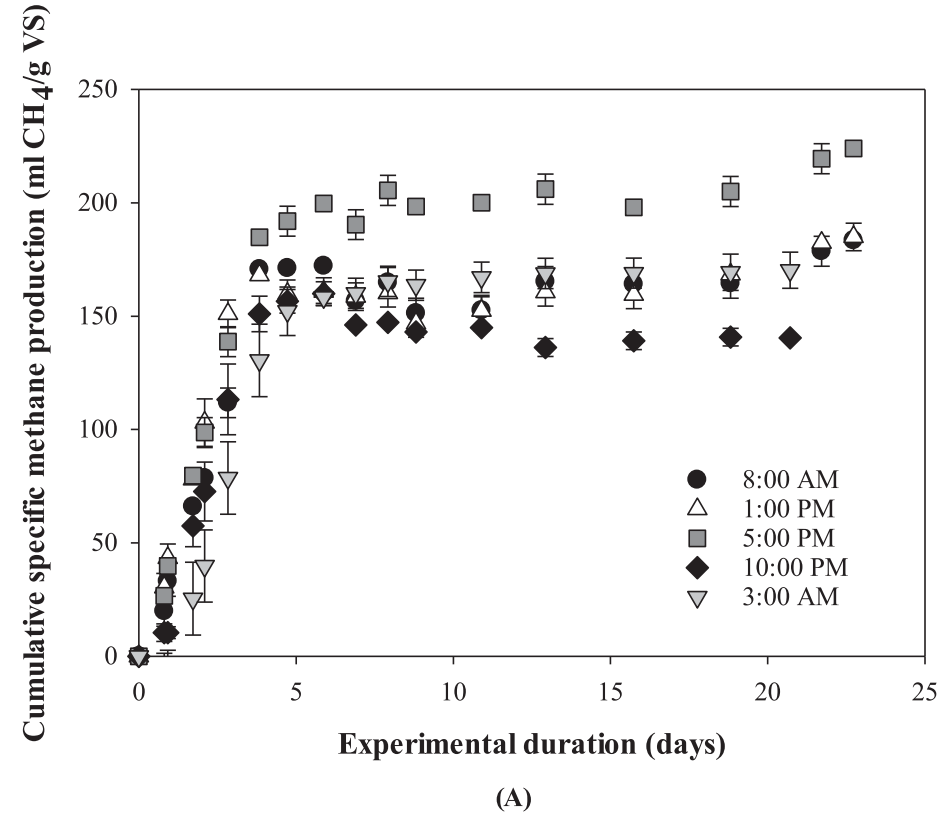
3.2. Relation between the harvesting time and the biomethane production

The variation of the methane yield coefficient throughout the experimental time for the anaerobic digestion of each microalga at each harvesting time is shown in Figure 1a and b. As it can be seen, the methane yield coefficient rapidly increased during the first 4-days, without any lag phase for both *C. sorokiniana* (Figure 1a) and *C. sorokiniana (strain S12/S13/S16)* (Figure 1b). The absence of a lag phase indicated that the hydrolysis was not a rate-limiting stage during the microalgae degradation due to readily degradable components in the substrate [29, 30]. The decrease in the methane yield coefficient observed for some samples was a consequence of the activity of the microorganisms in the blanks, which still produced a small amount of biogas when the biogas production in the samples was the exhaust.

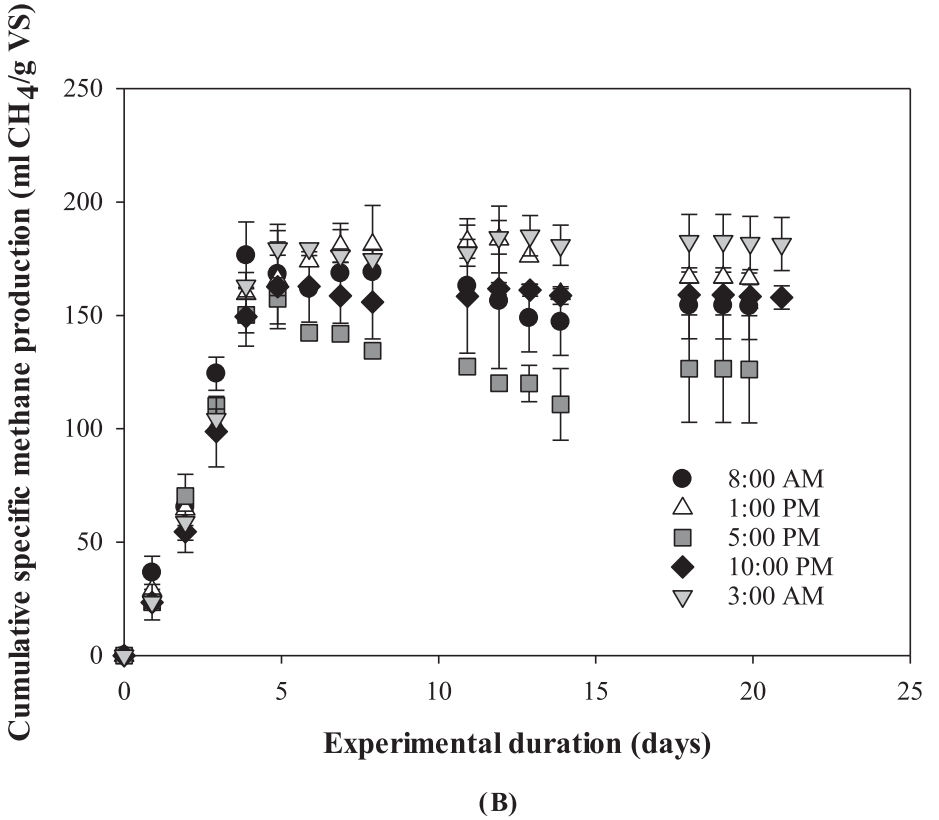
The first-order kinetic model was implemented to calculate the methane production rate and facilitate the comparison of the methane yield coefficient with other authors [31]. Figure 2 shows the ultimate specific methane production for each microalga harvested at each time. As it can be seen, the optimal harvesting time for *C. sorokiniana* was at 5:00 PM, with a G_{max} value of 214 ± 6 mL CH_4/g VS (Figure 2). This value was 42% higher than the value of G_{max} obtained for the same microalgae at 10:00 PM. The highest content of proteins would explain this difference in the samples of *C. sorokiniana* harvested at 10:00 PM (Table 1). The anaerobic degradation of proteins can increase the concentration of volatile fatty acids, ammonia, or, to a lesser extent, hydrogen and sulphur compounds which can inhibit the anaerobic digestion process at specific concentrations [32]. Samples of *C. sorokiniana (strain S12/S13/S16)* presented quite similar values of G_{max} to those obtained for *C. sorokiniana* (Figure 2), except for the sample harvested at 5:00 PM, which showed the lowest values of G_{max}, i.e. only 132 ± 6 mL CH_4/g VS.

This value was 30% lower than the highest value of G_{max} obtained for *C. sorokiniana (strain S12/S13/S16)* at 3:00 AM (Figure 2). Despite the lower value obtained at 5:00 PM, the values of G_{max} for *C. sorokiniana (strain S12/S13/S16)* remained almost constant, with a mean value of 173 ± 12 mL CH_4/g VS. Usually, the variation in the composition has been seen to influence the biodegrading of biomass [33, 34]. However, the variation in the composition of the biomasses at the different harvesting time was not very marked, being not possible to establish a clear relation with the variations in the methane production, which would be related to other factors. For example, differences in the cell wall microalga morphology and thickness, which has been reported for different growth phases, could explain variations in the anaerobic biodegradability [35]. Therefore, it is possible to conclude that the harvesting time has a strong influence in the methane yield coefficient obtained from the microalgae, although was not possible to establish a clear relationship this variation and the analysed characteristics of the microalgae.

Figure 3 shows the values of R_m for each microalga harvested at each time. For both microalgae, the highest R_m values were obtained during daylight hours (from 8:00 AM to 5:00 PM), while the lowest R_m values corresponded to samples harvested at night (Figure 2). It is worth noting that this trend was more marked for *C. sorokiniana* than for *C. sorokiniana (strain S12/S13/S16)*. Concretely, the values of R_m for *C. sorokiniana* varied from only 41 mL CH_4/(g VS·d) at 3:00 AM to 80 mL CH_4/(g VS·d) at 5:00 PM, i.e. an increment of around 100%. These methane production rate values were in the same range as those reported by Beltrán et al. [36], which reported a methane production rate of 76.6 mL CH_4/(g VS·d) during the batch anaerobic digestion of *C. sorokiniana* at mesophilic temperature.



(A)



(B)

Figure 1. (**A**) Variation of the methane production with time for *C. sorokiniana* harvested at different times, and (**B**) Variation of the methane production with time for *C. sorokiniana (strain S12/S13/S16)* harvested at different times, where VS, total volatile solids.

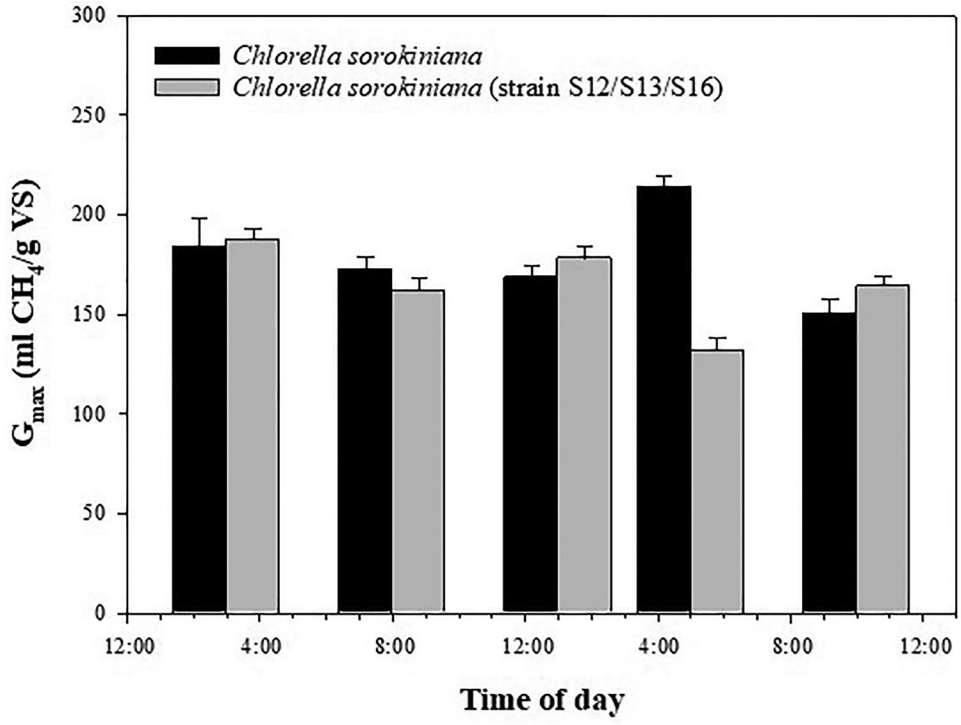


Figure 2. Variation of the ultimate specific methane production (G_{max}) for *C. sorokiniana* and *C. sorokiniana (strain S12/S13/S16)* harvested at different times, where VS, total volatile solids.

The enhancement in the values of R_m achieved at varying the harvesting time was similar to one described by Córdova et al. [37], which reported a variation from 11.56 ± 0.15 mL CH_4/(g VS·d) to values between 18 and 28 mL CH_4/(g VS·d) at applying different physical pretreatment methods before the anaerobic digestion of

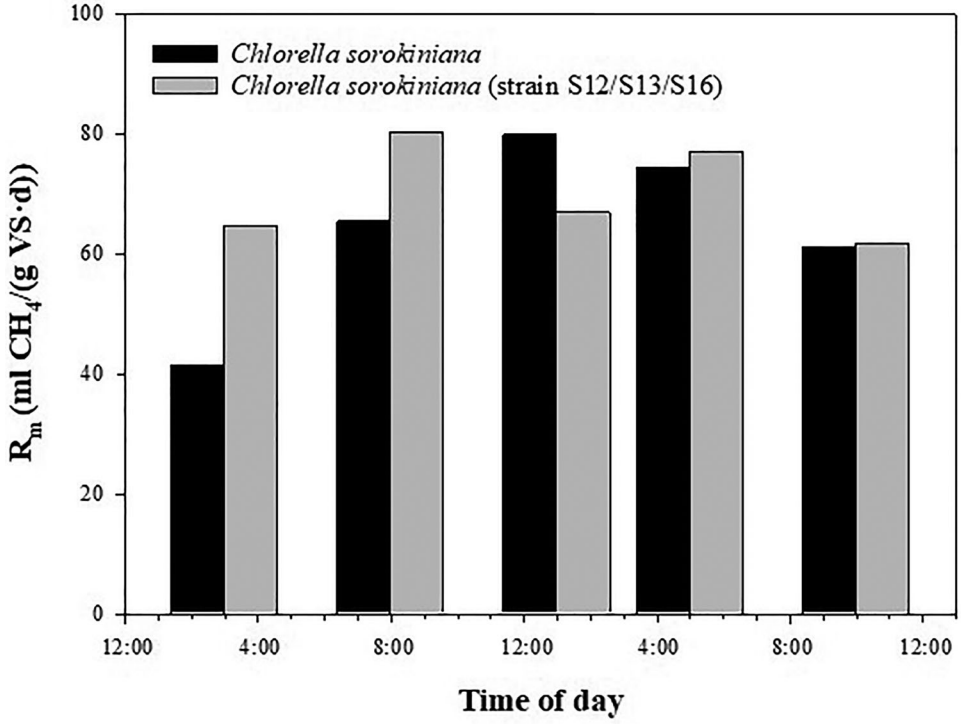


Figure 3. Variation of the methane production rate (R_m) for *C. sorokiniana* and *C. sorokiniana (strain S12/S13/S16)* harvested at different times, where VS, total volatile solids.

C. sorokiniana. Therefore, it was impossible to determine a clear relation between the microalgae composition and the anaerobic digestion behaviour at the different harvesting times. However, the harvesting time strongly influenced R_m, resulting in variations like the obtained by other authors through the application of pre-treatments to the microalgae [33].

4. Conclusions

The anaerobic digestion process evaluated the methane production and methane production rate from *C. sorokiniana* than for *C. sorokiniana (strain S12/S13/S16)*. The microalgae have been harvested at one natural time every 5 h and evaluated through a biochemical methane potential test. The influence of sunlight in harvesting both microalgae has been demonstrated. In the other harvesting times, both microalgae present a similar methane production, i.e. 173 ± 12 mL CH_4/ g VS. The methane production rates have been the most optimal at 1:00 PM and 8:00 AM for *C. sorokiniana* and *C. sorokiniana (strain S12/S13/S16)*, respectively.

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