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Cyanobacterial Biomass Produced in the Wastewater of the Dairy Industry and Its Evaluation in Anaerobic Co-Digestion with Cattle Manure for Enhanced Methane Production

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Abstract: The unique perspective that microalgae biomass presents for bioenergy production is currently being strongly considered. This type of biomass production involves large amounts of nutrients, due to nitrogen and phosphorous fertilizers, which impose production limitations. A viable alternative to fertilizers is wastewater, rich in essential nutrients (carbon, nitrogen, phosphorus, potassium). Therefore, *Arthrospira platensis* was cultivated in 150 mL photobioreactors with 70% (*v*/*v*) with the wastewater from a dairy industry, under a regime of light:dark cycles (12 h:12 h), with an irradiance of 140 µmol m^{-2} s^{-1} photon. The discontinuous cultures were inoculated with an average concentration of chlorophyll-*a* of 13.19 ± 0.19 mg L^{-1}. High biomass productivity was achieved in the cultures with wastewater from the dairy industry (1.1 ± 0.02 g L^{-1} d^{-1}). This biomass was subjected to thermal and physical treatments, to be used in co-digestion with cattle manure. Co-digestion was carried out in a mesophilic regime (35 °C) with a C: N ratio of 19:1, reaching a high methane yield of 482.54 ± 8.27 mL of CH_4 g^{-1} volatile solids (VS), compared with control (cattle manure). The results demonstrate the effectiveness of the use of cyanobacterial biomass grown in wastewater to obtain bioenergy.

Keywords: anaerobic co-digestion; nutrient removal efficiency; dairy industry; photobioreactor

1. Introduction

Cyanobacteria and microalgae are photosynthetic microorganisms, capable of adapting to various physical and chemical factors, such as light, salinity, pH, temperature, and nutrient concentration [1]. This metabolic plasticity gives them great potential to treat wastewater and industrial water, bioremediation of aquatic and terrestrial habitats, and applications, such as biofertilizers, biofuels, food supplements, or bioenergy [2]. The nutrient assimilation capacity (nitrogen and phosphorus) of these photosynthetic microorganisms has aroused the interest of the scientific community in the environmental field for its possible use in the biological treatment of wastewater, reducing the risk of contamination of water bodies by effluent discharges with the presence of suspended solids, biodegradable organic compounds and pathogenic microorganisms [3–5]. The accumulation of nitrogen and phosphorus in water bodies is the primary source responsible for eutrophication [6].

According to its biochemical composition, these photosynthetic microorganisms can grow and assimilate nutrients from the wastewater and biomass production that could have a bioenergetic profile [7]. Urban and industrial effluents are considered nutrient-rich culture media due to the organic and inorganic load [8]. The cultivation of photosynthetic microorganisms in wastewater represents several advantages, such as low energy cost process, improvement of water quality through the use of nutrients in wastewater, and obtaining nutrient-rich biomass (nitrogen and phosphorus), and oxygen generation [4]. Microalgae and cyanobacteria are an essential component in many wastewater treatment systems around the world. In many countries with developing economies, wastewater is generally treated in facultative and oxidation ponds [9,10]. Microalgae and cyanobacteria spontaneously colonize these, but due to the deep and low mixing of this system, dry biomass productivity is low, 10 tons ha^{-1} year^{-1}. For these reasons, the biomass is not harvested, and the removal of nutrients from the wastewater by microalgae and cyanobacteria is inefficient [11]. In the late 1950s, high-rate algal ponds (HRAPs) were developed as an alternative to facultative ponds [12]. HRAPs are raceways in which a paddlewheel mixes the water, as a result of this better mixing, high productivity of microalgae and cyanobacteria biomass are obtained, about 30 tons dry biomass ha^{-1} year^{-1} [13]. Likewise, the productivity of microalgae and cyanobacteria grown in agro-industrial wastewater in closed systems (photobioreactors) is high. Thus, Álvarez and Otero [14] obtained a biomass concentration of 5 g L^{-1} with *Arthrospira* sp. grown in anaerobic centrate from anaerobic digestion of wastewater from fish processors.

The food industries generate large volumes of wastewater for all the processes that involve the processing of their products, water with a high nutrient load, as is the dairy industry characterized by its wastewater rich in carbohydrates and proteins [15]. World milk production increased to 829 million tons in 2018, growing annually by 16.7 million tons, or 2.1% as of 2017, with an accelerated increase since 2014. However, production growth is expected world milk by 22% until 2027 [16]. A third of milk production is consumed as fresh milk, a fourth processed in cheese making; one fifth used in butter, milk powder, and casein [17]. However, this process consumes significant amounts of water, with an average consumption of 1.44 ± 0.20 L per L of raw milk processed in milk, cheese, or commercial powdered products. This high-water consumption causes the dairy industry to present problematic aspects in eliminating specific residues, such as acid whey, crystallized lactose mother liquor, and cleaning in situ. Due to its characteristics, high chemical oxygen demand (COD) and biological oxygen demand (BOD), lipids, nutrients, lactose, this type of industrial waste can cause eutrophication in water bodies if it does not receive adequate treatment [18]. Dairy industry waste disposal is reported as a significant environmental problem due to soil or water contamination. Possible use of this type of wastewater would be to cultivate microalgae or cyanobacteria; thus, the sewage nutrients would be recycled into microalgal or cyanobacterial biomass, biomass that, according to its biochemical and nutritional composition, could have a possible application.

One of the emerging applications of microalgae and cyanobacteria biomass is to use them as a source of biofuels due to their high productivity and the fact that cultivation systems do not compete for land or freshwater with terrestrial crops [19,20]. The low concentration of microalgal biomass in the culture system, and its high-water content (80–90%) have an unfavorable impact on the energy balance of the process that depends on dry biomass [21–24]. For these reasons, the use of microalgae and cyanobacteria biomass in the energy field is not feasible for direct combustion, pyrolysis, and gasification that require dry biomass due to the high-energy input to remove intracellular water but is viable for processes that use wet biomass [25–27]. Anaerobic digestion (AD) is the primary biological process to obtain bioenergy from biomasses with high-water content, such as microalgae and cyanobacteria biomass. It easily supports biomass high water content without the energy drawbacks of centrifugation and drying [28,29]. AD is eliminating some of the costs generated in microalgae-based biofuel production systems. The integration of AD to the biofuel production systems from microalgae, higher efficiency, and sustainability of the process are obtained [30]. The AD of the biomass of microalgae and cyanobacteria is catabolized in the absence of an external electron acceptor by strict or facultative anaerobic microorganisms through a redox reaction. The product generated during the process accepts the electron released during the decomposition of the organic matter; therefore, the organic matter acts as a donor and acceptor of electrons [31].

Golueke et al. in 1957 [32] recognized the importance of generating energy through the methane produced by the fermentation of the obtained microalgae biomass; they investigated AD of biomass from *Chlorella* spp., and *Scenedesmus* spp., grown in wastewater. However, little research has been done on the fermentation of microalgae biomass for methane production, and unlike what was done with macroalgae on the same topic, only today is this important energetic source receiving attention again [33–35]. Later Oswald et al., between, 1959 and 1998, continue with the research of the AD of the biomass of microalgae and cyanobacteria produced in "Advanced Integrated Wastewater Pond Systems" [36–42].

The co-digestion of microalgae and cyanobacteria biomass has been carried out with various substrates. Thus, Solé-Bundó et al. evaluated the co-digestion of the biomass of *Chlorella* sp. and wheat straw in different proportions, the maximum methane yield was obtained with the 50% microalgae –50% wheat straw [43]. The biomass of another chlorophyte, *Scenedesmus* sp., has been evaluated in co-digestion with pig manure, reaching a maximum methane yield of 245 mL CH_4 g^{-1} volatile solids (VS) [44]. Another material frequently used as co-substrate is waste activated sludge (WAS). Thus Wang et al. obtained a maximum methane yield of 296 mL CH_4 g^{-1} VS in the co-digestion of the biomass of *Chlorella* sp. and WAS [45]. A poorly evaluated co-substrate is lipid-rich fat, oil, and grease waste (FOG). However, it generally produces high methane yields in co-digestion with microalgae biomass. Thus, Park and Li evaluated co-digestion anaerobic biomass of *Nannochloropsis salina* D.J. Hibberd 1981, and FOG, reaching a methane yield of 540 mL CH_4 g^{-1} VS [46].

Anaerobic co-digestion is one of the areas of most considerable investigation. Thus Fernández-Rodríguez et al. [47] used the microalgae *Dunaliella salina* (Dunal) Teodoresco 1905 as a co-substrate in the AD of olive mill solid waste (OMSW). They evaluated different C/N ratios, the maximum methane production 330 mL CH_4 g^{-1} VS was obtained for the co-digestion mixture 75% OMSW-25% *D. salina* with a C:N ratio of 26.7:1. While Gonzalez-Fernandez et al. [48] evaluated anaerobic co-digestion of microalgal biomass of *Scenedesmus* sp. and *Chlorella sorokiniana* Shihira and R.W. Krauss 1965, grown in urban wastewater and sewage sludge, they operated biodigesters at psychrophilic (15–25 °C), mesophilic (35 °C) and thermophilic (50 °C) conditions. The highest methane yield occurs in thermophilic conditions, 90.2 ± 1.9 mL CH_4 g^{-1} VS.

The main objective of this research was to evaluate the culture and biomass production of *Arthrospira platensis* Gomont 1892 (*Spirulina*) in tubular photobioreactors, using wastewater from a dairy industry as a source of nutrients, and its subsequent use in anaerobic co-digestion with cattle manure with the premise of increasing methane production. During the investigation, the characteristics of both substrates were determined before co-digestion.

2. Materials and Methods

2.1. Dairy Industry Wastewater Characteristics

The Dairy Industry Wastewater (DIWW) utilized was obtained from a dairy industry specialized in gourmet cheeses and milk derivatives, which are made with Italian technology. Samples of DIWW were taken at the outlet of the distribution channels, presenting the following characteristics: total nitrogen (TN) 2.22 mM, total phosphorus (TP) 0.20 mM, and pH 5.5.

2.2. Photobioreactors and Culture Conditions

The cyanobacterium *A. platensis* was obtained from the culture collection of the Laboratorio de Biotecnología Microbiana (BiotemLab) of the Universidad de Guayaquil (Universidad de Guayaquil, Guayaquil, Guayas, Ecuador). Cultures were acclimated to the wastewater by increasing the concentration of DIWW for several months, reaching a maximum concentration of 70% (*v*/*v*).

Acclimated cultures from log-phase were centrifuged before starting the experiment and resuspended in DIWW 70% and 30% of seawater (35%). A nutritional correction was made with the foliar nutrient Bayfolan®, to reach a final concentration of N 20.80 mM and P 1.67 mM, with an N:P ratio of 12.5: 1; further, it was supplemented with 47.6 mM $NaHCO_3$, and due to the pH of the residual water (5.5) it was adjusted to 10.2. The control cultures were performed with standard Zarrouk media (N:P 10:1), with the following composition: $NaHCO_3$ 214.3 mM; $NaNO_3$ 29.4 mM; K_2HPO_4 2.9 mM; K_2SO_4 5.7 mM; NaCl 17.1 mM; $MgSO_4{\cdot}7H_2O$ 0.8 mM; $CaCl_2{\cdot}2H_2O$ 0.5 mM; $FeSO_4{\cdot}7H_2O$ 0.04 mM; EDTA 0.3 mM; H_3BO_3 0.046 mM; $MnCl_2{\cdot}4H_2O$ 0.009 mM; $ZnSO_4{\cdot}7H_2O$ 0.0008 mM; Na_2MoO_4 0.00009 mM; and $CuSO_4{\cdot}5H_2O$ 0.0003 mM (Sigma-Aldrich, Saint Louis, MO, USA) [49].

Cultures were carried out in triplicate and initiated with a high concentration of chlorophyll-*a* (13.19 ± 0.19 mg L^{-1}) from acclimated cultures from log-phase, to avoid the inhibitory effect of DIWW on growth [50], and kept in batch culture for five days. Cultures were carried out indoors in six glass bubble-column photobioreactors (PBRs-0.3 in height, 0.05 in diameter), and 150 mL volume, at 30 °C in a thermostatic bath under a circadian lighting regime 12 h light/12 h dark with an irradiance of 140 µmol photon m^{-2} s^{-1} (Figure 1a). The bioreactors were continuously aerated with air filtered with cellulose acetate filters of 0.2 µm pore diameter (Minisart® Sartorius, Hamburg, Germany), supplemented with pulses of CO_2 to maintain the pH 9.9 ± 0.3 during the light cycle. Evaporation (less than 6% of the culture volume) was corrected daily with sterilized distilled water before the harvesting for analysis.

(**a**) (**b**) (**c**)

Figure 1. Flow diagram of the cultivation of cyanobacterial biomass and its anaerobic co-digestion: (**a**) Cultivation in column photobioreactors (PBRs) of *Arthrospira platensis* in wastewater from a dairy industry; (**b**) harvested and pre-treated cyanobacterial biomass, and raw cattle manure; (**c**) anaerobic co-digestion of cyanobacterial biomass and raw cattle manure in a mesophilic regimen (35 °C).

2.3. Anaerobic Co-Digestion

At the end of the batch culture (day five), these were harvested to collect the cyanobacterial biomass to determine the biochemical composition, VS, TN, TP, and total organic carbon (TOC) were analyzed. The cyanobacterium biomass was subjected to a thermal pre-treatment at 120 °C for 40 min; subsequently, the biomass was ultrasonicated at 20 kHz for two minutes and was used in anaerobic co-digestion with cattle manure (Figure 1b). The raw cattle manure was collected at the El Algarrobo farm in the Engabao commune (2°36′37′′ S; 80°26′12′′ W) of the General Villamil Playas canton during September, October, and November 2019 (dry season). The manure collected was transported to the Microbial Biotechnology Laboratory, where it was sieved (5 mm) to separate large particles, weighed, and finally, the content of VS, TN, and TOC was determined. Pre-treatment of the cyanobacterium biomass before anaerobic co-digestion allows to the improvement of its biodegradability significantly. This step makes the organic matter more accessible to anaerobic microbiota and, therefore, it degrades more efficiently [51]. Pre-treatment methods to disrupt microalgal cell walls, such as thermal, chemical, and ultrasonic methods, can significantly improve the digestibility of crude proteins and guarantee maximum methane production [52]. Anaerobic co-digestion was conducted in batch mode. Digesters were in glass bottles with 1000 mL capacity. Each bottle contained a final concentration of substrates (cyanobacterium biomass (CB) + cattle manure (CM)) of 7 g VS L^{-1}, the ratio of the percentage of biomasses was 15% CB and 85% CM, with a final carbon: nitrogen ratio of 19:1. Finally, the bottles were filled up to 700 mL with distilled water and incubated at 35 °C (Figure 1c). CH_4 concentration was determined every 24 h for 40 days. Similarly, anaerobic digestion of each substrate (CB and CM) was performed out separately under the same conditions described above.

2.4. Analytical Methods

Samples (5 mL) were taken daily and centrifuged at 17,100 g for 15 min at 4 °C. For the measurement of chlorophyll-*a*, Talling and Driver method [53] modified by Álvarez [54] was applied: the pellet was resuspended in methanol 95% (*v*/*v*) and sonicated at 20 kHz for 2 min followed by 4-h extraction in the dark at 4 °C. Cell debris was removed by centrifugation, and chlorophyll-*a* absorption was measured spectrophotometrically at 665 nm. The methanolic extract was read at the optical density of 665 nm, and the chlorophyll-*a* content was quantified using the following equation:

$$\text{Chl-}a\ (\text{mg L}^{-1}) = \text{DO}_{665\text{nm}} \times 13.9 \quad (1)$$

where OD is the optical density (665 nm), and 13.9 a constant value.

TN and TP from DIWW, biomass, and cell-free supernatant fraction were analyzed, with an adaptation of the persulfate digestion method [55] and vanadate-molybdate methods [56], respectively, with a Water Testing and Color Measurement Photometer MD 600 (Lovibond®, Dortmund, North Rhine-Westphalia, Germany). The carbohydrates of the biomass were quantified spectrophotometrically by the phenol-sulfuric acid method using glucose as standard [57]. Protein and phycobiliprotein were also analyzed in the pellets on day five. Protein was determined by the Lowry method [58] modified by Herbert et al. [59], using bovine serum albumin as standard, and phycobiliproteins were measured according to the Bennett and Bogorad method [60] after re-suspending the pellet in 6.0 mL of saline buffer (0.01 M Na_3PO_5-0.15 M NaCl, pH 7.0). Dry weight was measured per diem by filtering 5 mL of biomass and washing with 5 mL HCO_2NH_4 0.5 M three times [61]. The total nitrogen and total organic carbon content of the organic residues (cyanobacterial biomass and cattle manure) used for anaerobic co-digestion were analyzed with an adaptation of the persulfate digestion method proposed by D'Elia et al. [55], and the chromic acid oxidation method proposed by Degtjareff [62], and modified by Walkey and Black [63], respectively. VS were measured according to Standard Methods 2540 G [64]. Methane was determined with a Digital Handheld Gas Leakage Detector model 7899 (*E*-instruments® Langhorne, PA, USA). All analytics were done in triplicate.

2.5. Statistical Analyses

The graphs and statistical analyses were performed with the GraphPad PRISM version 8.4.3 (GraphPad Software, Inc.®, San Diego, CA, USA). The results were analyzed using variance (ANOVA double and one-way) parametric test and the Tukey test post hoc with a significance level of $p \leq 0.05$.

3. Results

3.1. Growth and Productivity of Arthrospira Platensis in Batch Cultures with Wastewater from Dairy Industries

The growth of *A. platensis* measured as chlorophyll-a concentration did not show significant differences between the cultures maintained in DIWW (70% *v*/*v*) (32.31 ± 1.01 mg L^{-1}) and the cultures maintained in standard medium (Zarrouk) (31.37 ± 0.95 mg L^{-1}) (test Tukey's $P \leq 0.05$) (Figure 2). The DIWW with the respective nutritional correction supported the growth of *A. platensis*.

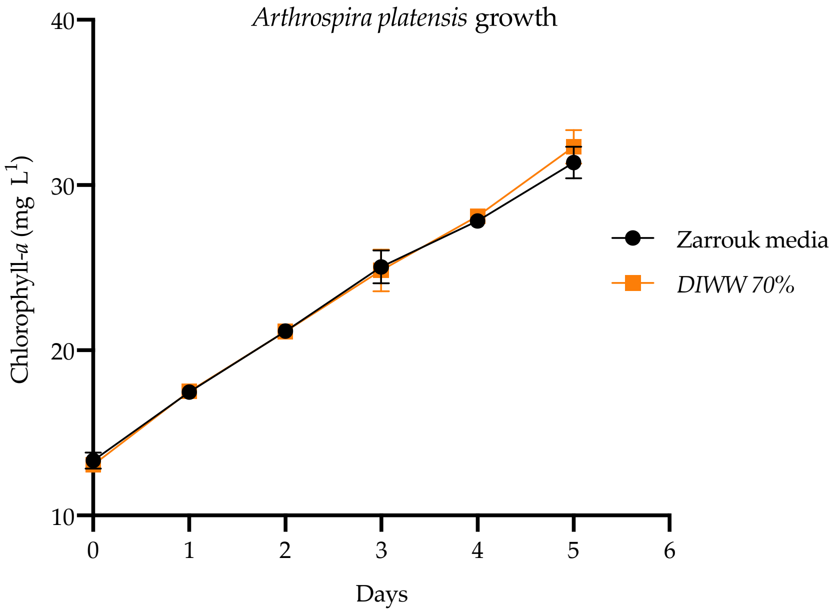

Figure 2. Growth, measured as the chlorophyll-*a* concentration of *Arthrospira platensis* cultivated in Zarrouk media (—●—), and wastewater from the dairy industry (—■—). Values are average ±SD (n = 3).

The productivity values of the cultures maintained in DIWW (1.26 ± 0.31 g L^{-1} d^{-1}) not present significant differences concerning the control (Zarrouk medium) (1.20 ± 0.38 g L^{-1} d^{-1}) (Tukey P test ≤ 0.05) (Figure 3).

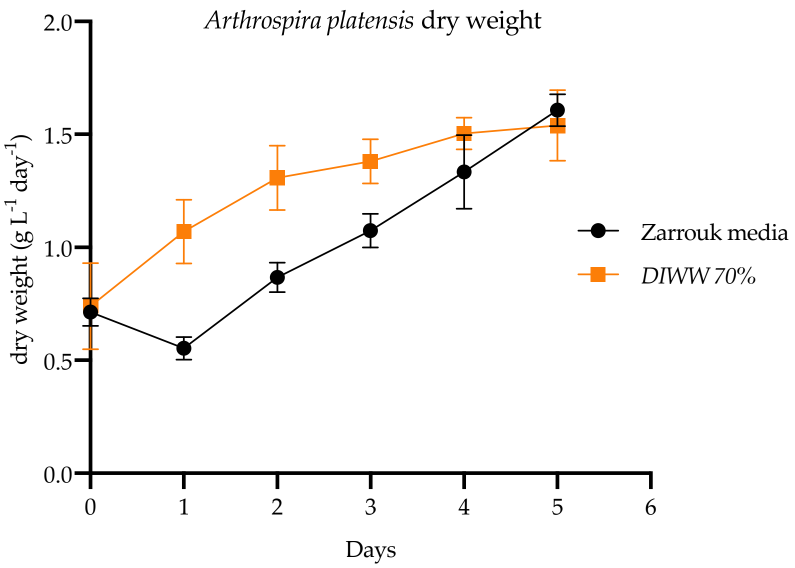

Figure 3. Productivity (g L^{-1} $days^{-1}$ of dry biomass) of Arthrospira platensis in cultures with Zarrouk medium (—●—), and with wastewater from a dairy industry (—■—). Values are average ± SD (n = 3).

3.2. Carbohydrates, Protein, and Phycobiliprotein of Arthrospira Platensis in Batch Culture with Wastewater from Dairy Industries

The biomass carbohydrates did not show significant differences between the cultures maintained in DIWW (253.35 ± 0.01 mg g^{-1}) and the cultures maintained in Zarrouk medium (261.22 ± 0.01 mg g^{-1}) (Tukey P test ≤ 0.05) (Table 1). However, the protein concentration showed significant differences between the cultures with DIWW 487.50 ± 31.14 mg g^{-1} concerning the cultures with Zarrouk: 400.45 ± 32.01 mg g^{-1} (Tukey P test ≤ 0.05) (Table 1). The cultures maintained in DIWW obtained a 21.74% more protein concentration of the dry biomass than the control (Zarrouk Medium). In contrast to protein content, phycobiliproteins: allophycocyanin (APC) and phycocyanin (PC) did not show significant differences in their concentration (mg g^{-1} dry biomass) between the cultures maintained in Zarrouk medium and the cultures maintained in DIWW (Table 1) (Tukey test $P \leq 0.05$). The control presented the following values: 105.09 ± 9.44 mg g^{-1} and 82.12 ± 7.16 mg g^{-1}; for APC and PC, respectively. The cultures with the DIWW showed the following average values: 94.27 ± 5.23 mg g^{-1} and 77.40 ± 4.69 mg g^{-1}; for APC and PC, respectively.

Table 1. Biomass carbohydrate, protein, and phycobiliprotein (allophycocyanin [APC] and phycocyanin [PC]) of *Arthrospira platensis* in batch cultures. Values are average ± SD (n = 3).

Biochemical Composition	Zarrouk Medium	Dairy Industry Wastewater (DIWW)
Biomass carbohydrate	261.22 ± 0.01 mg g^{-1}	253.35 ± 0.01 mg g^{-1}
Protein	400.45 ± 32.01 mg g^{-1}	487.50 ± 31.14 mg g^{-1}
Phycobiliprotein		
Allophycocyanin	105.09 ± 9.44 mg g^{-1}	94.27 ± 5.23 mg g^{-1}
Phycocyanin	82.12 ± 7.16 mg g^{-1}	77.40 ± 4.69 mg g^{-1}

3.3. Nutrients Removal

In the discontinuous culture of A. platensis with DIWW, the concentration of TN was reduced from 20.80 mM to 2.41 mM, a removal efficiency (RE) of this nutrient of 88.41% was obtained. In comparison, for the TP, the concentration decreased from 1.67 mM to 0.05 mM, reaching an RE of 97.01%.

3.4. Content of VS, TN, TP, OC, and Mesophilic Anaerobic Co-Digestion (CB and CM)

The content of VS, TN, TP, and TOC of the CB and CM substrates submitted to anaerobic co-digestion was, for CB: 85.03 ± 10.76% for the VS, 105.70 ± 7.60 mg g^{-1}, 3.00 ± 0.25 mg g^{-1}, and 566.90 ± 76.20 mg g^{-1} for the TN, TP, and TOC, respectively. For CM the VS it was 70.70 ± 17.87%, and 5.65 ± 1.18 mg g^{-1}, 1.75 ± 0.45 mg g^{-1}, and 270.65 ± 10.13 mg g^{-1} for the TN, TP, and TOC, respectively (Table 2).

Table 2. Volatile solid, total nitrogen, total phosphorus, and organic carbon of cyanobacterium biomass and cattle manure. Values are average ± SD (n = 3).

Substrate	VS % (Volatile Solid)	TN mg g^{-1} (Total Nitrogen)	TP mg g^{-1} (Total Phosphorus)	OC mg g^{-1} (Organic Carbon)
Cyanobacterium biomass	85.03 ± 10.76	105.70 ± 7.60	3.00 ± 0.25	566.90 ± 76.20
Cattle manure	70.70 ± 17.87	5.65 ± 1.18	1.75 ± 0.45	270.65 ± 10.13

Mesophilic anaerobic co-digestion was performed for 40 days. The accumulated methane yield profiles obtained for the evaluated substrates are shown in Figure 4. The previously treated CB showed a yield of 299.63 ± 7.53 mL of CH_4 g^{-1} VS; for CM, the values obtained were 191.93 ± 2.22 mL of CH_4 g^{-1} VS, while for the mixture (80:20) of CB and pretreated CM, the maximum methane production was obtained, 482.54 ± 8.27 mL of CH_4 g^{-1} VS (Figure 4).

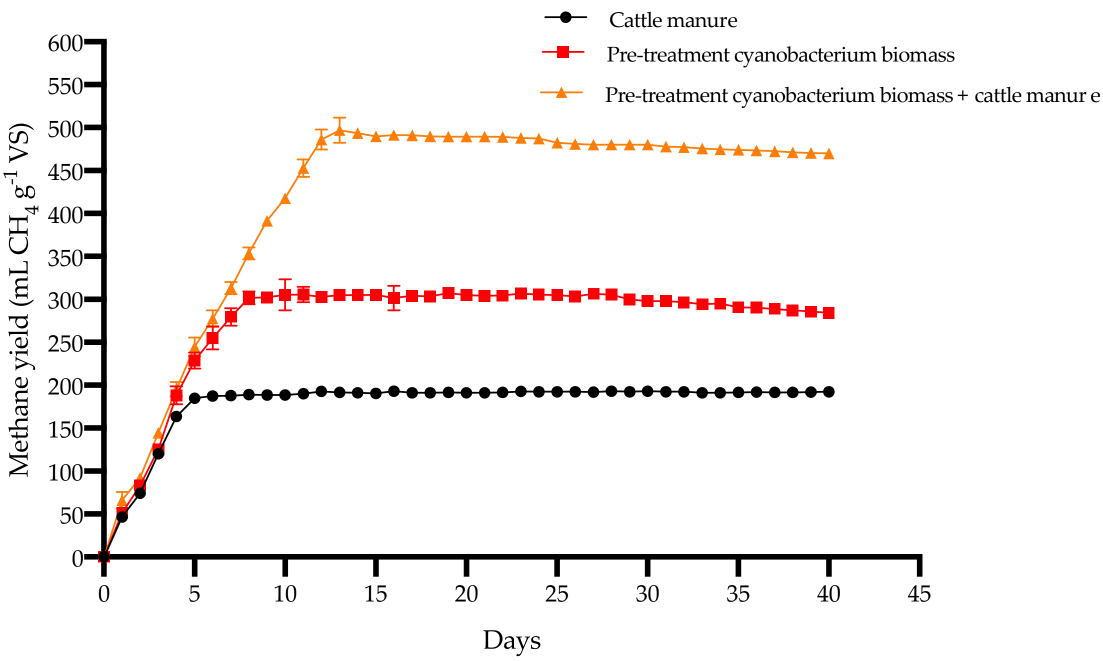

Figure 4. Cumulative methane yield of anaerobic digestion of cattle manure ( ); anaerobic digestion of pre-treated Arthrospira platensis biomass ( ); and anaerobic co-digestion of bovine manure + of pretreated Arthrospira platensis biomass ( ). Values are average ± SD (n = 3).

4. Discussion

The result obtained demonstrated the feasibility of maintaining a stable batch culture of the cyanobacteria *A. platensis* using wastewater from a dairy industry as a culture medium, and the subsequent use of the cyanobacteria biomass obtained in this culture system in anaerobic co-digestion with cattle manure, resulting in high methane yield. The cultivation of this type of photosynthetic microorganisms in agro-industrial wastewater is presented as an exciting alternative to develop as tertiary wastewater treatments, avoiding the environmental impact that arises with the discharge of this type of sewage into bodies of water. Additionally, due to the elemental composition (C:N:P), presented by the biomass of *A. platensis*, it can be used as a biofertilizer or a biostimulant in agriculture [65–67].

The results of this work with the wastewater from a dairy industry demonstrate the viability of using *A. platensis* in the bioremediation of this type of effluent, with the potential to become an alternative to standard tertiary treatments its high removal capacity of nutrients. Added to the high production of biomass, which can be applied for multiple purposes, including pharmaceuticals, organic fertilizers, biofuels, and animal feed [68–72]. This work was possible to obtain removal efficiencies of 88.41% for TN and 97.01% for TP in cultures with wastewater from the dairy industry.

The use of microalgae and cyanobacteria for the removal of N and P in agro-industrial wastewater is an area of permanent study. Therefore, Ouhsassi et al. [73] evaluated the treatment of wastewater from dairy plants with the cyanobacterium *Pseudanabaena galeata* Böcher 1949, cultivated the microorganisms with different concentrations of wastewater (25, 50, and 75% *v*/*v*), with a final concentration of TN of 1.1, 2.2 and 3.2 mM, respectively. The highest RE was with the concentration of TN 1.1 mM, with 86.44%. Marazzi et al. [74] evaluated the treatment of wastewater from whey processing with the microalgae *Scenedesmus acuminatus* (Lagerheim) Chodat 1902, and a consortium of microalgae consisting of *Chlorella*, *Scenedesmus*, and *Chlamydomonas* spp. The wastewater had a TN concentration of 3.71 mM and a TP of 0.42 mM. The RE for *S. acuminatus* was 88% and 69% for nitrogen and phosphorus, respectively, and for the microalgal consortium, it was 90% and 73% for TN and TP, respectively. In comparison, Daneshvar et al. [75] evaluated a freshwater microalgae *Scenedesmus quadricauda* Chodat, nomenillegitimun 1926, and a seawater microalgae *Tetraselmis suecica* (Kylin) Butcher 1959, in the treatment of dairy wastewater (DWW). The initial concentration of TN and TP in the DWW was 6.14 and 0.09 mM, respectively, for the culture of the marine species, they added 34 g L^{-1} of sea salt. The RE percentages for TN and TP were 86.21% and 89.83% for *S. quadricauda*, and 44.92% and 42.18 for *T. suecica*. We must emphasize that the significantly lower TN and TP concentrations are used in the studies mentioned above than the ones used in our research. Cyanobacteria are characterized by their high consumption and accumulation of nutrients, nitrogen in cyanophycin granules, and carbon in carboxysomes. These characteristics make them strong candidates for the removal of nutrients in agro-industrial wastewater.

The growth of *A. platensis* measured as the concentration of chlorophyll-*a* obtained in the cultures with DIWW is similar to that described in other investigations. Álvarez and Otero [16], in semi-continuous cultures of *Arthrospira* sp. with an anaerobic centrate rich in ammonium, they obtained a chlorophyll-*a* concentration of 27.6 ± 0.5 mg L^{-1}. Markou et al. [76] obtained a chlorophyll-*a* concentration of 23.74 mg L^{-1} in *A. platensis* cultures with oil mill wastewater. Olguín et al. [77] cultivated *Arthrospira maxima* Setchell and N.L. Gardner 1917, in raceways at 30 °C, with seawater (salinity 30%) supplemented with 2% (*v*/*v*) of pig purine concentrate with a final ammonium concentration of 1.4 mM, obtaining a maximum concentration of chlorophyll-*a* of 8.1 mg L^{-1}, a concentration significantly lower than the concentration of this work (32.31 ± 1.01 mg L^{-1}). The chlorophyll-*a* concentration obtained in our research is higher than the works mentioned above. This difference is explained by the high light intensity used (140 µmol photon m^{-2} s^{-1}) and the CO_2 supplement that our cultures had. The mechanisms of production and accumulation of pigments in cyanobacteria, chlorophyll-*a*, and phycobiliproteins, are activated by ambient light conditions [78], this confirms our high production of both photosynthetic pigments.

The productivity of the *Arthrospira platensis* cultures maintained with DIWW is among the highest (1.26 ± 0.31 g L^{-1} d^{-1}), thus Olguín et al. [79] with cultures of *Arthrospira* sp. in seawater supplemented with anaerobic effluents of digested swine waste, they reached productivity of 0.144 g L^{-1} d^{-1}. Rizzo et al. [80], using the Zarrouk medium in *A. platensis* cultures with a light intensity of 150 µmol photons m^2 s^{-1}, obtained maximum productivity of 0.104 g L^{-1} d^{-1}. Kim et al. [81] cultivated *A. maxima* for one year in semi-open raceway systems, the lowest productivity obtained in these systems was 0.142 g L^{-1} d^{-1}, and the highest was 0.314 g L^{-1} d^{-1}. However, studies with higher productivity, such as that described by Michael et al. [82], who cultivate *Arthrospira fusiformis* (Voronichin) Komárek and J.W.G. Lund 1990, in a medium formulated based on the commercial fertilizer NPK10-20-20, obtained productivity of 2.54 g L^{-1} d^{-1}. The high productivity values obtained here may be due to the high effective irradiance available in tubular systems (diameter 4.6 cm) and the strict pH control.

The concentrations of carbohydrates, proteins, and phycobiliproteins obtained in *A. platensis* cultures maintained with DIWW are similar to those obtained by other researchers. In batch cultures of *Spirulina platensis* with Zarrouk medium supplemented with 2.5%, 5%, and 10% (*v*/*v*) of cheese whey (CW), varied concentrations of proteins and carbohydrates were obtained, for 2.5% CW 60.62% and 23.29%, for 5% CW 44.56% and 47.83%, and for 10% CW 39.62 and 40.65% for proteins and carbohydrates, respectively [82]. Olguín et al. [83] evaluated the light intensity of 66 (low) or 144 µmol photon m^2 s^{-1} (high) in batch cultures of *Spirulina* sp. maintained in seawater enriched with 2% (*v*/*v*) of anaerobic effluents and 23.81 mM $NaHCO_3$. The cultures with low light intensity presented concentrations of 39.0% and 3.41% for proteins and carbohydrates, respectively, while the cultures with high light intensity, the values obtained were 33.0% and 28.41% for proteins and carbohydrates, respectively. However, the biochemical composition of the biomass is directly correlated with the species used and the cultivation conditions. Thus, Markou et al. [76] obtained ranges in carbohydrate concentration from 16.52% to 63.75%. The protein content varied from 22.04% to 38.13% in *A. platensis* cultures in oil mill wastewater (OMWW); this variation depended on the treatments used to decrease the concentration of phenolic compounds turbidity of the OMWW. The values obtained for the phycobiliproteins are among the highest for the cultivation of cyanobacteria in wastewater (171.67 mg g^{-1}). Chaiklahan et al. [84], in semi-continuous cultures of *A. platensis* with 20% of the anaerobic centrate of porcine slurry, supplemented with 53.6 mM $NaHCO_3$ and 3.3 mM NH_2CONH_2, with an ammonium concentration of 1.6 mM, obtained a concentration of 195.0 mg g^{-1} PC and 192.0 mg g^{-1} for the control (Zarrouk medium). While in the semi-continuous culture of *Arthrospira* sp. in the anaerobic centrate of the fish and shellfish industry, phycobiliprotein values of the order of 158.1 mg g^{-1} were obtained, with a final NH_4^+/NH_3 concentration of 14.3 mM [14].

The content of TN, TP, and OC of *A. platensis* maintained in DIWW presented values between those obtained in other works. Mahdy et al. [85] determined the content of OC of 486.0 mg g^{-1} and TN of 106.0 mg g^{-1} in the biomass of *Chlorella vulgaris* Beyerinck [Beijerinck] 1890. OC values 526.0 mg g^{-1}, and TN, 82.0 mg g^{-1}, are described for the same species [86]. Biller et al. [86] obtained similar values for *A. platensis* to those obtained in our work, for OC 557.0 mg g^{-1} and TN 112.0 mg g^{-1}, while *Scenedesmus dimorphus* (Turpin) Kützing 1834, presented OC 534.0 mg g^{-1} and TN 79.0 mg g^{-1}. The OC content of the biomass of microalgae and cyanobacteria present similar values between the different species; the TN content is significantly higher in *A. platensis* correlated with the high protein percentage that this species can present (40–70%) [16,86]. Regarding the percentage of VS in the biomass of microalgae and cyanobacteria, low values such as 45% have been obtained for the *Nannochloropsis oculata* (Droop) D.J. Hibberd 1981 [87], intermediate values, 77%, for *Nannochloropsis salina* [87], and high values, 90%, in *Chlorella vulgaris* [88]. For *Arthrospira platensis*, SV values of 86–90% have been obtained [89–91]. However, in more detailed work on *Arthrospira maxima*, values that fluctuated between 80–92.7% were obtained. VS variation is correlated with differences in *A. maxima* biomass composition due to cultivation conditions, so the percentage of protein presented a range of 60–71%, carbohydrates 13–16%, and lipids 6–7% [92].

The high methane yields for the different substrates used in anaerobic co-digestion in our work presented values between those already described by other researchers. For the microalgae, *Chlorella vulgaris*, the methane yield by the anaerobic digestion of its biomass is between 189–403 mL CH_4 g^{-1} VS [88,93,94]. This wide variation in this chlorophyte is presented using different cultivation systems, reflected in the variation in the biomass composition. Similar results are described in the methane yield by anaerobic digestion of the biomass of *Scenedesmus*, with a production range between 170–354 mL CH_4 g^{-1} VS [95–97]. This methane yield variation is related to the composition of the biomass and the C / N ratio that it presents, which is generally low [98]. For the genus *Spirulina* (*Arthrospira*), the methane yield product of the anaerobic digestion of its biomass, in the same way, presents values between 50–150 mL g^{-1} VS [99]. The CH_4 yield values of the anaerobically digested *A. platensis* biomass in our work are significantly higher (299.63 ± 7.53 mL of CH_4 g^{-1} VS); this is explained by the thermal and ultrasonic pre-treatment given to it, which significantly increases the digestibility of the high protein fraction contained [52]. The co-digestion of the pre-treated cyanobacterial biomass with cattle manure in our work presented high yield values of CH_4 (482.54 ± 8.27 mL of CH_4 g^{-1} VS), higher than those described in other works. Thus, Solé-Bundó et al. [43], in the co-digestion of pre-treated biomass of *Chlorella* sp. (20%) with wheat straw (80%), obtained a methane yield of 304.7 mL CH_4 g^{-1} VS. In comparison, the co-digestion of the *Scenedesmus* sp. (30%) with pig manure (70%) produced a yield of 245 mL CH_4 g^{-1} VS [46]. The cyanobacterium *Spirulina* (*Arthrospira*) has been evaluated in co-digestion (50% –50%) with sewage sludge with a methane yield of 360 mL CH_4 g^{-1} VS [90]. The high methane yield achieved in the co-digestion of C.B. and CM is directly related to:

- The thermal and sonic pre-treatments that break the cell wall of the cyanobacteria and significantly improve its biodegradability;
- The improvement in the carbon-nitrogen ratio of both substrates;
- The thermophilic regime used (35 °C).

5. Conclusions

The results obtained in this work show that the filamentous cyanobacterium *Arthrospira platensis* can be cultivated using wastewater from the dairy industry, with high biomass productivity, representing an interesting alternative for the treatment of this type of wastewater that, with its discharge, causes environmental impacts. Additionally, the biomass obtained by this system presents an interesting biochemical composition, with a high protein percentage (48.8%) that would be suitable for animal nutrition. It was found that this biomass has a high methane yield when used in anaerobic co-digestion with cattle manure, becoming a bioenergetic alternative.

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