energies

MDPI

Article

Evaluation of the use of static electric fields for the intensification of microalgae culture as biomass for biogas production

Marlena Owczuk [1], Anna Matuszewska [2,*], Piotr Wieczorek [3]

[1] Łukasiewicz Research Network—Automotive Industry Institute; Jagiellońska Str. 55, 03-301 Warsaw, Poland; marlena.owczuk@pimot.lukasiewicz.gov.pl
[2] Łukasiewicz Research Network—Automotive Industry Institute; Jagiellońska Str. 55, 03-301 Warsaw, Poland; anna.matuszewska@pimot.lukasiewicz.gov.pl
[3] Łukasiewicz Research Network—Automotive Industry Institute; Jagiellońska Str. 55, 03-301 Warsaw, Poland; piotr.wieczorek@pimot.lukasiewicz.gov.pl

* Correspondence: anna.matuszewska@pimot.lukasiewicz.gov.pl

Abstract: The move toward a closed-loop economy and the implementation of non-competing bio-raw materials are becoming a major challenge. One source of biomass can be microalgae. As shown in the article, these microorganisms can be a good feedstock for biogas production, while requiring pretreatment due to the structure of the cell wall. With climatic conditions preventing microalgae culture in open tanks, the limitation of their implementation into the economic system is their high cost. The authors of the article showed that the application of a static electric field during microalgae culture can stimulate cell growth and thus increase the efficiency of culture. The biomass yield depends on the microalgae species, exposure time, and current intensity value.

Keywords: microalgae; biogas; static electric field; growth intensification; biochemical methane potential.

1. Introduction

Microalgae are unicellular, autotrophic microscopic algae with photosynthetic abilities, inhabiting aquatic environments (salty or fresh). There are many species of microalgae, which differ in structure, composition of cells and cell walls, growth rate, and resistance to changes in culture conditions (temperature, pH, access to nutrients, light, or carbon source) [1, 2]. Depending on the species and culture conditions of the microalgae, they contain 6 to 52% of proteins, 7 to 23% of fats, 5 to 23% of carbohydrates, and various mineral compounds [3-5].

These organisms are used in various industries [6], e.g. in the food, pharmaceutical, or cosmetics industry as a source of the various types of pigments, protein supplements, lipids or carbohydrates, in the production of feed, nutritional supplements, and cosmetics [7-10]. They are also used to purify post-process gases (including biogas) from carbon dioxide in the photosynthesis process [11, 12], wastewater from organic pollutants in treatment plants [13-15] or post-fermentation sludge from biogas plants [16-18]. Such solutions are characterized by both economic and environmental benefits [19-21]. Microalgae can also produce biohydrogen themselves in the photolysis process [22, 23] which is a green biofuel or a valuable biosubstrate for many chemical syntheses. In addition to the above-mentioned applications, they are a good material in the energy industry, as a raw material for the production of biofuels and biochemicals, using the following methods:

- biochemical (e.g. biodiesel, bioethanol) [24, 25],
- chemical and thermochemical (e.g. bio-oil, synthesis gas) [26-29],
- electrochemical (e.g. biohydrogen) [30].

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Received: date
Revised: date
Accepted: date
Published: date

One of the areas of frequent use of microalgae is their use as a raw material for the biogas production process [31]. In this process, microalgae can be a substrate or co-substrate and fermented with other organic raw materials [2, 32, 33]. The methane content in biogas obtained from microalgae biomass depends, among others, on the conditions of the methane fermentation process and the type of microalgae. For example, in the work [34], biogas was produced from the *Tetraselmis* species, in a continuous process, at a temperature of 35°C, obtaining 72-74% methane (0.31 m^3 CH_4/kg d.m.o.). In turn, the authors of the work [35] examined the methanogenic potential of *Spirulina*, conducting the process at a temperature of 30°C, using the sequential method. They obtained biogas containing 68-72% methane, with an efficiency of 0.26 m^3 CH_4/kg d.m.o. In studies on methane fermentation of *Chlorella* [36], biogas containing 68-75% methane was obtained, with the efficiency of 0.31-0.35 m^3 CH_4/kg d.m.o. The process was carried out in a batch method, at a temperature of 28-31°C. Co-fermentation of this microalgae with *Scenedesmus*, at a temperature of 35°C, obtained a similar concentration of methane in biogas (at the level of 69% in the case of continuous fermentation [37] and 70% for batch fermentation [38]). The processes differed in efficiency - 0.09-0.14 m^3 CH_4/kg d.m.o. and 0.16 m^3 CH_4/kg d.m.o., for continuous and batch fermentation, respectively.

The use of microalgae in the fermentation process has its limitations, including the presence of cell walls that are resistant to decomposition by microorganisms at the hydrolysis stage. The structure of the cell walls may limit the efficiency or even completely inhibit the fermentation process [31, 39]. Such species include, for example, *Scenedesmus* and *Chlorella* [40]. The cell walls of these algae have a multilayer structure, composed mainly of cellulose and hemicellulose, which are more difficult to biodegrade. Algae species that lack a cell wall (e.g. *Dunaliella, avlova_cf*) or have a cell wall composed of glycoproteins (e.g. *Chlamydomonas, Euglena*) produce higher methane yields than species with a complex wall. The solution to the problem of the difficultly degradable cell wall of microalgae is their pre-treatment [41-43]. As a result, the microorganism cell walls disintegrate. This increases the availability of the contents of the interior of the cells to methanogenic bacteria and affects the increase in biogas efficiency. For example, in the work [44] the influence of microalgae species and their pretreatment conducted by three methods was studied to increase their susceptibility to anaerobic decomposition. It was shown that enzymatic treatment was the most effective, followed by thermal treatment - the obtained increase in biogas efficiency was over 270% and 60-100%, respectively. The least effectiveness was shown in the case of the treatment using ultrasound, obtaining a 30-60% increase in biogas efficiency.

Microalgae are most often cultivated in special systems: open ponds and various types of photobioreactors [45-47]. However, their industrial use is limited due to the high costs of cultivation [48]. The main challenge for researchers is to develop new technologies for intensifying microalgae cultivation while at the same time reducing costs and thus increasing economic profitability [49, 50].

Many research works have been conducted on the possibility of using magnetic fields [51-53] or electromagnetic fields [54, 55] to improve biotechnological processes and the growth of microorganism cells. Recently, scientists have been very interested in using electric fields of varying intensity for this purpose.

There are three types of electric fields used in the literature:

- pulsed (PEF), with a voltage above 1000 V/cm and duration from nano to milliseconds, used mainly in medicine and food technology, less frequently in environmental and biotechnological processes [56, 57],
- moderated (MEF), a variable electric field with an intensity of up to 1000 V/cm, where the process usually lasts several minutes and involves non-thermal action (combined action of electrical energy and temperature), used mainly in food technology [58, 59],
- static (SEF), a constant electric field applied to two electrodes for a unidirectional flow of charges and particles, used mainly for separating or concentrating particles from a liquid mixture [60, 61].

These technologies may constitute one of the methods of stimulating the growth of microalgae cells in the processes of their cultivation, however, such application is still in the initial phase of research, and the effect of the electric field on microorganism cells is not yet fully understood. The use of an electric field with parameters causing damage to the cell membrane of microorganisms or changing its structure as a result of electroporation (reversible or irreversible) can also be used in the process of their disintegration before methane fermentation [62].

In the research works described in the literature, the most frequently studied is the effect of the electric pulse field (PEF) on microorganisms. In terms of the use of SEF and MEF, there are few reports in the available literature on the possibility of using them to stimulate algae cultivation. In the work [63], the authors assessed the effect of a nanosecond PEF on the ability of cyanobacteria *Arthrospira platensis* to reproduce. The study revealed an increased increase in cell biomass by approx. 13% due to increased proliferation, which was most visible after five days of the experiment, with the input energy of 256.22 ± 67.53 J/kg. Increased production of pigments and proteins in cells was also observed, which could be caused by the cell reaction to stress induced by the electric field. The work [64] presents the effect of nanosecond PEF on microorganism cells. It was found that the phase of the fastest proliferation of microorganism cells exposed to the electric field is mainly observed in the early phase of exponential cell growth. This phenomenon occurs after 5 days from the moment of PEF exposure, which confirms the results of the authors' research presented in the work [63].

Nanosecond pulsed electric field PEF was also used in the work of [65] to increase the biomass yield in a biorefinery based on microalgae *Chlorella vulgaris*. The increase in the algae biomass yield was found by about 17% at a frequency of 5 Hz and an intensity of 10 kV/cm for 100 ns, due to intracellular and cell membrane changes. Other authors also indicate that PEF technology has a positive effect on cell proliferation, without negatively affecting cellular components (non-thermal and non-invasive method) [66, 67].

In the work [68], a pulsed electric field was used for cell disintegration and extraction of intracellular components from freshwater microalgae *Auxenochlorella protothecoides*. The effect of suspension treatment energy (52-211 kJ/kg), electric field intensity (23-43 kV/cm), pulse frequency (1.0-5.5 Hz), and biomass concentration (36-167 g dry weight per kg of suspension) on the degree of microalgal cell disintegration was studied. It was found that the efficiency of cell disintegration increased with increasing energy, while the field intensity had practically no effect. Significant cell disintegration was obtained, and the use of a pulsed electric field promoted the release of soluble cellular components from *Auxenochlorella protothecoides* microalgae cells. PEF treatment did not cause spontaneous release of lipids but enhanced their extraction with a solvent.

In the work [61], the authors assessed the effect of the application time of a static electric field (for 10–70 min) with an intensity of 2.7 kV/cm. It was found that within 50 min of the field's impact on algae, the concentration of *Chlorella vulgaris* algae biomass increased by up to 51% due to the increased permeability of the cell membrane. Further increase in the exposure time resulted in a decrease in the concentration of biomass caused by the accumulation of harmful components in the culture medium.

The authors of another work [69] used a constant electric field (at 60, 100, and 120 mA and voltages of 15, 25, and 30 V) to cultivate *Haematococcus pluvialis* microalgae in OHM medium and intensify the production of astaxanthin pigment. Cells exposed to SEF at 60 mA and 100 mA showed an increase in biomass density by 16% and 20%, respectively, and increased pigment production compared to the control sample. Increasing the current intensity to 120 mA resulted in a decrease in cell number. The differences between the test and control samples were most visible on the fourth day of the study, after which they decreased. It was shown that periodic exposure of the culture to electric current brings better effects than a single exposure.

In the available literature, the effect of electric field on microorganisms, including microalgae, mainly concerns PEF. Works covering the use of MEF and SEF are still few

and far between and this area is still poorly understood. Therefore, the research presented in this paper aims to investigate the biochemical methanogenic potential (BMP) of selected photosynthetic microorganisms and to assess the effect of using a constant electric field (SEF) on the intensification of microalgae biomass cultivation.

2. Materials and Methods

2.1. BMP methodology

Live saltwater microalgae (mixture of *Nannochloropsis sp, Tetraselmis sp, Isochrysis sp*) and freshwater microalgae were used to carry out the research: 276-4D - *Desmodesmus armatus* and CC16-90 - *Chlamydomonas reinhardtii* obtained from the Department of Plant Physiology and Biotechnology at the Faculty of Biology of the University of Gdańsk. Additionally, lyophilized microalgae strains were used as a comparative substrate for BMP studies: *Chlorella sp., Spirulina sp. and Scenedesmus sp*. The research was conducted in the Łukasiewicz-PIMOT Biogas Laboratory.

A single research station consisted of a fermenter with a capacity of 500 dm^3 equipped with biogas collection nozzles, placed in a water bath (at a temperature of 42±1°C). Biogas generated during the process was collected into graduated receivers filled with an aqueous solution of NaCl. The receiver was connected to a 5 dm^3 surge tank filled with the same barrier solution. Individual elements of the system were connected using gas-tight tubing (Tygon).

For the BMP studies, microalgae were subjected to thermal and mechanical pretreatment to increase the accessibility of cells for methane microorganisms. Live and lyophilised (hydrated in a 1:2 biomass:water ratio) microalgae cultures were stored for 4 days at -20°C and ground in a mortar with the addition of quartz sand (in a 6:1 ratio). The prepared biomass was analyzed in terms of dry matter content (drying method) and chemical oxygen demand (dichromate method). Microalgae were placed in fermenters together with inoculum (in a 1:6 ratio) and placed in a water bath. The contents of the fermenters were mixed daily. The amount of biogas produced from a given substrate was measured and its composition was analyzed using the GMF 416 portable biogas analyzer from GAS DATA, which allows for the quantitative and qualitative determination of the content of methane (0-100)% v/v, carbon dioxide (0-100)% v/v, oxygen (0-25)% v/v, hydrogen sulphide (0-5000) ppm v/v, and hydrogen (0-1000) ppm v/v.

2.2. Electrostimulation methodology

Freshwater microalgae cultures were used to carry out the research: 276-4D - *Desmodesmus armatus* and CC16-90 - *Chlamydomonas reinhardtii*. The microalgae culture electrostimulation research station consisted of 4 systems equipped with 3-liter beakers, each with aerators. Spiral stainless steel electrodes, to cover the whole volume of the culture, connected to DC power supplies were connected to 3 beakers. The fourth system was the so-called blank test (sample 0), which was not exposed to the current. It was the base to which the results for algae in beakers 1-3 (marked as samples 1-3) subjected to electrostimulation were referred. The culture systems were illuminated for 12 h with LED lighting and the same temperature conditions were maintained. The microorganisms were fed with a BBM medium. Figure 1 shows a view of the research station.

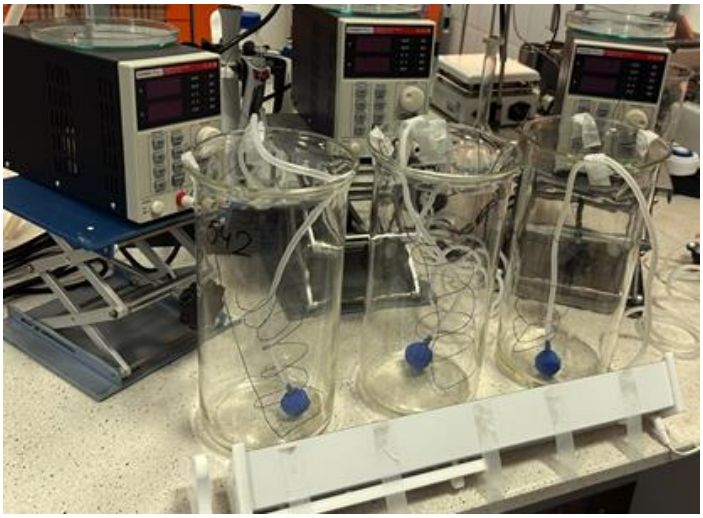

Figure 1. View of the electrostimulation research station.

Stimulations were performed at constant current voltage for all systems 24 hours a day, but at different intensities (Table 1).

Table 1. Experiment parameters.

Test No.	**0**	**1**	**2**	**3**
Voltage [V]	0.00	5.00	5.00	5.00
Current [mA]	0.0	25.0	50.0	100.0

Electrostimulation was maintained throughout the controlled growth period of 4 weeks. To determine the growth rate of the culture, dry matter content was measured at weekly intervals using the gravimetric drying method.

To check the effect of electrostimulation on the increasing availability of microalgae cells to methane fermentation microorganisms, BMP tests were performed. The study was carried out on samples of *Desmodesmus armatus* microalgae after 4 weeks of electrical stimulation (with three different current intensities) and a reference sample (without exposure to the electric field). Microalgae cells were not subjected to any additional disintegration.

3. Results

3.1. Methanogenic potential of microalgae biomass

Table 2 presents the results of the analysis of the dry matter content and the chemical oxygen demand (COD) values of the substrates used for the tests.

Table 2. Dry matter content and chemical oxygen demand of substrates used for research experiment parameters.

Substrate	**DM content [%]**	**COD [g O_2/g s.m.]**
inoculum	1.49	668.22
Spirulina	32.75	2 693.93
Scenedesmus	33.31	1 692.70
Chlorella	32.16	2 554.99
Desmodesmus armatus	5.25	4 639.12
Saltwater algae	9.77	1 503.99

Chlamydomonas reinhardti	6.32	3 357.24

Figure 2 shows the average volumes of biogas and methane obtained from individual systems.

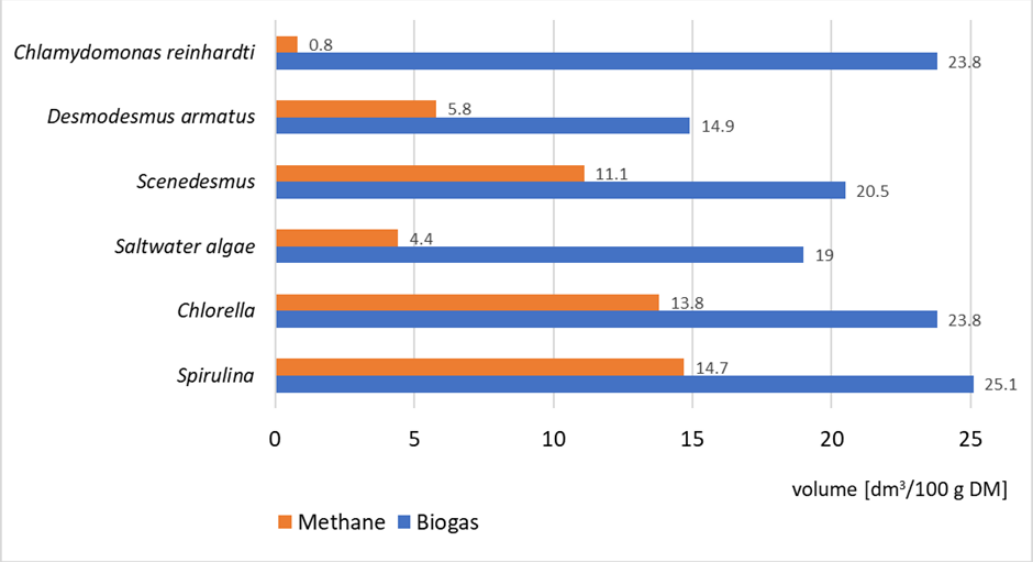

Figure 2. Volume of biogas and methane obtained from microalgae biomass.

Based on the conducted studies on the methanogenic potential of various microalgae, it can be stated that the largest volume of biogas and methane contained in it was obtained from lyophilised algae *Spirulina sp.* (from 100 grams of dry mass, 25.1 dm^3 the biogas with 14.7 dm^3 of methane was obtained). *Chlorella sp*. and *Scenedesmus sp*. were characterized by slightly lower efficiency. Moreover, the biogas obtained from all lyophilized algae contained 54-59% methane. According to the authors, the increased efficiency in terms of biogas and methane, concerning the efficiency from cultivated microalgae, could have been caused by the increased availability of the microalgae cell content for methanogens due to the cracking of cell walls under the conditions of the additional lyophilised process or other processes to which they could be subjected before lyophilised.

In the case of microalgae obtained from our own culture, the efficiency in terms of biogas yield was lower and this gas contained less methane (up to 39% methane for *Desmodesmus armatus*). The above results indicate that the pre-treatment of live microalgae cells was less effective (lower availability of cell content for bacteria participating in the methane fermentation process was observed). The lowest efficiency in terms of methane was observed for the freshwater algae *Chlamydomonas reinhardti* and the fermentation process of these microalgae proceeded with visible disturbances. In the case of saltwater microalgae, the process could be additionally inhibited by a high concentration of salt ions present in the algal biomass.

3.2. Electrostimulation study

Figures 3 and 4 present the results of the dry matter content tests in the algae suspension before the electrostimulation process and after 1, 2, 3 weeks, and at the end of the experiment.

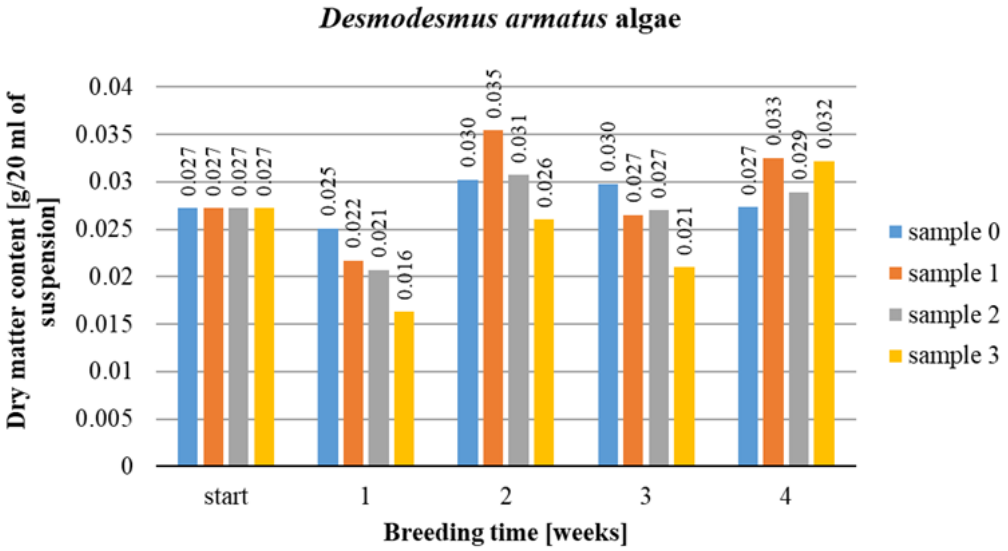

Figure 3. Results of dry matter content of suspended *Desmodesmus armatus* algae subjected to electrostimulation.

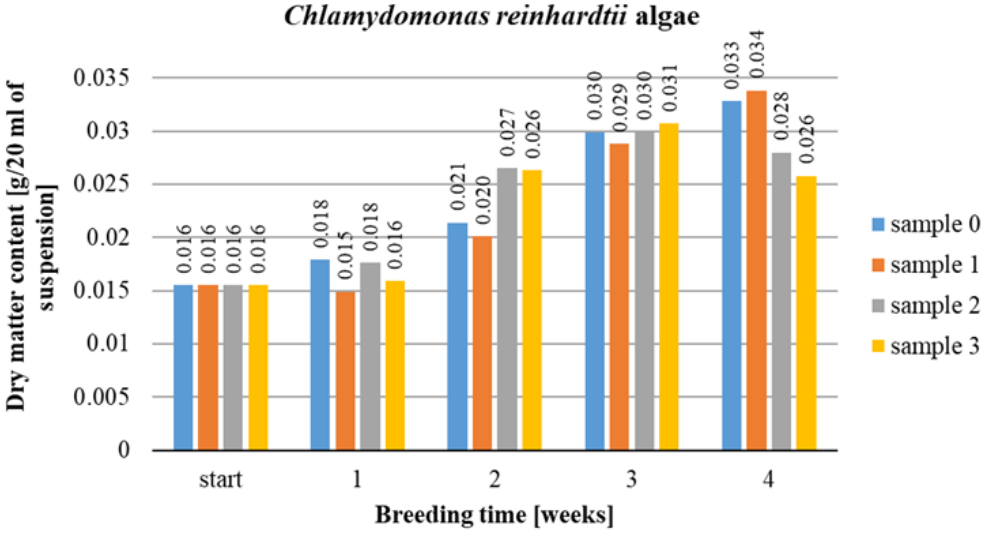

Figure 4. Results of dry matter content of suspended *Chlamydomonas reinhardtii* algae subjected to electrostimulation.

Based on the obtained results of the dry mass content tests in the cultures subjected to electrostimulation, the highest increase in biomass was found:

- *Desmodesmus armatus* algae in the 2nd week of the experiment: for sample 0 by 11% and for samples 1 (intensity 25 mA) and 2 (intensity 50 mA), by 30% and 13%, respectively. For sample 3 (intensity 100 mA), the highest increase of 18% was found in the 4th week of the experiment. Compared to sample 0, the greatest increase in biomass was obtained after 4 weeks of testing, when the algae were exposed to a current of 25 mA (increase of 22%).
- *Chlamydomonas reinhardtii* algae in the 4th week of the experiment: for sample 0 by 112% and for sample 1 (intensity 25 mA) by 118%. For sample 2 (intensity 50 mA) and 3 (intensity 100 mA) the largest increase of 81% and 67% respectively was observed in the 3rd week of the experiment. Compared to sample 0, the greatest increase in biomass was obtained after 2 weeks of testing, when the algae were exposed to a current of 50 mA (an increase of 29%).

The obtained results indicate that the applied electric field intensity in the range of 25-100 mA had a positive effect on the intensification of algal cell multiplication processes, increasing their biomass yield. The magnitude of intensification depends on the microalgae species, the time of exposure to electrostimulation, and the magnitude of the current intensity.

To test the effect of electrostimulation on the availability of *Desmodesmus armatus* microalgae cells for methane fermentation microorganisms, BMP tests were carried out without pretreatment. Based on the tests, it was found that regardless of the magnitude of the electric field strength applied, similar results were obtained in terms of biogas and methane volume compared to algae that were not electrostimulated. Thus, the structure of the cell walls was not altered during electrostimulation to such an extent that they did not act as a barrier to hydrolysing microorganisms for biogas production. The use of such cultures for biogas production will require an additional cell disintegration process. Increasing the electric field strength to a value that increases the permeability of the algal cell wall may also be a possible solution, but there is a risk of complete cell destruction and death of the culture. However, this requires additional research into optimising the parameters of direct current electrostimulation.

4. Conclusions

Microalgae are a valuable raw material not competing with food production, for many industries, including biofuel production. The use of such biomass as a feedstock for the methane fermentation process is one of the most promising and effective methods of energy use of microalgae. However, their cells often require pre-treatment to increase their availability to microorganisms participating in the methane fermentation process. As a result of the BMP tests, it was found that biogas obtained from lyophilized algae contained 54-59% methane. In the case of microalgae obtained from own cultivation, the efficiency in terms of biogas yield was lower and this gas contained up to 39% methane. In the case of lyophilized organisms, the higher efficiency of the methane fermentation process could be caused by better accessibility of the microalgae cell content for fermenting microorganisms, as a result of the cracking of cell walls under the conditions of the lyophilized process or other processes before lyophilised. In climatic conditions, where it is not possible to cultivate algae in open waters, their cultivation involves large financial outlays. To reduce costs various methods are being sought to increase the profitability of cultivating these microorganisms. Studies conducted by the authors have shown that the use of a constant electric field with an intensity of 25, 50, and 100 mA, acting on algae 24 hours a day, increased their biomass yield. This yield was dependent on the species of microalgae, exposure time, and current intensity value. The most effective for *Desmodesmus armatus* algae was the intensity of 25 mA, for which an increase in biomass of 22% was found compared to the sample not exposed to the electric field, after 4 weeks of testing. In the case of *Chlamydomonas reinhardtii* algae, the best results in biomass yield compared to the sample not exposed to the electric field were found after 2 weeks of exposure to a current of 50 mA (increase of 29%). The use of a constant electric field, although it influenced the intensification of cell multiplication processes and increased algae biomass yield, did not affect increasing the availability of cell content for methane fermentation microorganisms. For this reason, for biogas production, it is necessary to use cell disintegration methods.

Author Contributions: Conceptualization, M.O., A.M. and P.W.; methodology, M.O., A.M. and P.W.; formal analysis, M.O. and A.M.; investigation, M.O. and A.M.; writing—original draft preparation, M.O. and A.M.; writing—review and editing, M.O., A.M. and P.W. All authors have read and agreed to the published version of the manuscript

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Leong, Y.K., Chang, J.-S. Integrated role of algae in the closed-loop circular economy of anaerobic digestion. *Bioresource Technology* **2022**, *360*, 127618.
2. Vargas-Estrada, L., Longoria, A., Arenas, E. et al. A Review on Current Trends in Biogas Production from Microalgae Biomass and Microalgae Waste by Anaerobic Digestion and Co-digestion. *Bioenergy Researc*. **2022**, *15*, 77–92.
3. Grobbelaar, J.U. *Handbook of microalgal culture: biotechnology and applied phycology*, ed. Richmond A.; Wiley-Blackwell: United States, 2004; pp. 95–115.
4. Brown, M.R.; Jeffrey, S.W.; Volkman, J.K.; et al. Nutritional properties of microalgae for mariculture. *Aquaculture* **1997**, *151*, 315–331.
5. Droop, M.R. 25 years of algal growth kinetics. *Botanica Marina* **1983**, *26*, 99–112.
6. Zhang, Q.; Li, X.; Guo, D.; et al. Operation of a vertical algal biofilm enhanced raceway pond for nutrient removal and microalgae-based byproducts production under different wastewater loadings. *Bioresource Technology* **2018**, *253*, 323–332.
7. Mehariya, S.; Goswami, R.K.; Karthikeysan, O.P.; Verma, P. Microalgae for high-value products: A way towards green nutraceutical and pharmaceutical compounds. *Chemosphere* **2021**, *280*, 130553.
8. Almomani, F.; Hosseinzadeh-Bandbafha, H.; Aghbashlo, M.; Omar, A.; et al. Comprehensive insights into conversion of microalgae to feed, food, and biofuels: Current status and key challenges towards implementation of sustainable biorefineries. *Chemical Engineering Journal* **2023**, *455*, 140588.
9. Pereira, S.G.; Pereira, R.N.; Rocha C.M.R.; Teixeira, J.A. Electric fields as a promising technology for the recovery of valuable bio compounds from algae: Novel and sustainable approaches. *Bioresource Technology Reports* **2023**, *22*, 101420.
10. Pereira, R.N.;, Jaeschke, D.P.; Rech, R.; et al. Pulsed electric field-assisted extraction of carotenoids from Chlorella zofingiensis. *Algal Research* **2024**, *79*, 103472.
11. Wang, K.; Khoo, K.S.; Chew, K.W.; et al. Microalgae: the future supply house of biohydrogen and biogas. *Frontiers in Energy Research* **2021**, *9*, 660399.
12. Yang, W.; Li, S.; Qv, M.; et al. Microalgal cultivation for the upgraded biogas by removing CO2, coupled with the treatment of slurry from anaerobic digestion: A review. *Bioresource Technology* **2022**, *364*, 128118.
13. Saravanan, A.; Kumar, P.S.; Varjani, S.; et al. A review on algal-bacterial symbiotic system for effective treatment of wastewater. *Chemosphere* **2021**, *271*, 129540.
14. Liu, R.; Li, S.; Tu, Y.; Hao, X. Capabilities and mechanisms of microalgae on removing micropollutants from wastewater: a review. *Journal of Environmental Management* **2021**, *285*, 112149.
15. Chen, X.; Hu, Z.; Qi, Y.; et al. The interactions of algae-activated sludge symbiotic system and its effects on wastewater treatment and lipid accumulation. *Bioresource Technology* **2019**, *292*, 122017.
16. Zhou, L.; Niu, X.; Zhang, D.; et al. Insight into an efficient microalgae co-culture system for biogas slurry treatment: Nutrients recovery and valuable biomass production. *Journal of Water Process Engineering* **2024**, *60*, 105111.
17. Yan, H.; Lu, R.; Liu, Y.; et al. Development of microalgae-bacteria symbiosis system for enhanced treatment of biogas slurry. *Bioresource Technology* **2022**, *354*, 127187.
18. Zhang, Y.; Yin, Z.; Xie, J.; et al. Efficient treatment of actual biogas slurry by Chlorella sorokiniana: Nutrient recovery and biomass production. *Journal of Water Process Engineering* **2024**, *66*, 106007.
19. Kohlheb, N.; van Afferden, M.; Lara, E.; et al. Assessing the life-cycle sustainability of algae and bacteria-based wastewater treatment systems: high-rate algae pond and sequencing batch reactor. *Journal of Environmental Management* **2020**, *264*, 110459.
20. Scarcelli, P.G.; Ruas, G.; Lopez-Serna, R.; et al. Integration of algae-based sewage treatment with anaerobic digestion of the bacterial-algal biomass and biogas upgrading. *Bioresource Technology* **2021**, *340*, 125552.
21. Wei, C.; Xu, Y.; Xu, L.; et al. Comparative life-cycle assessment of various harvesting strategies for biogas production from microalgae: Energy conversion characteristics and greenhouse gas emissions. *Energy Conversion and Management* **2023**, *289*, 117188.
22. Goswami, R.K.; Mehariya, S.; Obulisamy, P.K.; Verma, P. Advanced microalgae-based renewable biohydrogen production systems: A review. *Bioresource Technology* **2021**, *320*, 124301.
23. Jiao, X.; Tsigkou, K.; Elsamahy, T.; et al. Recent advances in sustainable hydrogen production from microalgae: Mechanisms, challenges, and future perspectives. *Ecotoxicology and Environmental Safety* **2024**, *270*, 115908.
24. Liu, H.; Liu, T-J.; Guo, H-W.; et al. A review of the strategy to promote microalgae value in CO2 conversion-lipid enrichment-biodiesel production. *Journal of Cleaner Production* **2024**, *436*, 140538.
25. El-Mekkawi, S.A.; Abdo, S.M.; Samhan, F.A.; et al. Optimization of some fermentation conditions for bioethanol production from microalgae using response surface method. *Bulletin of the National Research Centre* **2019**, *43*, 164.
26. Amin, S. Review on biofuel oil and gas production processes from microalgae. *Energy Conversion and Management* **2009**, *50*, 1834–1840.
27. Mustapha, S.I.; Mohammed, U.A.; Rawat, I.; et al. Production of high-quality pyrolytic bio-oils from nutrient-stressed Scenedesmus obliquus microalgae. *Fuel* **2023**, *332*, 126299.
28. Raheem, A.; Abbasi, S.A.; Mangi, F.H.; et al. Gasification of algal residue for synthesis gas production. *Algal Research* **2021**, *58*, 102411.

29. Shahi, T.; Beheshti, B.; Zenouzi, A.; et al. Bio-oil production from residual biomass of microalgae after lipid extraction: The case of Dunaliella sp. *Biocatalysis and Agricultural Biotechnology* **2020**, *23*, 101494.
30. Ayub, H.M.U.; Nizami, M.; Qyyum, M.A.; et al. Sustainable hydrogen production via microalgae: Technological advancements, economic indicators, environmental aspects, challenges, and policy implications. *Environmental Research* **2024**, *244*, 117815.
31. Zabed, H.M.; Akter, S.; Yun, J.; et al. Biogas from microalgae: Technologies, challenges and opportunities. *Renewable and Sustainable Energy Reviews* **2020**, *117*, 109503.
32. El Nemr, A.; Hassaan, M.A.; Elkatory, M.R.; et al. Enhancement of biogas production from individually or co-digested green algae Cheatomorpha linum using ultrasound and ozonation treated biochar. *Ultrasonics Sonochemistry* **2022**, *90*, 106197.
33. Giwa, A. Comparative cradle-to-grave life cycle assessment of biogas production from marine algae and cattle manure biorefineries. *Bioresource Technology* **2017**, *244*, 1470–1479.
34. Asinan, D.I.; Marcano, C.M.; Legros, A.; et al. Biomethanation of the marine algae Tetraselmis. *International Journal of Sustainable Energy* **1982**, *1*, 263–272.
35. Samson, R.; LeDuy A. Improved performance of anaerobic digestion of Spirulina maxima algal biomass addition of carbon-rich wastes. *Biotechnology Letters* **1983**, *5*, 677–682.
36. Hernandez, E.P.S.; Cordoba, L.T. Anaerobic digestion of Chlorella vulgaris for energy production. *Resources, Conservation and Recycling* **1993**, *9*, 127–132.
37. Yen, H.W.; Brune, D.E. Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresource Technology* **2007**, *98*, 130–134.
38. Gonzales-Fernandez, C.; Molinuevo-Salces, B.; Garcia-Gonzales, M.C. Evaluation of anaerobic codigestion of microalgal biomas and swine manure via response surface methodology. *Applied Energy* **2011**, *88*, 3448–3453.
39. Ding, L.; Gutierrez, E.C.; Cheng, J.; et al. Assessment of continuous fermentative hydrogen and methane co-production using macro- and micro-algae with increasing organic loading rate. *Energy* **2018**, *151*, 760–770.
40. Okuda, K. Structure and phylogeny of cell coverings. *Journal of Plant Research* **2002**,*115*, 283–288.
41. Ramaraj, R.; Junluthin, P.; Dussadee, N.; et al. Potential evaluation of biogas production through the exploitation of naturally growing freshwater macroalgae Spirogyra varians. *Environment, Development and Sustainability* **2022**.
42. Parakh, S.K.; Tian, Z.; Wong, J.Z.E.; et al. From Microalgae to Bioenergy: Recent Advances in Biochemical Conversion Processes. *Fermentation* **2023**, *9*, 529.
43. Lee, J.; Jang, H.; Kang, S.; et al. Shockwave pre-treatment enhances the physicochemical availability and anaerobic mono- and co-digestion of highly concentrated algae. *Journal of Environmental Chemical Engineering* **2022**, *10*, 108993.
44. Ometto, F.; Quiroga, G.; Pšenička, P.; et al. Impacts of microalgae pre-treatments for improved anaerobic digestion: Thermal treatment, thermal hydrolysis, ultrasound and enzymatic hydrolysis. *Water Research* **2014**, *65*, 350–361.
45. Suparmaniam, U.; Lam, M.K.; Uemura, Y.; et al. Insights into the microalgae cultivation technology and harvesting process for biofuel production: a review Renew. *Renewable and Sustainable Energy Reviews* **2019**, *115*, 109361.
46. Anto, S.; Mukherjee, S.S.; Muthappa, R.; et al. Algae as green energy reserve: technological outlook on biofuel production. *Chemosphere* **2020**, *242*, 125079.
47. Ijaola, A.O.; Akamo, D.O.; George, T.T.; et al. Algae as a potential source of protein: A review on cultivation, harvesting, extraction, and applications. *Algal Research* **2024**, *77*, 103329.
48. Deamici, K.M.; Silva, P.G.P.; Costa, J.A.V.; et al. Low Electromagnetic Fields Applied to Chlorella fusca Cultivation to Increase Production of Microalga-Based Carbohydrates. *Bioenergy Research* **2023**, *16*, 1548–1555.
49. Rozenberg, J.M.; Sorokin, B.A.; Mukhambetova, A.N.; et al. Recent advances and fundamentals of microalgae cultivation technology. *Biotechnology Journal* **2024**, *19*, 2300725.
50. Park, W.K.; Min, K.; Yun, J.H.; et al. Paradigm shift in algal biomass refinery and its challenges. *Bioresource Technology* **2022**, *346*, 126358.
51. Deamici, K.M.; Dziergowska, K.; Silva, P.G.P.; et al. Microalgae cultivated under magnetic field action: insights of an environmentally sustainable approach. *Sustainability* **2022**, *14*, 13291.
52. Santos, L.O.; Deamici, K.M.; Menestrino, B.C.; et al. Magnetic treatment of microalgae for enhanced product formation. *World Journal of Microbiology and Biotechnology* **2017**, *33*, 169.
53. Deamici, K.M.; de Morais, M.G.; dos Santos, L.O.; et al. Magnetic Field Action on Limnospira indica PCC8005 Cultures: Enhancement of Biomass Yield and Protein Content. *Applied Sciences* **2022**, *12*, 1533.
54. Kubar, A.A.; Jin, N.; Cui, Y.; et al. Magnetic/electric field intervention on oil-rich filamentous algae production in the application of acrylonitrile butadiene styrene based wastewater treatment. *Bioresource Technology* **2022**, *356*, 127272.
55. Lingvay, I.; Vranceanu-Jipa, M.; Chihaia, R.-A.; et al. Impact of 50 Hz Electromagnetic Field on the Growth of Chlorella vulgaris. *Applied Sciences* **2024**, *14*, 6506.
56. Barba, F.J.; Parniakov. O.; Pereira. S.A.; et al. Current applications and new opportunities for the use of pulsed electric fields in food science and industry. *Food Research International* **2015**, *77*, 773–798.
57. Vorobiev, E.; Lebovka, N.I. Electrotechnologies for extraction from food plants and biomaterials. Springer, New York, 2009.
58. Sensoy, I.; Sastry, S.K. Extraction using moderate electric fields. *Journal of Food Science* **2004**, *69*, 7–13.
59. Sastry, S. Ohmic heating and moderate electric field processing. *Food Science and Technology International* **2008**, *14*, 419–422.
60. Geada, P.; Rodrigues, R.; Loureiro, L.; et al. Electrotechnologies applied to microalgal biotechnology–applications, techniques and future trends. *Renewable and Sustainable Energy Reviews* **2018**, *94*, 656–668.

61. Nezammahalleh, H.; Ghanati, F.; Adams II, T.A.; et al. Effect of moderate static electric field on the growth and metabolism of Chlorella vulgaris. *Bioresource Technology* **2016**, *218*, 700–711.
62. Pereira, R.N.; Jaeschke, D.P.; Mercali, G.; et al. Impact of ultrasound and electric fields on microalgae growth: a comprehensive review. *Brazilian Journal of Chemical Engineering* **2023**, *40*, 607–622.
63. Buchmann, L.; Frey, W.; Gusbeth, C.; et al. Effect of nanosecond pulsed electric field treatment on cell proliferation of microalgae. *Bioresource Technology* **2019**, *271*, 402–408.
64. Haberkorn, I.; Siegenthaler, L.; Buchmann, L.; et al. Enhancing single-cell bioconversion efficiency by harnessing nanosecond pulsed electric field processing. *Biotechnology Advances* **2021,** *53*, 107780.
65. Haberkorn, I.; Buchmann, L.; Hiestand, M.; et al. Continuous nanosecond pulsed electric field treatments foster the upstream performance of Chlorella vulgaris-based biorefinery concepts. *Bioresource Technology* **2019**, *293*, 122029.
66. Buchmann, L.; Böcker, L.; Frey, W.; et al. Energy input assessment for nanosecond pulsed electric field processing and its application in a case study with Chlorella vulgaris. *Innovative Food Science & Emerging Technologies* **2018**, *47*, 445–453.
67. Reberšek, M.; Miklavčič, D. Advantages and disadvantages of advantages and disadvantages of different concepts of electroporation pulse generation. *Automatika* **2011**, *52*, 12–19.
68. Goettel, M.; Eing, C.; Gusbeth, C.; et al. Pulsed electric field assisted extraction of intracellular valuables from microalgae. *Algal Research* **2013**, *2*, 401–408.
69. Kim, J.Y.; Lee, C.; Jeon, M.S.; et al. Enhancement of microalga Haematococcus pluvialis growth and astaxanthin production by electrical treatment. *Bioresource Technology* **2018**, *268*, 815–819.