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# Biogas Production from *Arthrospira platensis* Biomass

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**Abstract:** Biogas production by fermentation is a relatively low-cost and simple method for the transformation of a substrate into an energy carrier with a wide range of possible applications. The aim of this study was to determine the potential of *Arthrospira platensis* biomass as a source of bioenergy produced during anaerobic digestion (AD). The studies were carried out on a fractional-technical scale. Biogas yield and composition were analyzed as a function of the amount of biomass subjected to anaerobic digestion, the substrate dosing frequency in the digester and the use of biomass pre-hydrolysis in the mixing compartment. The energy efficiency of the process was also compared for each sample. In addition, a biomass conversion power index was developed and determined. It was found that *A. platensis* biomass had significant energy potential, and the amount of biogas obtained and its calorific value changed depending on the applied treatments. The maximum cumulative biogas production was 505 L $kg^{-1}$ volatile solids (VS), while the maximum average methane ($CH_4$) content was 67.32%. A two-fold increase in the organic loading rate from 1 g VS·$L^{-1}$ volatile solids (VS) to 2 g VS·$L^{-1}$ had a positive effect on methane concentration. The highest energy efficiency of the AD process was obtained for 2 g VS·$L^{-1}$, with a single feedstock input into the digester, in a single-stage process (2/s/-), while the highest conversion power ratio was for a feedstock of 1 g VS·$L^{-1}$, under the same process conditions (1/s/-). Moreover, the energy efficiency of the microalgae fermentation process obtained in the study is higher compared to conventional substrates used in biogas plants. This energy analysis can support the selection of cogeneration power engines in a biogas plant and help to determine the potential output of the biogas plant, especially with varying energy and heat demand.

**Keywords:** *Arthrospira platensis*; microalgae; biomass; biogas; bioenergy; power index



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## 1. Introduction

The combustion of fossil fuels has negative environmental impacts and is responsible for the greenhouse effect [1,2], so alternative energy must be provided. A renewable and sustainable source of energy is biogas. It can be used in energy cogeneration to produce both heat and electricity [3] and as a transportation fuel [4]. Biogas used as a fuel significantly reduces greenhouse gas (GHG) emissions (25–100 g $CO_2$-eq./km) compared to conventional fuels (185–220 and 210–220 g $CO_2$-eq./km, for diesel and gasoline) [5].

Biogas is produced during the anaerobic digestion (AD) of different types of biomass and waste. Agricultural residues [6,7], food processing waste [8,9], forest residues [10], and municipal solid waste [11] are used as biogas feedstocks. There are some less conventional substrates, such as microalgae [12–15]. The main components of their biomass are proteins, lipids, and carbohydrates used by microorganisms for methane production [16]. Microalgal biomass as a substrate for biogas plants does not require dehydration, which reduces costs compared to the production of other biofuels [17].

The structure and chemical content of the microalgae cell wall determines the biomass decomposition during anaerobic digestion, the biogas yield and its composition [18–20]. The efficiency of biogas production can be increased by pre-treating microalgal biomass

using physical [21], chemical [22] or biological [23] methods. The amount of energy required for these processes cannot be higher than the amount of bioenergy obtained.

Available data suggest that microalgal biomass has significant energy potential [24–26]. There is wide variability among the different microalgae species for their potential as substrates for methane production [27]. The average yield, for example, ranges between 140.48 $mL \cdot g^{-1}$ VS for *Microcystis* spp. and 408 $mL \cdot g^{-1}$ VS for *Isochrysis* spp. [28]. Even for the same strain, significant differences in methane production (153–600 $NL_{CH4} \cdot kg_{VS}{}^{-1}$) and kinetics can be observed [29]. The biogas yield from microalgal biomass is similar to [30] or higher than the amount obtained from other substrates [12].

Biogas production efficiency depends on processing temperature, pH, organic loading rate (OLR) and hydraulic retention time (HRT) [31–33]. Methane yields increase with increasing lipid content in the feedstock [34], while the content of other macromolecules decreases. Among these, the protein content is particularly important due to the potential for significant amounts of ammonia to be released during anaerobic digestion [35]. Protein content has an impact on the biomass C/N ratio. This can be balanced by co-fermentation with high-carbon substrates [36]. Co-digestion of algal biomass with other feedstocks increases biogas production [37]. In some studies, productivity increased only when the proportion of microalgal biomass in the feedstock mixture did not exceed 40% VS [38]. For *A. platensis* biomass, high methane yields were observed during mono-fermentation, but with low organic loading rates of about 1.0 g $VS \cdot L^{-1} \cdot d^{-1}$ [36].

Biogas, produced from microalgal biomass and converted to methane, is a valuable and complete fuel for powering combustion engines in cogeneration systems for transport or injection into the gas grid [39]. Methane fermentation of microalgal biomass into biogas is the simplest method to produce engine fuel [30] and is more energy efficient than transesterification into biodiesel [40]. However, the highest efficiency of microalgal biomass transformation is represented by combined technologies that allow the simultaneous use of biogas and biodiesel in a direct injection diesel engine [41].

With the unstable energy situation, especially in recent times, a strategy to control the supply of energy has become more important. This also includes biogas plants, which are part of the electricity system. It is important that biogas production rates can be adjusted to cover energy requirements. Biogas production rate is determined, for example, by the pretreatment of substrates during hydrolysis [42,43]. The level of variability in the production of energy from biogas depends on a range of parameters, including the digester construction type and its size, the extent of the additional capacity of the cogeneration system, or the availability of biogas storage. Apart from increasing biogas storage capacity, one possible solution for improving the biogas plant's flexibility is direct control of the biological process, i.e., fermentation. Bioprocess control is cost-effective since it does not require investment in additional biogas storage tanks [44,45]. In modern biogas plants, series-connected, two-stage digestion systems consisting of a high-load primary digester and a low-load secondary digester processing the digestion feedstock from the first stage are preferred. Two-stage fermentation can be characterized by higher efficiency and more stable biogas production [46].

The aim of our study was to analyze the energy potential of the untreated biomass of the *Arthrospira platensis* microalgae on a fractional-technical scale using a methane digester. The biogas yield, composition, energy efficiency, and energy conversion factor were estimated.

## 2. Materials and Methods

### *2.1. Microalgae Biomass as Feedstock for Methane Fermentation—Substrate Preparation*

The biomass of the microalgae *Arthrospira platensis* was used as substrate in the anaerobic digestion. The biomass was produced at a technical scale in vertical tubular photobioreactors with a total volume of 100 L. A modified F/2 medium [47] was used for cultivation, with the addition of sodium bicarbonate ($NaHCO_3$) at 0.5 $g \cdot L^{-1}$ and sodium chloride (NaCl) at 4%. The cultivation used LED lighting with red, blue, and white LEDs

(70:20:10 ratio) at 240 W. The culture illumination time (photoperiod) was set at 12/12 h on a light/dark cycle. A Hailea 175 W membrane pump with a capacity of 275 L $min^{-1}$ was used to provide aeration of the culture. The culture medium pH was adjusted to 7 (stabilized if necessary at the start with 1 N NaOH). Batch cultures were carried out for 10 days at around 28 °C, and then biomass was separated in a rotary centrifuge at 8000 rpm·$min^{-1}$. The obtained pulp provided the feedstock for the methane digester. Total solids (TS), volatile solids (VS), protein, carbohydrate, and lipid contents were determined in the substrate, as well as crude fiber content.

### *2.2. Experimental Setup*

The anaerobic digestion process was carried out in an installation consisting of a digester with a total capacity of 115 L (working capacity of 100 L), additionally equipped with a preliminary mixing chamber providing the substrate to the digester (Figure 1). The bioreactor has an electric motor-driven stirrer regulated by a system of control and measurement. The temperature inside the digester is stabilized by a water mantle and a Peltier system (Peltier cell). Besides temperature sensors, the installation is equipped with electrodes for measuring the redox potential and pH.

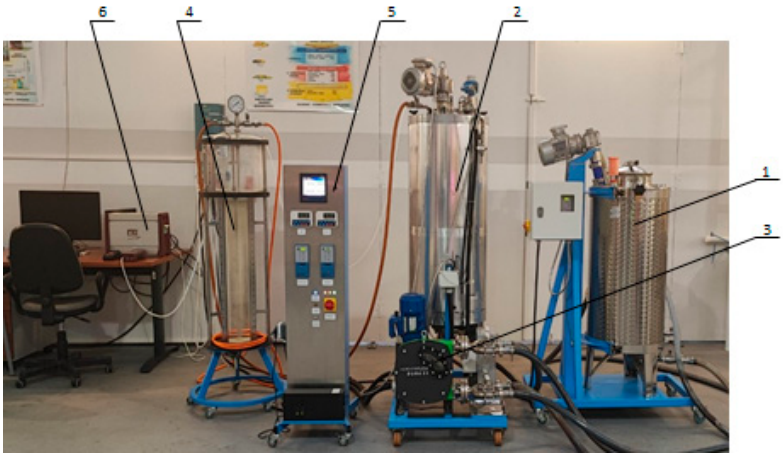



**Figure 1.** Scheme of anaerobic digestion installation: 1—preliminary mixing chamber, 2—digestion chamber, 3—peristaltic pump, 4—gas meter, 5—control and measurement system, 6—biogas analyzer.

The study was concerned with biogas yields and the level of microalgal biomass conversion to methane, the main energy carrier. Biogas production was analyzed depending on: the amount of the feedstock (1 g VS·$L^{-1}$ or 2 g VS·$L^{-1}$), use of the mixer chamber for preliminary substrate hydrolysis (single-stage or multi-stage technology), and the frequency of feedstock dosage into the digester (periodic mode: single dosage; continuous mode: multiple dosage, in equally divided ratios, daily for a 1 g VS·$L^{-1}$ feedstock and every 2 days for a 2 g VS·$L^{-1}$ feedstock). The experimental objects are shown in Table 1.

**Table 1.** Scheme of experiments.

| Objects | Microalgal Biomass [g VS·$L^{-1}$] | Preliminary Mixing Chamber | Biomass Batching Frequency |
|---|---|---|---|
| 1/s/- | 1 | - | single |
| 1/s/+ | 1 | + | single |
| 1/m/- | 1 | - | multiple |
| 1/m/+ | 1 | + | multiple |
| 2/s/- | 2 | - | single |
| 2/m/- | 2 | - | multiple |

The substrate was transferred from the mixing chamber to the digester using a Verderflex Dura25 peristaltic pump with a capacity of 1193.6 $L \cdot h^{-1}$. The volume of the digester was kept constant. The amount of input was balanced with the amount of digestate discharged. Homogeneous substrate distribution inside the digester was provided by a paddle mixer. The operating speed of the mixer was set at 35.43 rpm. The fermentation was carried out under mesophilic conditions (temperature 37 ± 0.1 °C). The mesophilic inoculum to initiate the digestion of microalgal biomass in the bioreactor was taken from a digester at a municipal wastewater treatment plant.

A water level meter that was attached to the digester and had two tanks—the upper one used as a compensatory tank and the lower one serving as a temporary storage tank with a millimeter scale—was used to measure the total amount of methane. The biogas composition was determined using a multigas analyzer (MRU OPTIMA BIOGAS, Neckarsulm—Obereisesheim, Germany). The content of methane ($CH_4$), the amount of carbon dioxide ($CO_2$) and the calorific value of the gas (w $MJ \cdot m^{-3}$) were analyzed. Quantitative and qualitative analysis of the biogas was carried out once a day. The $H_2S$ content was also determined.

The efficiency of microalgal cell digestion during fermentation was analyzed periodically by analyzing the digestate using a camera-equipped optical microscope (Delta Optical, Mińsk Mazowiecki, Poland).

### *2.3. Analytical Methods*

Solids content (TS) was determined by the gravimetric method. The biomass was dried in a moisture analyzer (AXIS ATS60, Gdańsk, Poland) at 105 °C to a constant mass. Volatile fractions (VS) were determined at 550 °C according to PN-EN 12879:2004.

Based on the nitrogen content determined through the Kjeldahl technique and the standard PN-EN ISO 5983-2:2009, the crude protein content was calculated. The sample was mineralized in a mineralization block with sulfuric acid in the presence of a catalyst, followed by alkalinization with sodium hydroxide.

Carbohydrate content was determined from the content of reducing sugars and after inversion by the Luff–Schoorl method (with ethanol extraction) according to PN-R-64784:1994.

The crude lipid was determined after extraction of the biomass with petroleum ether in a Soxhlet apparatus according to PN-ISO 6492:2005. The extraction solution was distilled, and the residue was dried and weighed.

The crude fiber was determined according to PB-02/PS edition 5 of 01.11.2020. The sample was subjected to lipid extraction by rinsing in acetone, followed by digestion in sulfuric acid solution, and successively in sodium hydroxide solution. Samples were dried and ashed in an oven at approx. 550 °C. An automatic ANKOM fiber analyzer was used for the determination.

The energy efficiency ($E_e$) of microalgal biomass was determined from the following Equation (1):

$$E_e = \frac{Q \cdot C_v}{VS}, \quad (1)$$

where:

$E_e$—energy efficiency of microalgal biomass [$kWh \cdot kg^{-1}$ VS],
Q—volume of produced biogas [L],
$C_v$—unit calorific value of the produced biogas [$kWh \cdot L^{-1}$],
VS—mass of converted microalgae [kg VS].

The conversion power index ($P_c$) was determined from the following Equation (2):

$$P_c = 1000 \times \frac{E_e}{\tau}, \quad (2)$$

where:

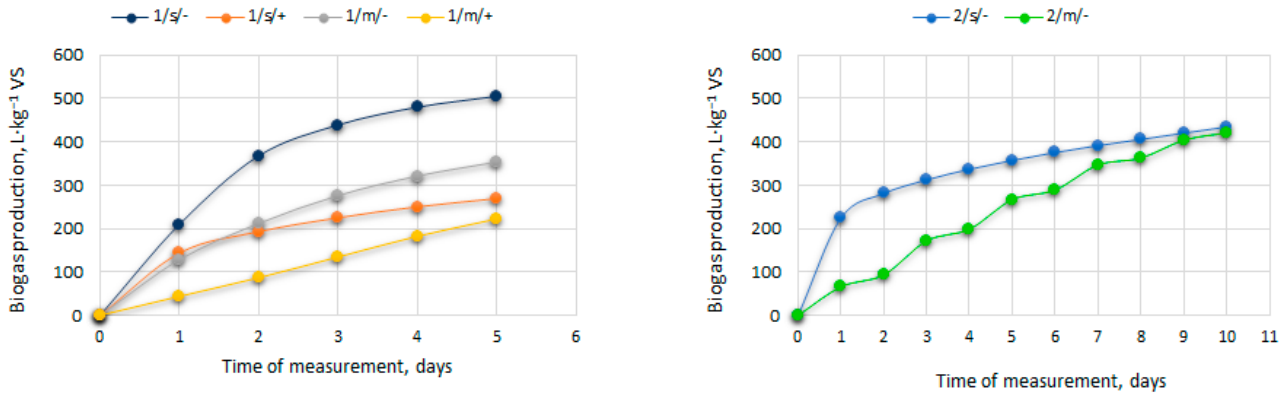
$P_c$—conversion power index of microalgal biomass to biogas [$W \cdot kg^{-1}$ VS],

$E_e$—energy efficiency from of microalgal biomass [kWh·kg$^{-1}$ VS],
τ—conversion time of microalgal biomass to biogas form [h].

## 3. Results and Discussion

### *3.1. Biogas Yield*

The cumulative biogas production from microalgal biomass under anaerobic digestion is shown in Figure 2a for a 1 g VS·L$^{-1}$ input and Figure 2b for a 2 g VS·L$^{-1}$ input. For the lower dose of microalgae biomass introduced into the digester, the highest biogas yield was recorded in the 1/s/- object, where biogas production increased successively up to 505 L·kg$^{-1}$ VS. During the experiments, the highest average values were determined for both variants 1/s/- and 1/m/-, 400.4 L·kg$^{-1}$ VS and 257.2 L·kg$^{-1}$ VS, respectively. Varol and Ugurlu [48] in a single-stage biogas production system obtained between 728 and 4662 mL·L$^{-1}$ from biomass with a VS of 0.6 to 5% (per dry matter). El-Mashad [49] after 40 days of *A. platensis* biomass fermentation obtained 355 mL of methane·g$^{-1}$ VS at 35 °C and 358 mL·g$^{-1}$ VS under thermophilic conditions (50 °C). These differences may be due to the different chemical compositions of the biomass destined for anaerobic digestion, which determines the susceptibility to biological decomposition and affects the potential for biogas production [50]. The chemical composition of biomass varies according to environmental conditions [51], which are also influenced by the parameters analyzed in this study. The production of *A. platensis* biomass was carried out at about 20 °C, which has been found in previous studies to be optimal for the growth of this strain, although some authors suggest that this species prefers slightly higher temperatures about 35 °C [52,53].




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

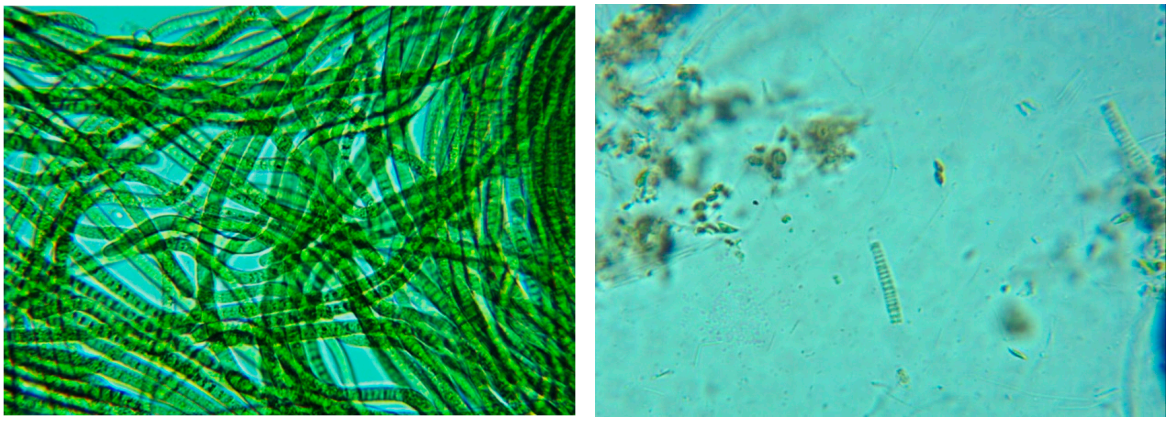
**Figure 2.** Cumulative biogas yield from anaerobic digestion of *A. platensis* biomass (**a**) 1 g VS·L$^{-1}$; (**b**) 2 g VS·L$^{-1}$.

Another important factor is the protein content of the biomass, which affects the C/N ratio. For microalgae, this value ranges from 4.16 to 10 [54], while the optimum value for anaerobic fermentation is 20 to 35 [55]. A low C/N ratio can negatively affect the digestion process and lead to the release of significant amounts of ammonium nitrogen and the accumulation of volatile fatty acids [56]. This is not confirmed by studies carried out by Guarino et al. [57] for manure digestion, where the C/N ratio was in the range of 9–50 while the methane volume fraction was always larger than 54%. According to Spínola et al. [58], the biomass of *A. platensis* contains up to 76% protein, whereas the chemical analyses carried out in the present study showed only 24.3% crude protein, in addition to 1.9% inverted sugars and 0.1% crude lipid. The $H_2S$ content of biogas produced from *A. platensis* biomass ranged from 325 to 625 ppm.

Considering the results from the first phase of the study, in the next stage, the process was carried out in a single step and the substrate was applied to the digester directly, bypassing the prechamber. A twofold increase in digester feedstock mass (2 g VS·L$^{-1}$) did

not increase biogas yield. Higher biogas production was observed for the 2/s/- variant (maximum 435 L·kg$^{-1}$ VS). The average biogas yield ranged from 262.2 L·kg$^{-1}$ VS to 354.6 L·kg$^{-1}$ VS in the 2/s/- and 2/m/- objects, respectively.

Not only protein content, but also the structure of the cell wall, can be barriers to efficient biogas production from microalgal biomass. No lag phase was observed after the substrate was introduced into the methane digester. It suggests that the microorganisms did not need to adapt to this type of organic matter. Such problems can be observed with microalgal biomass containing sodium ions used, as in the present study, as a component of culture media. This can result in osmotic pressure changes leading to a significant reduction in methane production [59], but no such effect was observed in our study. The composition and structure of the cell wall, which varies in different types of microalgae, determine its susceptibility to enzymatic degradation [60]. This could affect the commercial production of biogas [61]. Microscopic analysis of the digest confirmed that, for anaerobic fermentation microorganisms, microalgal biomass was a suitable substrate. Only single fragments of cellular structures were observed in the digestate, indicating that the hydrolysis process of the biomass introduced into the digester was proceeding properly (Figure 3).



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

**Figure 3.** Microscopic image of the biogas plant feedstock (**a**) and the digestate (**b**).

### *3.2. Efficiency of Microalgal Biomass Conversion to Methane*

The most important component of the biogas is methane, which determines its calorific value. According to Li et al. [62], for lignocellulosic biomass, the high content of fiber is an important parameter limiting methane yield. Different results were presented by Streitwieser and Cadena [63], who analyzed the production of biomethane from different organic wastes and recorded the highest values (above 30% and 50%) for substrates with a high fiber content. In the presented study, the crude fiber content was 12.7%, while the methane content of the different variants ranged from 42.2% to 67.3% (Figure 4). Using a prechamber resulted in a lower methane content compared to analogous variants where the substrate was applied directly to the digester (single-stage digestion). The highest methane content for the 1 g VS·L$^{-1}$ dose was observed in the 1/s/- variant, and for 2 g VS·L$^{-1}$ in the 2/s/- variant.

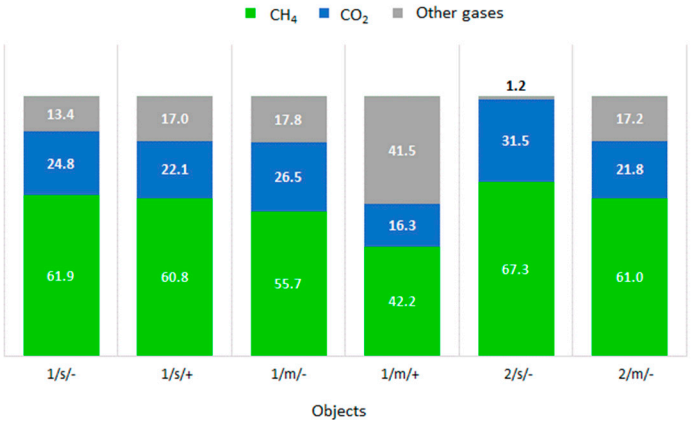



**Figure 4.** The percent of methane and carbon dioxide in biogas from microalgal biomass.

### *3.3. Energy Efficiency and Conversion Power Index for Microalgal Biomass*

The energy efficiency of microalgal biomass was expressed in units of energy included in the biogas produced from one kilogram of dry matter. This is a completely novel approach. In most papers, this parameter is expressed as the volume of biogas or methane in relation to the microalgal biomass [61,64]. Relating energy production efficiency to a kWh allows for considering the calorific value of biogas. This value is not constant and depends on the quality and quantity of the biogas plant feedstock.

Algae biomass at the 2/s/- object had the highest energy efficiency (Figure 5a). After ten days of fermentation, from one kg of VS, 2.9 kWh of energy in the biogas was obtained. The lowest value of this parameter (0.9 kWh·kg$^{-1}$ VS) was observed in the 1/m/+ variant. In comparison, Collet et al. [40] demonstrated in their study that up to 2 kWh of energy could be obtained from one kilogram VS of *Chlorella vulgaris* microalgae converted to methane. Studies carried out by Fantozzi and Buratti [65] in a reactor with a working capacity of 17 L permit the conclusion that non-microalgal substrates under methane fermentation have a significantly lower energy potential than algae. Thus, for chicken and pig manure, 1.4 kWh·kg$^{-1}$ VS was obtained, while for olive shells with inoculum, only 0.2 kWh·kg$^{-1}$ VS.

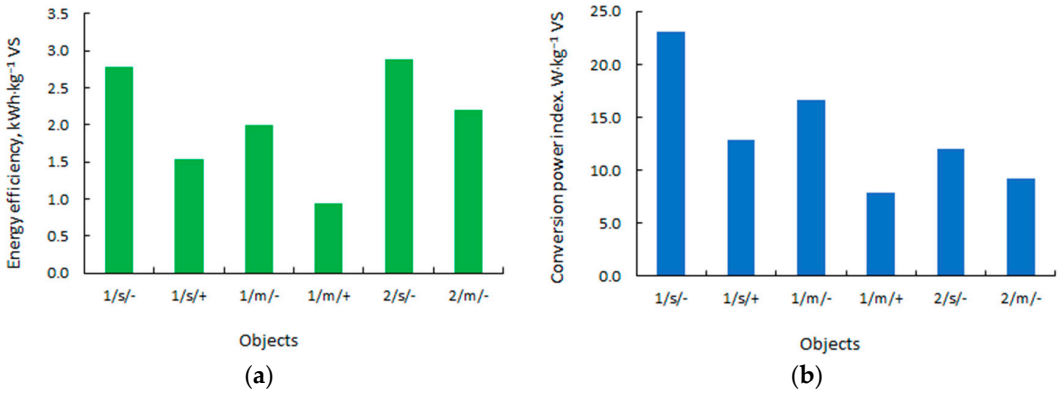



**Figure 5.** Energy parameters of microalgal biomass: (**a**)—energy efficiency; (**b**)—conversion power index.

In the presented study, the conversion power index of microalgae biomass was also determined. By assuming that both the device and the methane digestion conditions were the same for every situation, Figure 5b was obtained. This figure considers the rate of the conversion process and the calorific value of the produced biogas in relation to the time of the process. No publications on methods for determining a similar parameter were found in the available literature.

Figure 5b shows that the biomass in the 1/s/- variant had the highest conversion power of 23.1 W·kg$^{-1}$ VS, while the microalgae biomass in the 1/m/+ variant had the lowest, at 7.9 W·kg$^{-1}$ VS, respectively.

The relation of energy efficiency to the day of fermentation is shown in Figure 6. The increase in energy on the following day of digestion for the cases designated as a, b, c, and d, was logarithmic. This means that the energy gain gradient in biogas was getting lower and lower every day. For all biomass with a 10-day digestion period (e, f), the energy gain was linear. This is confirmed by the equations for the trend line with the determination coefficients shown in Figure 6. A similar curve for the fermentation process, but expressed as a function of the increasing volume of biogas obtained from non-microalgal substrates, was obtained by Fantozzi and Buratti [65]. Only chicken manure with pig manure and olive husks with inoculum showed an exponential function over time, meaning that the start of fermentation was very slow.

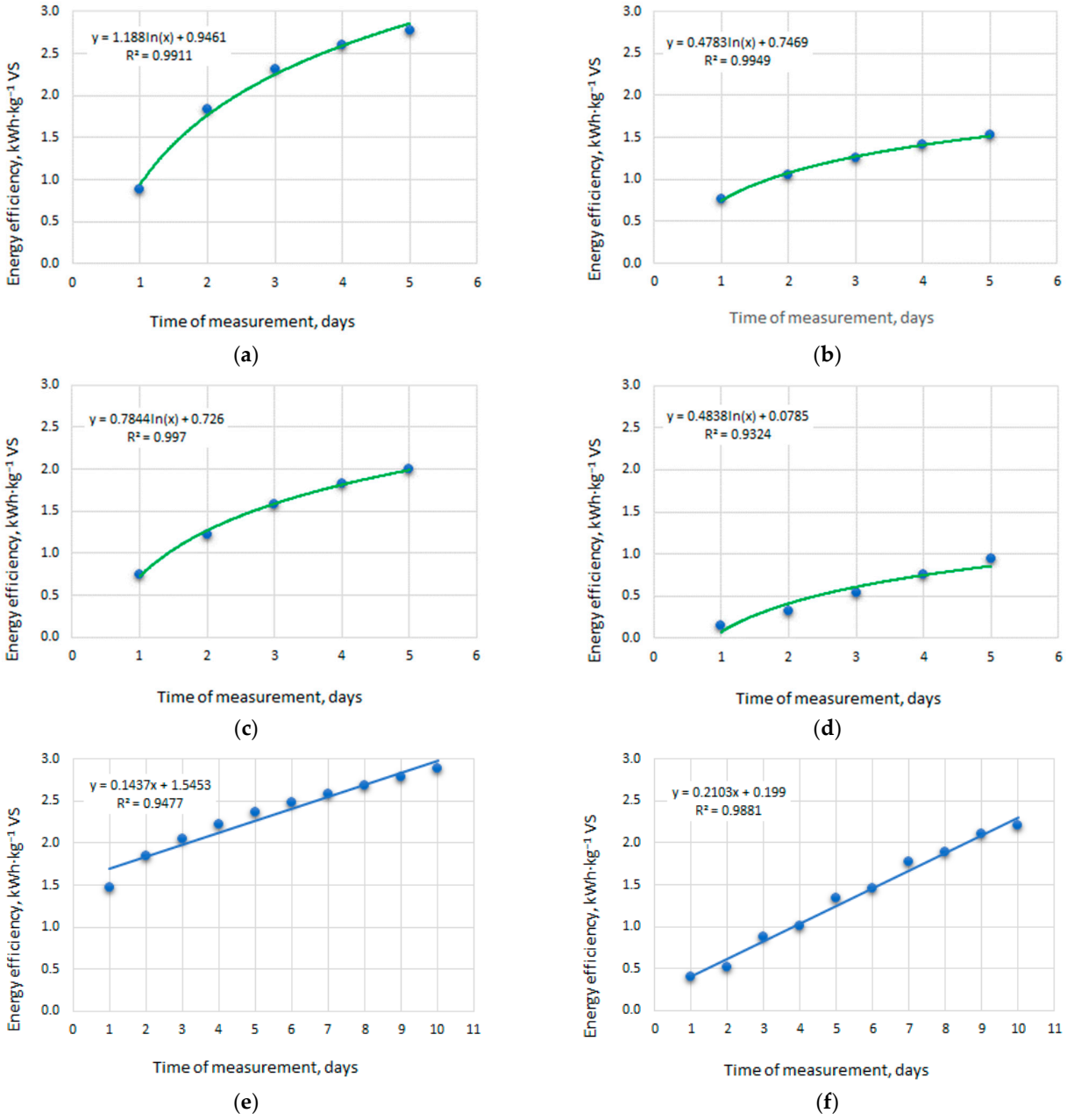
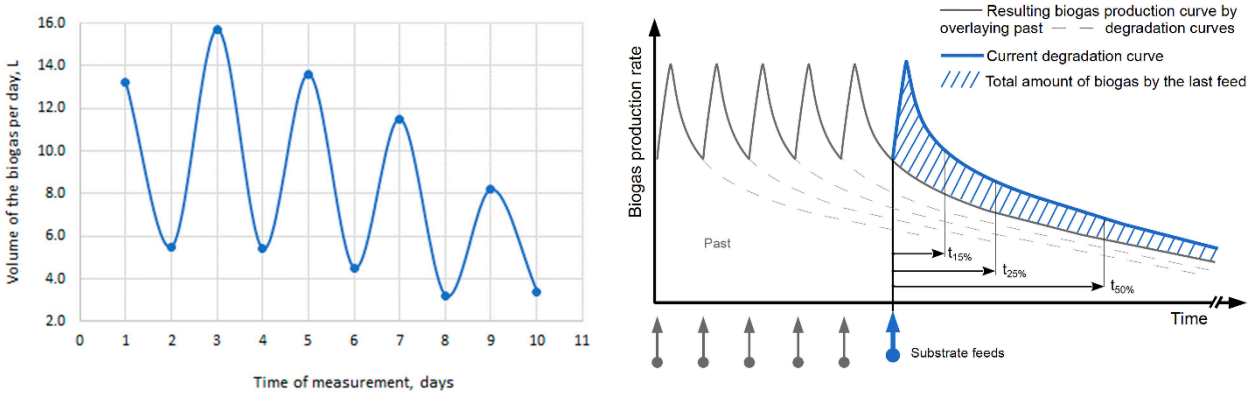



**Figure 6.** Energy efficiency of biomass on the following day of fermentation: (**a**)—1/s/-; (**b**)—1/s/+; (**c**)—1/m/-; (**d**)—1/m/+; (**e**)—2/s/-; (**f**)—2/m/-.

Depending on the technical capabilities of the digester, cyclic, regular supplementation of the feedstock at regulated time intervals and dosages can be used to steer the biogas output in accordance with its requirements. In the present study, the results for the microalgal biomass with the highest energy potential supplemented every two days, i.e., 2/m/- (Figure 7a), indicate that the fermentation process has a very similar character to that of the Mauky et al. [42] model, but the created sawtooth plot has a decreasing trend. According to the functional model developed by Mauky et al. [42] for a bioreactor with non-microalgal substrates (Figure 7b), 15-percent time intervals are most suitable to keep continuous biogas production. It should be noted that, in the present study, the selection of dose rate and the time interval for its addition was not derived from calculations and were dictated only by the ratio of the volume of a single application to the volume of the digester. To maintain the biogas production at the same level, it would be necessary to optimize both the time interval and the supplementary input rate, which will be the scope of the authors' further studies.




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

**Figure 7.** The level of biogas produced from microalgal biomass; (**a**)—obtained for a 2/m/- variant (feedstock supplemented every two days); (**b**)—according to the Mauky et al. [42] model with indication of 15%, 25% and 50% feedstock supplementation time.

A different way to maintain a constant level of biogas production is with a two-digester system that operates simultaneously, loading the substrates into the first digester and then transferring the semi-fermented feedstock to the second digester. Despite higher investment costs, this solution provides higher efficiency in the digestion process [66]. The determined energy efficiency and conversion power index allow comparing biomass according to the rate of decomposition and transformation to a unit of energy and power, not only to a unit volume of the produced biogas. These are important parameters for the receiving devices planned for connection to the biogas plant.

## 4. Conclusions

*A. platensis* biomass is a suitable substrate for high methane biogas production. A maximum of 505 L·kg$^{-1}$ VS of biogas for the 1 g VS·L$^{-1}$ dose and 435 L·kg$^{-1}$ VS for the 2 g VS·L$^{-1}$ dose were obtained in carried out studies. At a higher digester load rate, biogas production was lower while methane content increased. The highest $CH_4$ content was 67.32%. There was no positive correlation between the pre-hydrolysis of biomass in the mixing chamber and biogas yield. Improved results were obtained for single-stage fermentation. The highest energy efficiency was obtained for variant 2/m/- and the highest conversion power index was obtained for variant 1/s/-. Energy indicators for microalgal biomass are more favorable than for conventional substrates. The energy indicators determined in this study are important for the planning of future electricity

systems involving a biogas plant to compensate for the differences between energy demand and supply from uncontrolled renewable energy sources such as wind or solar.

**Author Contributions:** Conceptualization, M.H.-P.; methodology, M.H.-P. and A.K.; validation, M.H.-P. and A.K.; formal analysis, M.H.-P. and A.K.; investigation, M.H.-P., A.K., P.R., M.S. and A.G.; writing—M.H.-P. and A.K.; writing—review and editing, M.H.-P. and A.K.; visualization, M.H.-P. and A.K.; supervision, M.H.-P. and A.K. All authors have read and agreed to the published version of the manuscript.

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