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Cross-Comparison of the Impact of Grass Silage Pulsed Electric Field and Microwave-Induced Disintegration on Biogas Production Efficiency

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Abstract: Lignocellulosic biomass is included in the group of renewable energy sources. Its calorific value is high, owing to which it can be successfully used in the production of second-generation fuels, e.g., biogas. However, its complex structure makes it necessary to apply a pretreatment in order to increase the biogas output. This study presents the usability of a pulsed electric field in grass silage pretreatment in methane fermentation and compares it with microwave-induced disintegration. The experiment shows that substrate disintegration with a pulsed electric field (PEF) results in an increase in methane output. The productivity of methane from PEF pretreatment silage increased by 20.1% compared to the untreated control. The application of microwave disintegration, with the assumption that the same energy is used for the pretreatment, resulted in a methane output increase of 6% compared to the control. The highest biogas production output in PEF-pretreated samples was 535.57 NL/kg VS, while the highest biogas output from substrates pretreated with microwaves was 487.18 NL/kg VS.

Keywords: methane fermentation; PEF; pretreatment of lignocellulosic biomass; grass silage

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1. Introduction

The EU climate-and-energy-related policy and vision of climate neutrality to be achieved by 2050 have a crucial impact on the energy strategy of Poland and other EU member states. It is a collective goal of all the EU states to reduce greenhouse gas emissions by at least 55% by 2030 compared to the 1990 level. Another approved goal is to achieve a 32% share of renewable sources in gross final energy consumption [1].

In 2020, the whole world was affected by the COVID-19 pandemic; the war in Ukraine broke out in February 2022, and together these events had an impact on each of the world's economies. This extraordinary situation exposed the crucial role of the energy sector, including energy security, in the economies of European countries. The energy sector is facing multiple challenges, which have to be responded to in order to carry out an effective energy transformation. The heavy reliance of many EU member states on natural gas supply from one direction requires diversifying actions. One such action is to use biomass in biofuel production. Due to the current geopolitical situation, it is necessary to maximise biomass consumption, especially for biogas production, to stimulate gas market development and expand the gas transport and distribution network.

The effects of the fuel crisis can be alleviated, the greenhouse gas emissions can be reduced, and the requirements of the EU energy policy can be met by, among other measures, the production of second-generation biofuels from lignocellulosic feedstock [2,3].

Lignocellulosic biomass consists of three interconnected polymers: cellulose, hemicellulose, and lignin [4–7]. The latter affects the hardness of the whole complex and connects the other sugars to make a compact and durable compound, highly resistant to most enzymes and other substances. The properties of lignocellulosic biomass, such as the content of the main complex components, oxygen, hydrogen, nitrogen, sulphur, the dry matter and dry residue content, and the calorific value, play an important role in the processes of biomass conversion to energy [8,9]. Lignocellulose is successfully converted to biofuels, such as biogas, ethanol, and hydrogen. However, due to the factors that restrict lignocellulosic material biodegradation, it requires pretreatment. Lignocellulosic biomass disintegration destroys compact structures and releases the organic matter to the dissolved phase, resulting in an increase in the concentration of dissolved, easily decomposed organic substances [10,11]. Commonly applied pretreatment methods can be classified into physical, chemical, biological, and physicochemical [6,8,11–13]. Researchers apply various methods for biomass disintegration to find highly effective, productive, and profitable methods for biomass pretreatment before the conversion process. Such methods include pulsed electric field (PEF) and microwave radiation, which are presented in this paper. Compared with electromagnetic microwave radiation, the use of PEF is a novel method—the beneficial impact of which on biogas output has not been given much attention in the literature. The available reports deal with structural changes in the cell wall and membrane in plants, which directly increase the cell membrane permeability and, in consequence, accelerate and intensify the sugar hydrolysis process [14–17].

The application of microwave radiation, which is successfully applied in the pretreatment of lignocellulosic material, delignifies and partly removes hemicellulose and enhances the sugar hydrolysis [18,19]. Compared to conventional heating, microwave radiation is not based on surface heat exchange. Instead, heat is generated by an object interaction in an electromagnetic field. Microwave radiation destroys cellulose by molecular collisions caused by dielectric polarisation. The advantages of both methods include: a short process duration, a high selectivity, and a smaller amount of energy supplied compared to conventional heating [4,18,20–23].

Liquid fuel production from a feedstock containing polysaccharide complexes requires the choice of appropriate process conditions: pretreatment, hydrolysis, and fermentation. This paper shows the possibility of applying a novel, non-thermal method, i.e., the use of PEF for plant biomass destabilisation before methane fermentation, and also compares it with a different, commonly applied pretreatment technique, using electromagnetic microwave radiation.

2. Materials and Methods

2.1. Substrate

Grass silage was used as the substrate in this experiment. The raw substrate was cut up and subsequently hydrated to 95%. The substrate contained 47.53% dry matter (TS), whereas the content of the dry organic matter (VS) was 89.62% TS (Table 1).

Table 1. Parameters of grass silage.

Parameters	Value
Hydration [%]	52.47 ± 1.06
Dry weight [%]	47.53 ± 1.06
Dry organic weight [% TS]	89.62 ± 1.05
Total carbon (TC) [mg C/g TS]	478.99 ± 18.99
Total organic carbon (TOC) [mg C/g TS]	390.54 ± 11.67
Total nitrogen (TN) [mg N/g TS]	20.94 ± 3.89
C/N	22.87

2.2. Equipment

The PEF disintegration installation contained a biomass shredder used for cutting up the substrate and a tank for increasing its hydration. The tank was fitted out with a paddle stirrer, which was used to make the substrate homogeneous in its whole volume.

With a hopper mounted in the installation, the disintegrator was able to work in static conditions, whereas the use of the pump system allowed for operation under the flow-through conditions. The disintegrator was fitted out with a coaxial disintegration chamber with a capacity of 0.5 L, in which the electrodes made of stainless steel were 2 cm apart. Microwave processing was carried out using the MARS-Solvent Extraction System (CEM, Matthews, NC, USA).

2.3. Pretreatment

Due to the design of the disintegrator, and in particular the pumping system that feeds it with substrate, the substrate was cut up before disintegration and subsequently hydrated with distilled water to 95% in order to prevent the breakdown of the pumping system and to enable operation under flow conditions. The prepared material was put into the disintegration chamber through the charging hopper. The disintegration process was effected by electric impulses at an amplitude of 40 kV. The electric impulses were rectangular. Impulses with a width of 50 µs and a frequency of 5 kHz were applied. The disintegration was conducted in a coaxial chamber and, in consequence, the electric field was distributed unevenly. The maximum electric field strength in the chamber was 38.66 kV/cm, and the minimum was 11.66 kV/cm. The microwave treatment process was carried out with an output power of 400 W. The magnetron frequency was 2.45 GHz. The samples were placed in Easy Prep Teflon vessels with a capacity of 115 cm^3. The most significant difference between the use of microwave irradiation and PEF for substrate pretreatment was that, in the case of PEF, no significant temperature increase was observed. The essence of the application of electric waves with a high voltage amplitude for substrate pretreatment is the electric field induces plasmolysis (electroplasmolysis), which leads to changes in the cell membrane's thickness and consequently to its disruption. Microwave irradiation, on the other hand, heats the substrate due to electromagnetic radiation which, according to kinetic theories, accelerates chemical reactions. The research methodology was designed to treat the substrate with the same energy dose in both methods. The experiment was divided into nine series, whose classification criterion was the energy consumed in the disintegration process: M0, P0-0 Wh/kg TS—control sample; M1, P1-50 Wh/kg TS; M2, P2-100 Wh/kg TS; M3, P3-150 Wh/kg TS; M4, P4-200 Wh/kg TS; M5, P5-260 Wh/kg TS; M6, P6-320 Wh/kg TS; M7, P7-370 Wh/kg TS; M8, P8-420 Wh/kg TS (M—microwave pretreatment, P—PEF pretreatment).

2.4. Biochemical Methane Potential

The methane fermentation of the samples was conducted in an AMPTS II analyser (BPC Instruments AB, Lund, Sweden) in order to determine the methane potential of the substrates. Anaerobic fermentation was conducted under mesophilic conditions (37 °C) for 24 days. The biogas trials were conducted in 0.5 L glass reactors, fitted out with a stirring system (stirring every 10 min for 30 s at a speed of 100 rpm). The reactors contained 0.2 L of anaerobic inoculum (i) with the tested substrate (S). The ratio of the substrate sludge (I) organic matter in the prepared bed was I/S = 5. The reactors were flushed with pure nitrogen before the fermentation to remove oxygen. The dry weight of the inoculum was 5.35 ± 0.71 g/g, and that of the dry organic matter was 3.93 ± 0.68 g/g. A negative control sample was tested, with the substrate not subjected to disintegration. The experiment was performed in triplicate.

2.5. Analytical Methods

In order to determine the total organic carbon (TOC) content and the chemical oxygen demand (COD), samples before and after disintegration were centrifuged, and these compounds were determined in the supernatant. Chemical fractionation with a neutral and acidic detergent was also performed in the supernatant to determine the contents of cellulose, hemicellulose, and lignin. This was performed following the procedure developed by van Soest et al. [24] based on a measurement of the neutral detergent fibre (NDF), acidic-detergent fibre (ADF), and acidic-detergent lignin (ADL) content.

Determination of the cellulose content:

$$\text{Cellulose} = \text{ADF} - \text{ADL} \tag{1}$$

Determination of hemicellulose content:

$$\text{Hemicellulose} = \text{NDF} - \text{ADF} \tag{2}$$

Determination of lignin content:

$$\text{Lignin} = \text{ADL} \tag{3}$$

Moreover, samples before and after disintegration were examined under a scanning electron microscope (JSM-5310LV, JEOL, Tokyo, Japan) at 15 kV. To this end, the samples were fixed in a 2.5% solution of glutaraldehyde and subsequently washed for 20 min with phosphate buffer. The fixed samples were dehydrated in ethanol at a sequence of concentrations: 30%, 50%, 70%, 80%, and 96% for 10 min and twice at a concentration of 99.8% for 30 min. Subsequently, the samples were dried at the critical point of CO_2 and coated with gold in an argon atmosphere with an ion coater (Fine Coater, JCF-1200, JEOL, Tokyo, Japan).

The composition of the produced biogas was determined with a gas chromatography unit with a TCD detector (Agilent 7890 A, Santa Clara, CA, USA). The TS and VS content were determined by the gravimetric method and the carbon and nitrogen content in the substrate—with a Flash 2000 analyser (Thermo Fisher Scientific, Waltham, MA, USA). The TOC content was determined with a TOC-L analyser (Shimadzu, Kioto, Japan). Detergent chemical fractionation was performed with an ANKOM220 device (ANKOM Technology, Macedon, NJ, USA).

2.6. Statistical Analyses

The variance homogeneity for the results was determined with the Levene test. The significance of differences between the variants was determined with the Tukey test (HSD). The correlations between the groups were determined with the Pearson correlation (R). The level of significance was $\alpha = 0.05$. The statistical analyses were performed with Statistica 13 (TIBCO, Palo Alto, CA, USA).

3. Results and Discussion

3.1. Pretreatment Efficiency

The TOC in the liquid phase of the feedstock for methane fermentation was analysed to determine the effect of pulsed electric field and electromagnetic field on lignocellulosic material. The samples were analysed with a Shimadzu TOC analyser before and after the disintegration process. The initial TOC content in the grass silage was found to be 3126 ± 46 mg/L. The analyses of the charge after disintegration with PEF with the assumed hold times showed an increased TOC content in all the samples. The largest increase for the charge with grass silage was observed in series P7, where the mean TOC content was 3560 ± 52 mg/L. The highest TOC of 3367 ± 40 mg/L of all the samples after microwave disintegration was noted in M8 (Figure 1a,b). The initial COD level in the raw substrate was 9135 ± 101 mg O_2/L. The highest COD for samples after PEF pretreatment was achieved in P6 (10,554 ± 174 mg O_2/L). The highest COD of all the samples after microwave pretreatment was achieved in M8 (9505 ± 54 mg O_2/L) (Figure 1c,d). Kuşçu et al. [25] investigated the effect of PEF treatment on the content of dissolved COD in the liquid fraction of activated sludge. The use of PEF treatment increased the content of dissolved COD by 65%. The effect on waste activated sludge was also investigated by Deng et al. [26].

In their experiment, the use of PEF treatment increased the content of dissolved COD by almost 29%.

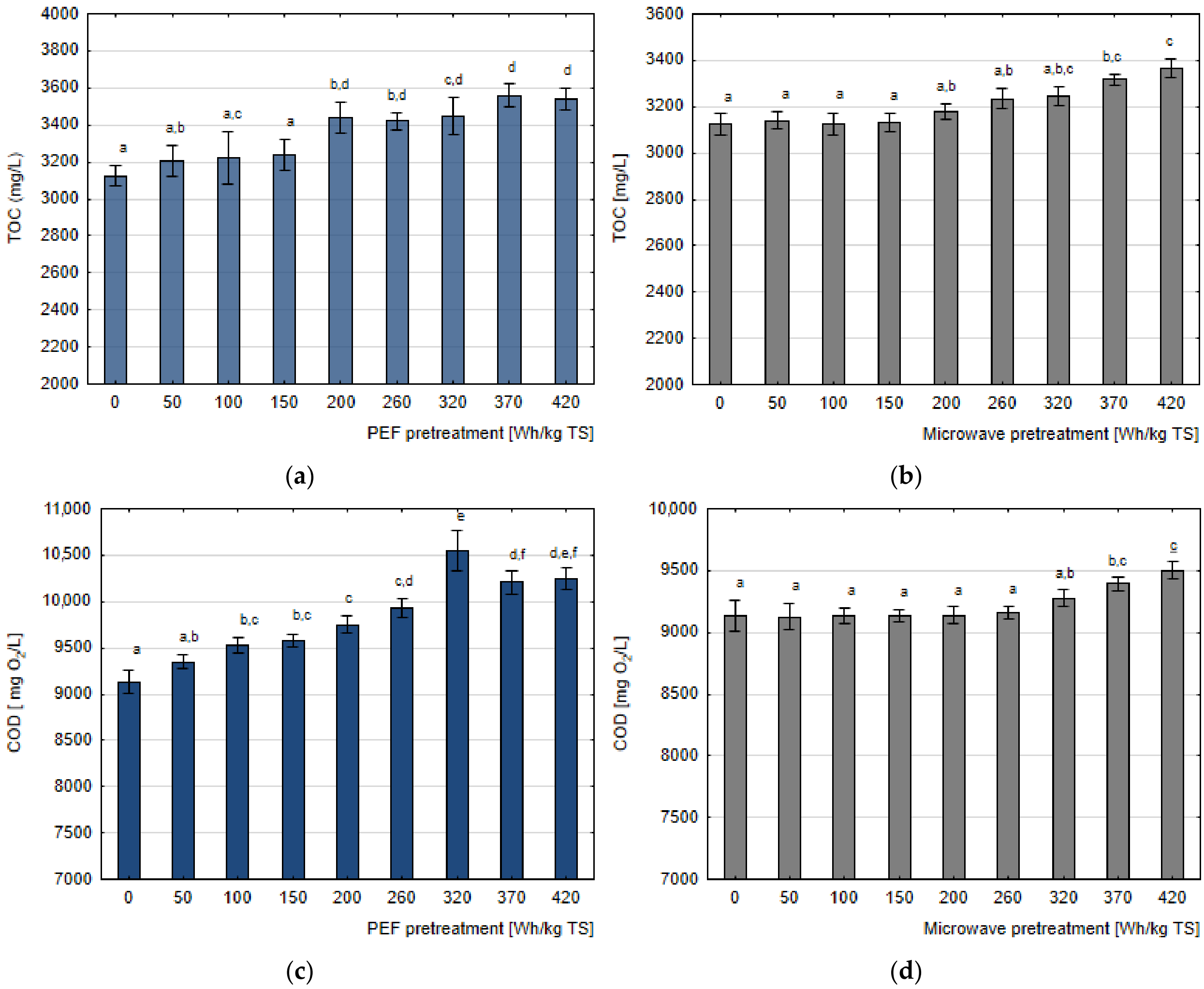

Figure 1. TOC (**a**,**b**) and COD (**c**,**d**) in the substrate liquid fraction. Superscript letters (a,b,c,d,e,f) denote significant differences (Tukey's RiR test, $p < 0.05$).

The depolymerisation effectiveness was also determined by analysing the cellulose, hemicellulose, and lignin contents. The results showed the cellulose content in the untreated grass silage substrate was 34.15 ± 0.90% TS, the hemicellulose content was 24.27 ± 0.97% TS, and lignin was 2.78% TS (Figure 2a–c). The lowest cellulose content after PEF disintegration was achieved in series P7 (32.22 ± 1.61% TS) (Figure 2a). The hemicellulose content was 20.29 ± 1.14% TS, and lignin was 2.49 ± 0.32% TS (Figure 2b,c). The lowest cellulose, hemicellulose, and lignin contents in samples following microwave disintegration were found in series M9 (32.98 ± 0.66, 23.56 ± 0.66, 2.71 ± 0.32% TS) (Figure 2a–c). Work performed by el Achkar et al. [27] described the detergent fractionation of fibres in PEF-treated grape marc to analyse the cellulose, hemicellulose, and lignin contents. The tests did not show any changes in the content of the above compounds under the influence of PEF treatment. Similar conclusions were presented by Zieliński et al. [28] studying the influence of hydrodynamic disintegration on silage of Virginia mallow.

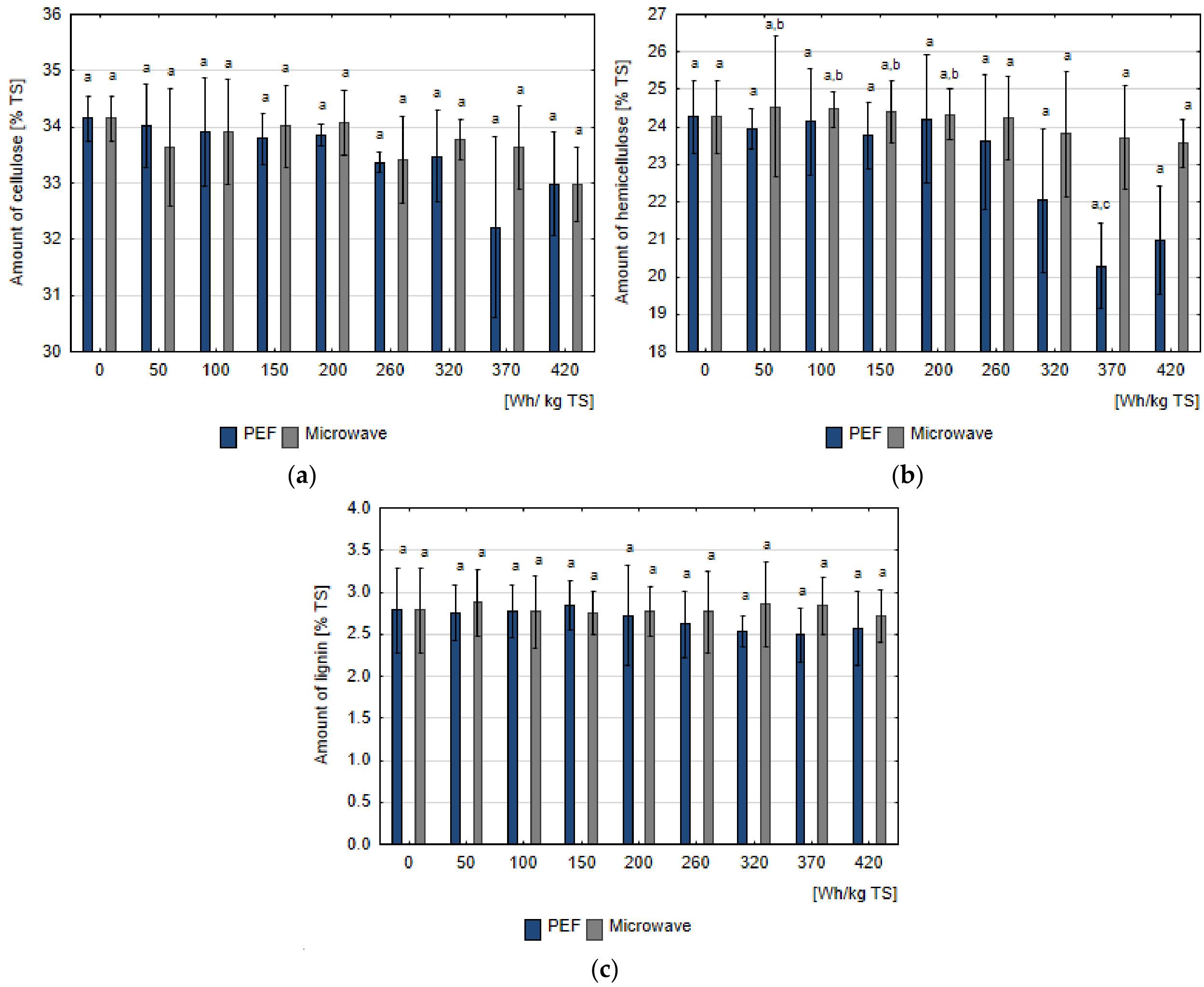

Figure 2. (**a**–**c**) The cellulose, hemicellulose, and lignin contents in the tested substrates. Superscript letters (a,b,c) denote significant differences (Tukey's RiR test, $p < 0.05$).

SEM imaging of the control sample and the samples following disintegration was performed, for which the best technological effect was achieved in methane production. The photographs clearly show the visible structures of monocotyledon leaf skin cells. Distinct deformation of the cell walls (marked with an arrow) was observed in sample P7 subjected to pretreatment with PEF. No such clear changes were observed in sample M8 after microwave treatment (Figure 3). Liu et al. [29] observed changes in tea leaf micromorphology following PEF disintegration. The analyses showed that PEF use results in the formation of unevenly distributed pores on the surface. The pretreatment also resulted in the formation of folded bulges on the material surface. Wang et al. [30] performed the observation of substrate micromorphology after microwave disintegration using SEM. They used corn straw as a substrate. They observed that the use of microwave disintegration caused the cell walls to appear somewhat damaged and the surface roughness to increase. Kovačić et al. [31] observed that the use of electroporation to pretreat the lignocellulosic substrate led to changes in the cell structure in the form of pore formation and damage to the cell membrane.

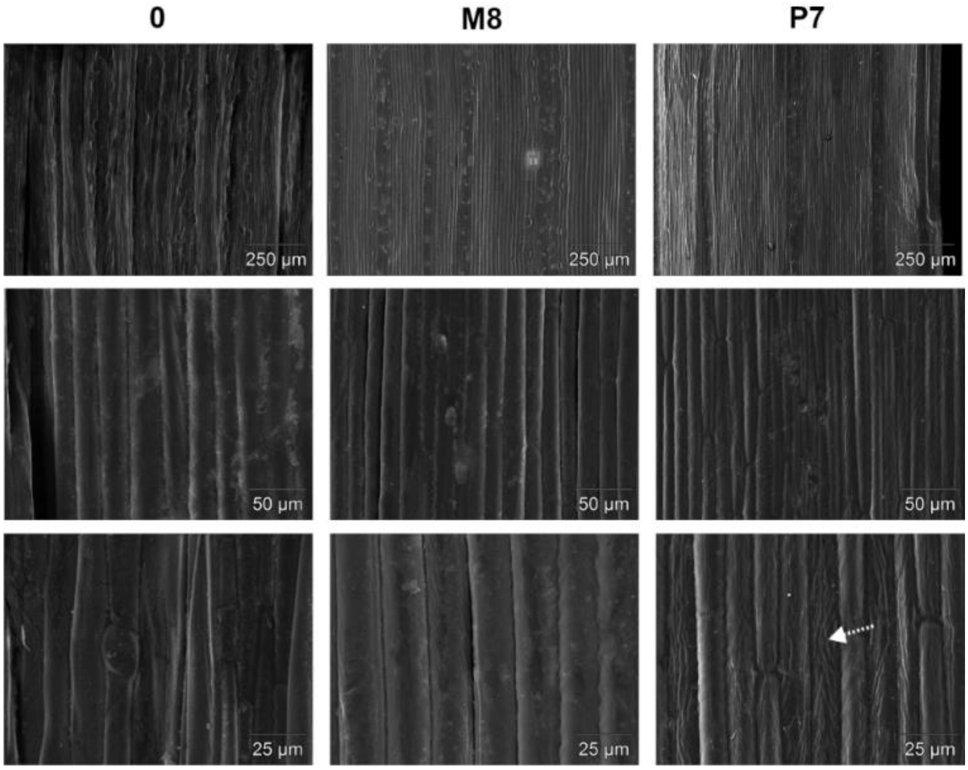

Figure 3. An SEM image of a sample following microwave and PEF disintegration. The white arrow shows the deformation of the cell walls.

3.2. Biogas and Methane Production

The analysis of grass silage biogas potential showed that the mean biogas production rate was 448.94 ± 16.54 NL/kg VS in a non-pretreated sample. The methane production rate for the control sample was 303.96 ± 11.17 NL/kg VS. The highest biogas production output in PEF-pretreated samples was obtained in series P7 (535.57 ± 18.91 NL/kg VS), whereas the methane productivity in this series was 364.95 ± 17.76 NL/kg VS. The highest biogas output from substrates pretreated with microwaves was obtained in series M9 (487.18 ± 13.55 NL/kg VS). The methane output obtained was 334.47 ± 7.11 NL/kg VS (Figure 4a,b). Wang et al. [32] examined the impact of PEF on biogas production from a Pennisetum hybrid. The experiments showed that the largest increase in biogas production was achieved under the following process conditions: 15 kV/120 Hz/60 min—26.95%. Kovačić et al. [31] examined the impact of PEF on biogas and methane productivity from maize stalks and soy straw. The use of PEF for the disintegration of maize stalks resulted in an 18% increase in biogas production and a 16% increase in methane production. Biogas production from soy straw also increased by 18%, whereas methane production increased by 17%. Reports from el Achkar [27] describe the application of PEF in the pretreatment of grape pomace, achieving a methane production increase of 4%. Budiyono et al. [33] investigated the effect of the microwave processing of fresh water hyacinth on biogas productivity. In their research, using microwave pretreatment, they achieved an increase in biogas production of almost 12%, with an energy consumption of 1025 Wh/g TS. Szwarc et al. [16,34] conducted research on the impact of PEF on the production of methane from maize silage and rapeseed straw. The pretreatment of maize silage with a pulsed electric field increased the production of methane by approximately 16%. The use of PEF treatment of rapeseed straw increased methane production by 14% compared to the control sample. Kuşçu et al. [25] applied PEF pretreatment in the methane fermentation of waste activated sludge. The obtained results showed that the biogas production increased by 70%. The application of PEF resulted in a 73% increase in methane production. Zou et al. [35] applied PEF treatment in the methane fermentation of food residues and obtained an increase in methane production of approximately 35%.

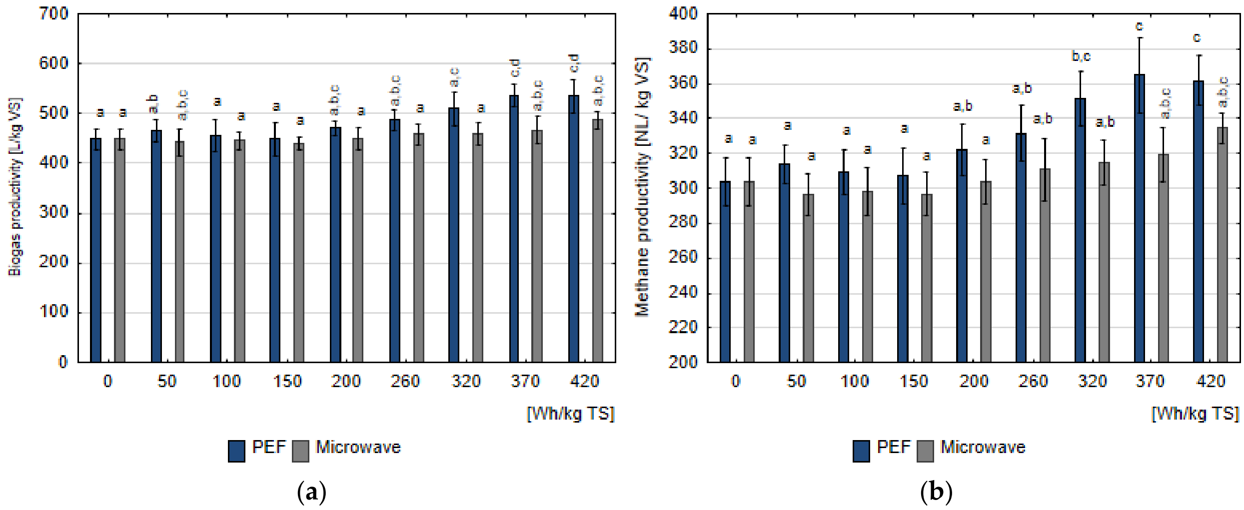

Figure 4. Productivity of biogas (**a**) and methane (**b**) from grass silage. Superscript letters (a,b,c,d) denote significant differences (Tukey's RiR test, $p < 0.05$).

4. Conclusions

The use of lignocellulosic biomass in the production of second-generation biofuels is economically viable and environmentally justified. This biomass is easily available, which is the main issue in energy production from renewable sources, e.g., biogas. Unfortunately, the complex structure of lignocellulosic biomass makes it necessary to apply biomass pretreatment before methane fermentation in order to increase biogas production. Therefore, pretreatment methods are still being sought, which will allow for biomass disintegration cost minimisation and production maximisation of the energy carrier-biogas. SEM images of the samples after disintegration by both methods showed that PEF causes distinct deformations of cell walls. No such changes were observed after microwave pretreatment. The application of PEF disintegration in series P6 resulted in a COD increase from 9135 ± 101 mg O_2/L to 10,554 ± 174 mg O_2/L. The application of microwave disintegration resulted in a smaller COD increase. The largest COD was noted in series M8 (9505 ± 54 mg O_2/L). The highest methane productivity from PEF-pretreated samples was noted in series P7—364.95 ± 17.76 NL/kg VS, which was a 20.1% increase compared to the control sample. The largest methane output from samples pretreated with microwaves was observed in series M8 (334.47 ± 7.11 NL/kg VS), which was a 10% increase compared to the control sample. According to the experimental results, PEF pretreatment can be applied in methane fermentation to increase biogas production. These findings show that PEF disintegration is more effective than that performed with microwaves, assuming that the same energy is used for pretreatment.

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