

Article

Influence of the Parameters of Used Biochar on the Dark Fermentation Process

Kamil Kozłowski ^{1,2,*}, Jacek Dach ¹, Wojciech Czekala ¹, Krystyna Malińska ³, Kacper Świechowski ⁴, Jakub Pulka ¹ and Andrzej Lewicki ¹

¹ Department of Biosystems Engineering, Poznan University of Life Sciences, Wojska Polskiego 50, 60-637 Poznan, Poland; jacek.dach@up.poznan.pl (J.D.); wojciech.czekala@up.poznan.pl (W.C.); jakub.pulka@up.poznan.pl (J.P.); andrzej.lewicki@up.poznan.pl (A.L.)

² Biogas Technology—Kamil Kozłowski, Jana Woźniaka Street 18, 62-330 Nekla, Poland

³ Institute of Environmental Engineering, Czestochowa University of Technology, Brzeznicza 60A, 42-200 Czestochowa, Poland; krystyna.malinska@pcz.pl

⁴ Department of Applied Bioeconomy, Wroclaw University of Environmental and Life Sciences, Chełmońskiego 37a, 51-630 Wroclaw, Poland; kacper.swiechowski@upwr.edu.pl

* Correspondence: kamil.kozlowski@up.poznan.pl; Tel.: +48-61-848-7156

Abstract: The aim of the work was to analyze the impact of biochar produced under various production conditions on the course of the dark (hydrogen) fermentation process. A series of experiments were planned, in which the starting material was digestate from a functioning agricultural biogas plant. Changes in the physicochemical properties and microstructure of biochar obtained in the manufacturing process with different parameters were also analyzed. Another issue analyzed was the size and dynamics of the gas production during dark fermentation with the use of various types of auxiliary material. This work showed that increasing the temperature and holding time during the production of biochar from digestion pulp improved the dynamics of biohydrogen production during the process of dark fermentation. The results of this research can be used in industrial research to optimize the process of biohydrogen production using biochar.

Keywords: biochar; dark fermentation; hydrogen production; biogas production



Citation: Kozłowski, K.; Dach, J.; Czekala, W.; Malińska, K.; Świechowski, K.; Pulka, J.; Lewicki, A. Influence of the Parameters of Used Biochar on the Dark Fermentation Process. *Energies* **2023**, *16*, 7484. <https://doi.org/10.3390/en16227484>

Academic Editor: Dimitrios Kalderis

Received: 14 September 2023

Revised: 28 October 2023

Accepted: 2 November 2023

Published: 8 November 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

In the last decades, research in Poland and in eastern and central Europe has focused primarily on using biomass for direct combustion (production of heat), the production of liquid biofuels in transesterification and esterification and the production of methane (in anaerobic digestion process (AD)) [1,2]. The constant increase in the demand for electricity and heat as well as fuels to power the engines of cars, ships and planes has led to further crises, not only energy ones. Hydrogen is currently considered one of the most promising energy carriers. It is characterized by a high content of energy per unit of mass ($142 \text{ kJ} \cdot \text{g}^{-1}$) and low emissions (its oxidation leads to the generation of water (in the form of water vapor) besides energy) [3–5]. An additional advantage of using hydrogen in energy and industry is the possibility of using it directly in combustion engines or fuel cells. Intensive work is underway around the world to introduce these technologies to the commercial market [6,7]. In other industrial sectors, hydrogen is commonly used as a reagent in fertilizer production, industrial ammonia synthesis and petroleum refining. It should also be noted that these industries still face many challenges related to hydrogen production, especially using ecological methods. The problem hindering the widespread use of hydrogen as a fuel is the high cost of its production. Another challenge involves the technical problems related to its temporary storage and distribution to end recipients. Currently, hydrogen is produced on an industrial scale in the process of steam reforming from natural gas and pipelines and on a small scale in the process of coal gasification and water electrolysis [8,9]. However, as

mentioned earlier, these processes are very energy-intensive and require non-ecological fossil fuels to produce the hydrogen. Therefore, hydrogen production using biological methods is a promising and future-proof solution because it is not an energy-intensive process [10].

Biological hydrogen production processes include methods dependent on light (direct biophotolysis of water carried out by algae and photofermentation with photosynthetic bacteria) and methods that do not require access to light (dark fermentation (DF) carried out by anaerobic bacteria and indirect biophotolysis of water using cyanobacteria) [10,11]. In the coming years, DF may become one of the most important technologies for generating green energy from organic waste, such as waste from the agri-food industry or municipal waste, which can also be used in AD and DF.

One of the serious problems related to the AD and DF processes is the production and maintenance of a digestate with appropriate nutritional parameters before its use as fertilizer. In most cases, the digestate has a high moisture content, and in an attempt to reduce it, phase separation equipment is used, which causes losses in terms of total nitrogen and phosphorus. Recovering nutrients from the digestate reduces the loss of nutrients but also reduces the economic value of the investment. There is growing interest in the use of biochar in AD and DF to both increase the recovery rates and reduce the nutrient losses before and after its application to soil [12].

In recent years, there has been a growing interest in biochar's properties and its applications in various fields of science and the economy (including the energy industry [13], agriculture and environmental protection [14]. This is due to the fact that the following material can be produced from different types of substrates (including plant biomass, animal waste, food waste and sewage sludge) and under various process conditions [15], which can contribute to the biochar having new chemical and physical properties. As a result of the spread of science, it is possible to determine the properties of biochar, e.g., in methane fermentation, more thoroughly [16,17]. Materials published in recent years confirm that the use of biochar increases the efficiency of AD [18,19].

Biochar itself is a material that, both in terms of its structure and basic properties, resembles traditional charcoal. According to the available literature, it is defined as a material resulting from the pyrolysis of biomass from a plant or animal origin and is characterized by a fine-grained form, a high carbon content and a slight susceptibility to degradation [20,21], and it is formed during thermal transformation of biomass at high temperature without the presence of oxygen. As a result of this process, other liquid and gas products with a high energy value are also generated [22].

The most important features of biochar include its high chemical stability, its developed specific surface area, its microporosity and the presence of functional groups that allow for its extensive application in environmental protection [23–25]. In addition, it can be utilized to increase the activity of microorganisms in various biochemical processes, such as being a supporting material in the methane fermentation process [16]

It should also be added that all stages of the methane fermentation process and the dark fermentation process have a correct course, unless the appropriate environmental conditions and parameters are met [26]. The most important include the pH, temperature, nutrient content and C/N ratio in the applied medium and the occurrence of inhibitors. The occurrence of process inhibitors particularly applies to substrates with high nitrogen or sulfur contents. According to sources in the literature, the toxic concentrations of ammonia, ammonia and sulfur for the fermentation process are >2.7 mg/L [27], >4000 mg/L NH_3 [28–30] and >100 mg/L S^{2-} , respectively [31]. However, due to its physical and chemical properties, biochar is one of the substances that is able to remove these inhibitors [12,32].

The main putative mechanisms for the effective performance of biochar are its favorable physicochemical properties, such as its high porosity and surface area [33], which enable surface complexation to interact with nutrient cycles, the precipitation of minerals for immobilization or adsorption and its modified symbiotic relationships with microbial communities [34].

The mechanisms of both the methane fermentation process as well as dark fermentation are well known and are described in the scientific literature. However, in particular, the latter requires an in-depth analysis at the basic level, including an examination of the impact of new supporting materials on the dynamics of hydrogen production, bacterial microflora development and the neutralization of existing process inhibitors. Supporting materials that are utilized in various fields of science include biochar produced from the pyrolysis of biomass from a plant or animal origin, which is characterized by a high content of organic carbon and a slight susceptibility to degradation. The primary feature is the developed specific surface area, microporosity and the presence of functional groups. However, there is not much information on the effect of the properties of this material on the process of hydrogen production during fermentation.

The aforementioned factors prompted us to conduct research into the impact of the parameters of the biochar production process on the course of the dark fermentation process, which was the subject of the proposed project. In connection with the project's defined goal, the impact of different types of biochar on the course of the dark (hydrogen) fermentation process were analyzed.

The research hypothesis of this study assumes that an increase in the temperature and retention time during the production of biochar from digested pulp will improve the dynamics of hydrogen production during the dark fermentation process. This article consists of main elements such as an Introduction (I), the Materials and Methods section (II), which describes the materials used in the research, a description of the production of biochar from digestate, the methods of physicochemical analysis used and an analysis of biohydrogen production during dark fermentation. Then, the Results and Discussion (III) are presented in relation to the available literature. The Conclusions (IV) are included at the end.

2. Materials and Methods

2.1. Materials

In the conducted research, the liquid fraction of digestion pulp obtained by the mechanical separation of digestion pulp from a functioning agricultural biogas plant located in Działyń (Poland) was used as a mesophilic bacterial inoculum. The installation operates at a temperature of 39 °C and is fed with maize silage, slurry and manure. The inoculum was stored under room-temperature conditions. However, for the tests carried out under thermophilic conditions (52 °C), the same inoculum was used but was previously adapted to higher thermal conditions by storing it in a water bath at the appropriate temperature. In both cases, before starting the research, the inoculations were subjected to thermal treatment in order to limit the development of methanogenic microflora (90 °C during 60 min).

Biochar produced from the solid fraction of post-fermentation pulp from a functioning biogas plant in Działyń (Poland, Wielkopolskie Voivodeship) was used in the research.

Throughout the experiment, the charge material (medium in the fermentation process) was crystalline dextrose. Crystalline glucose (α -D-glucose) in a fine crystalline form was obtained as a result of the enzymatic hydrolysis of starch. The manufacturer of the material was Przedsiębiorstwo Przemysłu Spożyczego PEPEES S.A. 18-402 Łomża, street Poznańska Street 121, Poland. The crystalline glucose used was in accordance with obligatory Polish and European Food Legislation. This substrate was characterized by a simple and homogeneous chemical structure, which allowed for maintaining optimal conditions for the growth of all the groups of microorganisms involved in the fermentation process. In addition, this material was characterized by high microbiological purity, thanks to which the risk of inhibiting the production of hydrogen by introducing undesirable microflora along with the substrate was limited.

2.2. Biochar Production

The biochar samples were produced following a previously described methodology [35]. The post-fermentation pulp was ground and dried to dry mass before the biochar production. For the grinding, a laboratory knife mill (Testchem, model LMN-100, Pszów, Poland) with a screen of 3 mm was used. Then, the ground (homogenized) material was dried in a laboratory dryer (Wamed, model KBC-65W, Warsaw, Poland) at 105 °C for 24 h. For the biochar production, a muffle furnace (Snol, model 8.1/1100, Utena, Lithuania) was used. For each process, a sample of ~130 g was used. The sample was placed in a heat-resistant glass vessel that was placed in the middle of the muffle furnace. Before heating, the furnace was flushed for 5 min with inert CO₂ gas (to facilitate an inert atmosphere). After the heating started, the CO₂ flow rate was ~5 dm³·min⁻¹ (to maintain the inert atmosphere). A heating rate of 50 °C·min⁻¹ was used. The biochar samples were produced at 200–600 °C (with 100 °C intervals) with residence times of 5–30 min (with 5 min intervals). After the process (end of residence time), the muffle furnace was left to cool down. The produced biochar samples were removed from the furnace when the temperature was ~100 °C (during the cooling stage, CO₂ gas also was supplied to prevent the biochar samples from self-igniting). For each temperature and residence time, one repetition was conducted.

2.3. Physical and Chemical Analysis

The produced biochar samples and the unprocessed material were subjected to a proximate analysis. The moisture content (MC) and dry matter content was determined following the PN-EN 14346:2011 standard [36] using a laboratory dryer (WAMED, model KBC-65W, Warsaw). The organic matter content (measured as a loss on ignition) (OM) was determined following the PN-EN 15169:2011 standard [37]. The ash content (*ash*) and combustible part (CP) were determined following the PN-Z-15008-04:1993 standard [38]. For the determination of the OM, *ash* and CP, a muffle furnace (Snol, model 8.1/1100, Utena, Lithuania) was used. All parameters were determined in three repetitions.

The contents of nitrogen, carbon, hydrogen and sulfur were determined using the dynamic combustion method in an EA Vario EL III elemental analyzer. An analysis of the presence of functional groups was performed on the basis of the spectrum based on FTIR-ART infrared spectroscopy. In addition, for a more accurate analysis of the structure of the auxiliary materials, microscopic examinations of the microstructure of the biochar samples were carried out using a scanning electron microscope.

2.4. Methodology of Hydrogen and Biogas Efficiency Research

The research on the methane efficiency of the substrates in batch culture technology was carried out in the Institute of Fluid-Flow Machinery of the Polish Academy Of Sciences on the basis of internal procedures, based on the adapted standards DIN 38 414-S8 and VDI 4630, which are commonly used in Europe. A detailed methodology of the performed research was presented by Cieřlik et al. [39] and Dach et al. [40]. Acid treatment was used as the pre-treatment process. The required amount of inoculum was acidified with 9M H₂SO₄ to a pH value of about 2.5. After a period of 24 h, the pH of the inoculum was raised with the use of a concentrated NaOH solution to a value of approx. 5.5.

The fermentation set-up consisted of 31 biofermenters. A total of 1200 mL of previously prepared inoculum, 2.4 g of carrier (biochar) and crystalline glucose in the amount of 15 g of VTS/L inoculum were placed in the 31 reactors. In each series, there was an additional 1 reactor containing inoculum and glucose (without the addition of biochar), which was a reference sample. Fermentation was carried out until the daily production of gases was stopped. Each individual fermenter (which was made from glass) had a volume of 1.8 dm³. The process was carried out under mesophilic conditions at 39 °C ± 1 °C and thermophilic conditions at 55 °C ± 1 °C. The produced biogas in each fermenter chamber was transported via a Teflon pipe to the gas storage. These reservoirs were made from plexiglass in the form of an inverted cylinder immersed in water. Between the water and gas areas, there was a liquid barrier preventing the dissolution of CO₂ in the water. The

shape of the test stand is presented in Figure 1, and its effectiveness has been confirmed during previous research [41].

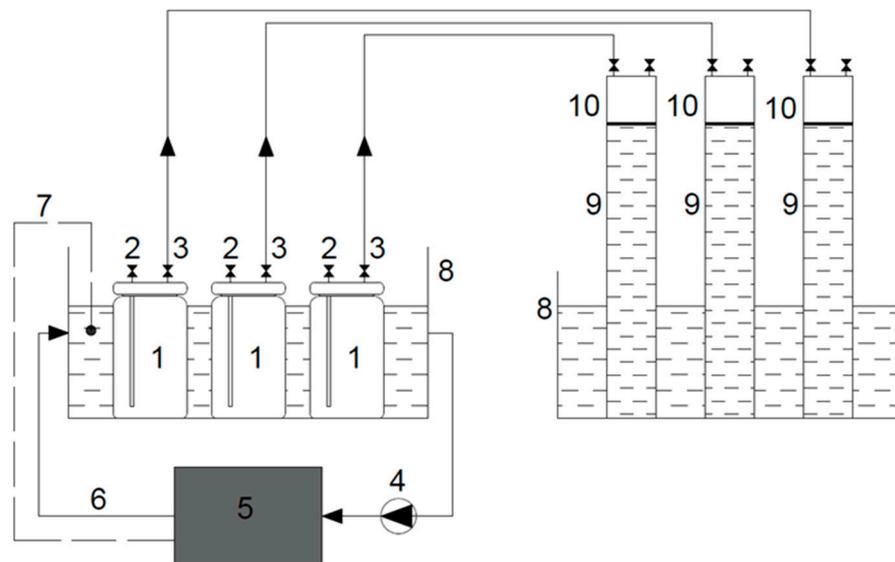


Figure 1. Shape of the AD reactors in BMP test (1. biofermenter with an input of 1.8 dm³ of capacity; 2. sampling tube; 3. tube for biogas flow; 4. water pump; 5. water heater with a temperature in the range of 20–70 °C; 6. isolated hot liquid tube; 7. temperature sensor; 8. reservoir; 9. biogas container made of poly (methyl methacrylate); 10. liquid barrier) [41].

The analysis of the composition of the generated biogas was carried out using the gas chromatography technique coupled with a thermal conductivity detector (GC-TCD). A column with a Restek[®] 2 m/2 mm ID 1/8'' OD silica packing and argon as a carrier gas was used. The final results of the biogas and hydrogen yields were converted into glucose VTS.

Figure 2 graphically presents the methodology of the conducted research.

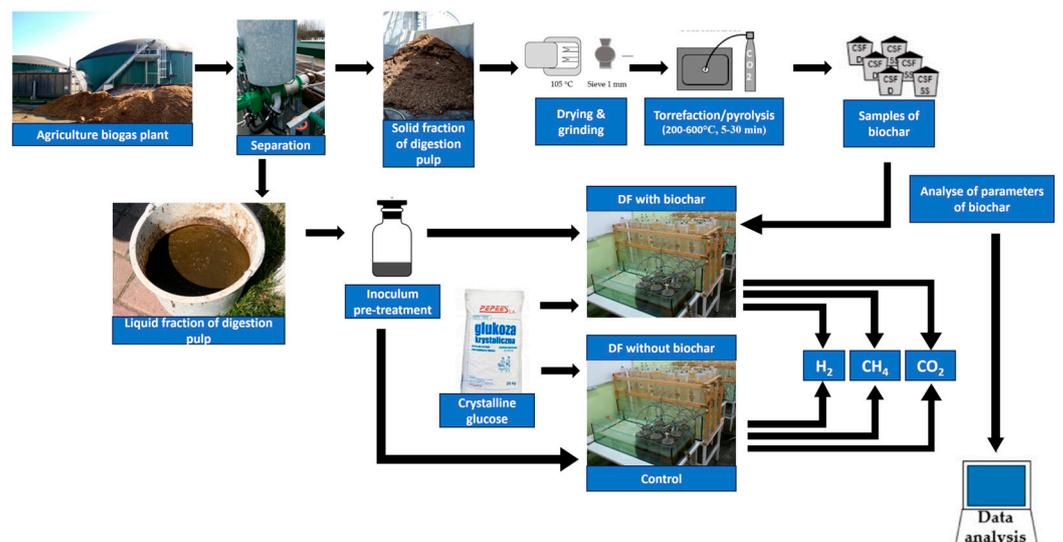


Figure 2. Shape of research methodology.

3. Results and Discussion

3.1. Inoculum

The project involved testing the impact of different types of biochar on the course of the dark fermentation process and the resulting H₂ and CO₂ production. In addition,

an analysis of changes in the properties of this material was conducted depending on the temperature and holding time during its production. In the first stage of the research, the basic physical and chemical parameters of the materials were determined. The solid fraction of the digestate used for the production of the biochar samples was characterized by the percentage content of dry matter and dry organic matter at the level of 5.06% FM and 82.67% DM, respectively. Table 1 contains the basic physicochemical parameters of the inoculum and monocrystalline glucose used in the further part of the project.

Table 1. Basic physicochemical parameters of inoculum and monocrystalline glucose.

Parameter	Mesophilic Inoculum	Thermophilic Inoculum	Crystalline Glucose
Dry matter (DM) [% FM]	4.50	4.84	91.11
Organic Dry Matter (ODM) [%]	70.12	70.75	99.77
DM after acid treatment [%]	6.58	6.34	-
ODM after acid treatment [%]	69.22	68.46	-
pH before acid treatment [—]	7.68	7.72	-
pH during acid treatment [—]	2.8	2.59	-
pH after acid treatment [—]	5.65	5.55	-

The research process that was carried out allowed for the determination of the usefulness of the dry fermentation pulp fraction for the biochar production process and for the determination of the appropriate proportions of the fermentation mixtures and substrate dosage in the further stages of the project.

3.2. Biochar Production and Analysis

During the research, 30 types of biochar were produced, which were characterized by different temperatures (200 °C, 300 °C, 400 °C, 500 °C and 600 °C) and residence times (5 min, 10 min, 15 min, 20 min, 25 min, 30 min) during the production process. The flow of carbon dioxide into the combustion chamber was kept constant in all cases in order to obtain anaerobic conditions during the pyrolysis process. Table 2 presents the basic physicochemical parameters of the produced biochar samples.

Table 2. The basic physicochemical parameters of the produced biochar samples.

Number	Name of Sample	Biochar Production Temperature (°C)	Biochar Holding Time (min)	Moisture (%)	VTS (% TS)
1	Control (RAW)	-	-	5.06	82.67
2	200/5	200	5	5.78	84.48
3	200/10	200	10	5.67	82.18
4	200/15	200	15	5.40	81.96
5	200/20	200	20	5.06	82.38
6	200/25	200	25	5.35	83.88
7	200/30	200	30	4.99	81.80
8	300/5	300	5	5.62	82.49
9	300/10	300	10	4.39	80.29
10	300/15	300	15	3.85	71.12
11	300/20	300	20	3.47	72.67
12	300/25	300	25	3.60	61.83

Table 2. Cont.

Number	Name of Sample	Biochar Production Temperature (°C)	Biochar Holding Time (min)	Moisture (%)	VTS (% TS)
13	300/30	300	30	3.71	64.21
14	400/5	400	5	4.27	79.33
15	400/10	400	10	4.50	67.69
16	400/15	400	15	4.37	67.21
17	400/20	400	20	4.87	66.95
18	400/25	400	25	5.05	66.65
19	400/30	400	30	5.34	68.38
20	500/5	500	5	4.12	74.69
21	500/10	500	10	4.73	69.79
22	500/15	500	15	4.92	66.53
23	500/20	500	20	5.10	66.84
24	500/25	500	25	5.14	60.53
25	500/30	500	30	4.56	56.97
26	600/5	600	5	4.46	79.97
27	600/10	600	10	4.70	61.28
28	600/15	600	15	4.39	63.25
29	600/20	600	20	4.22	54.08
30	600/25	600	25	5.04	53.26
31	600/30	600	30	5.43	57.31

The highest content of moisture and dry organic matter was found in the biochar samples produced at 200 °C. The highest moisture and organic matter contents were recorded for the biochar produced at 200 °C and with a holding time of 5 min. The above parameters were 5.78% and 84.48 TS, respectively. The obtained results confirmed the analyses conducted by Ajeng et al., which confirmed that wet roasting is preferred for the conversion of biomass with a high moisture content [42,43]. These raw materials require an energy-intensive drying process. It is believed to be a better pretreatment method for fast biomass pyrolysis because dry roasting causes severe cellulose degradation, and a reduced degree of crystallinity with more carbon residues is produced with increasing temperature, which was confirmed by this study [42].

When biomass undergoes pyrolysis, water loss through dewatering and the release of volatile compounds from the carbon matrix contribute to the formation of the biochar's pore structure, which also affects the development of primary pores [44]. In order to determine the active and adsorption surface of the resulting biochar, the iodine number was determined, specifying the available surface area in m²/g of pure coal. This was used to measure the amount of micropores in the biochar. The highest value of this parameter was obtained for the biochar produced at 200 °C and with a holding time of 5 min, i.e., 181.523 m²/g. The lowest value was obtained for the biochar produced at 600 °C and with a holding time 25 min, i.e., 84.515 m²/g. A decrease in the iodine number was also observed with the increase in the holding time during the production of the biocarbon from the digestate from a value of 149.580 ± 16.914 m²/g (for a holding time of 5 min) to 145.243 ± 22.760 m²/g. For the AD digestate fibers in the pyrolysis process (500 °C and 600 °C for 60 min), values of 134 and 142 m²/g were obtained, respectively [45]. However, for typical biogas substrates such as pig manure and sewage sludge, active surface values of

47.4 and 71.6 m²/g, respectively, were obtained during the pyrolysis carried out at 500 °C for 4 h [46].

For all the produced biochar samples and digestates, an analysis of the presence of functional groups was performed on the basis of spectra obtained from FTIR-ART infrared spectroscopy. In addition, in order to more precisely analyze the structure of the auxiliary materials, microscopic examinations were carried out. Microscopic photos of the produced biochar samples are shown in Figure 3.

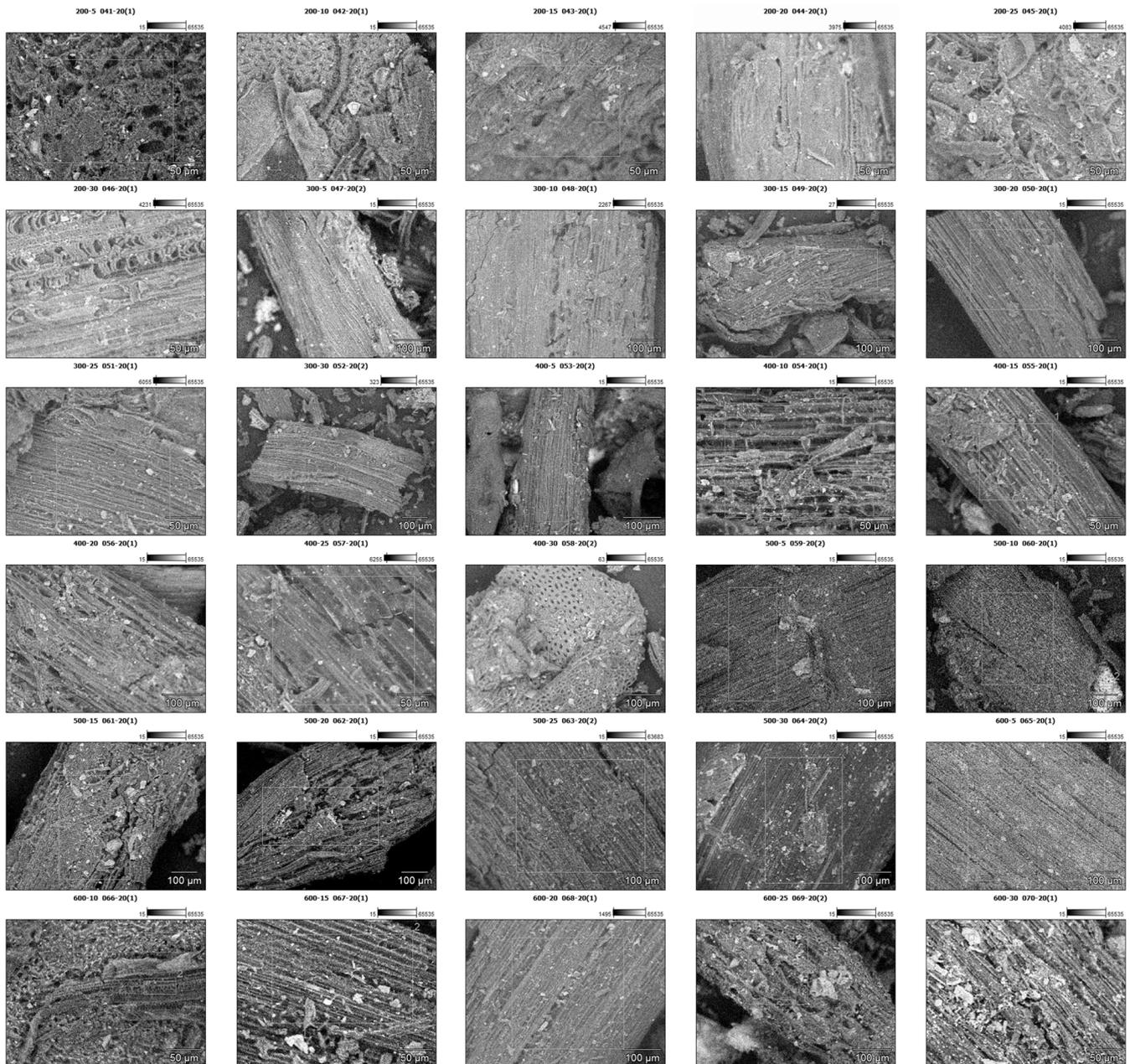


Figure 3. Microscopic photos of biochar samples produced during the project.

As part of determining the properties of the biochar samples, an elemental analysis was performed, the results of which are presented in the graphs shown in Figure 4.

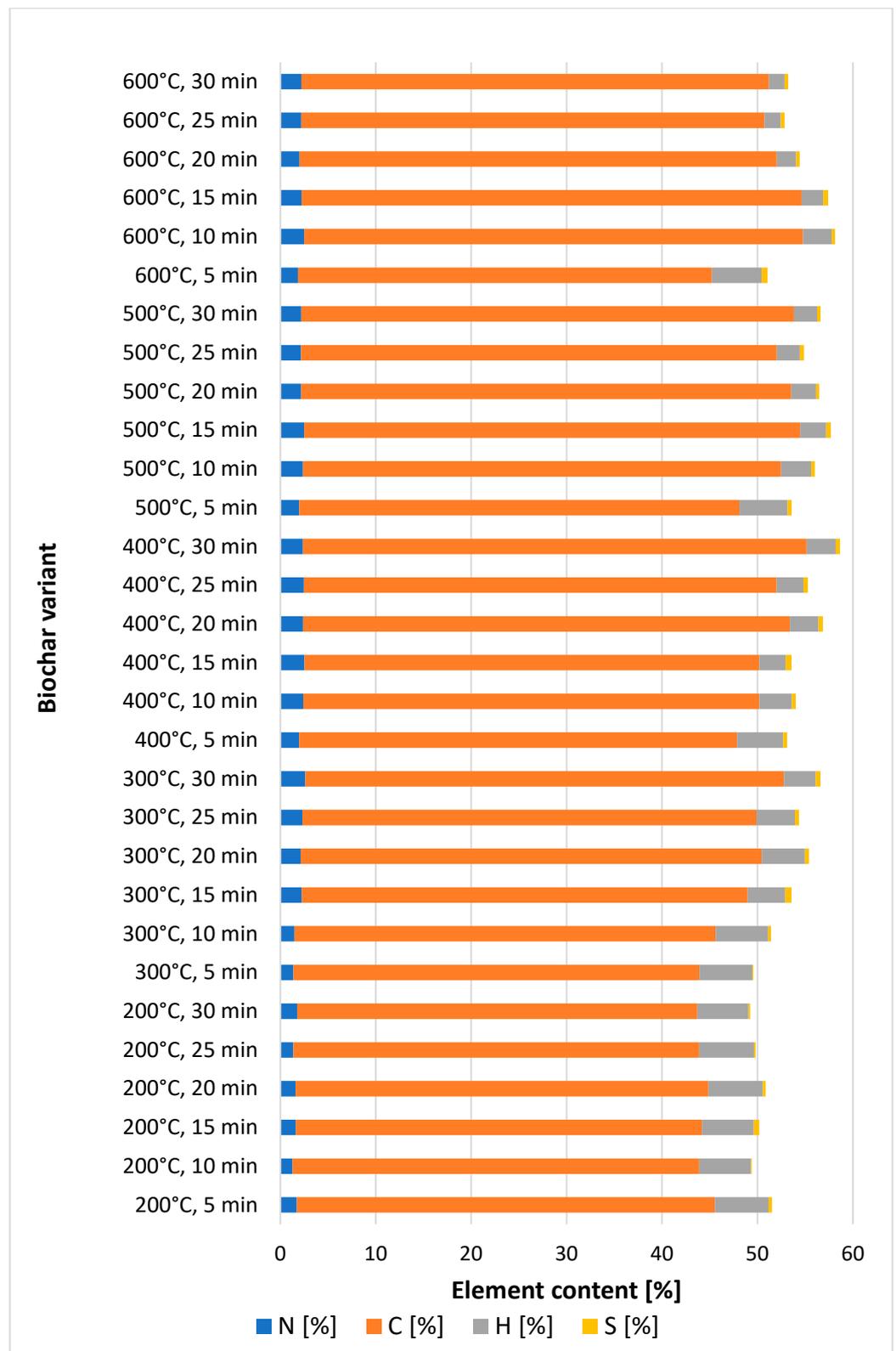


Figure 4. Charts of the content of elementary components of biochar samples (N, C, H and S) produced during the project.

Biochar samples with different physicochemical properties were prepared under different temperatures. According to the elemental analysis shown in Figure 4, the total N content and total C content increased with the increase in the pyrolysis temperature.

It should be assumed that this was mainly due to the higher degree of graphitization and carbonization of the material [47]. However, it should also be remembered that the elemental composition of biochar is inherently linked to the kinds of materials and the temperature at which it is created [48].

3.3. Hydrogen and Biogas Efficiency

The next stage of the project was to conduct research on the dark (hydrogen) fermentation process under mesophilic and thermophilic conditions using the produced biochar. Throughout the experiment, the charge material (medium in the fermentation process) was crystalline glucose. This substrate was characterized by a simple and homogeneous chemical structure, which allowed for the maintenance of optimal conditions for the growth of all groups of microorganisms involved in the fermentation process. In addition, this material was characterized by a high microbiological purity, thanks to which the risk of an inhibitory effect on the hydrogen production by introducing undesirable microflora along with the substrate was limited. Figures 5 and 6 present graphs of the cumulative daily production of hydrogen using the individual types of biochar under mesophilic and thermophilic conditions, respectively.

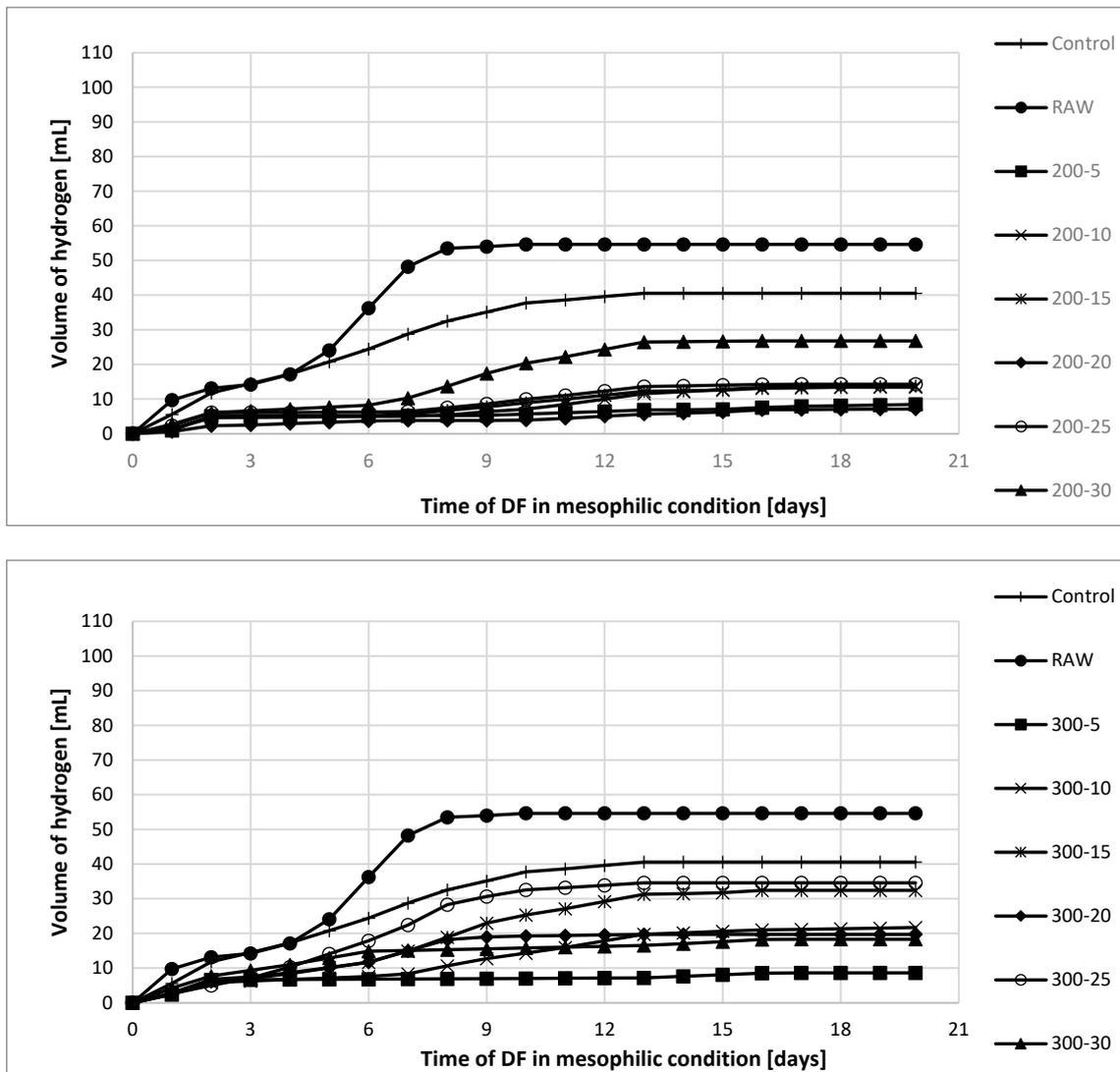


Figure 5. Cont.

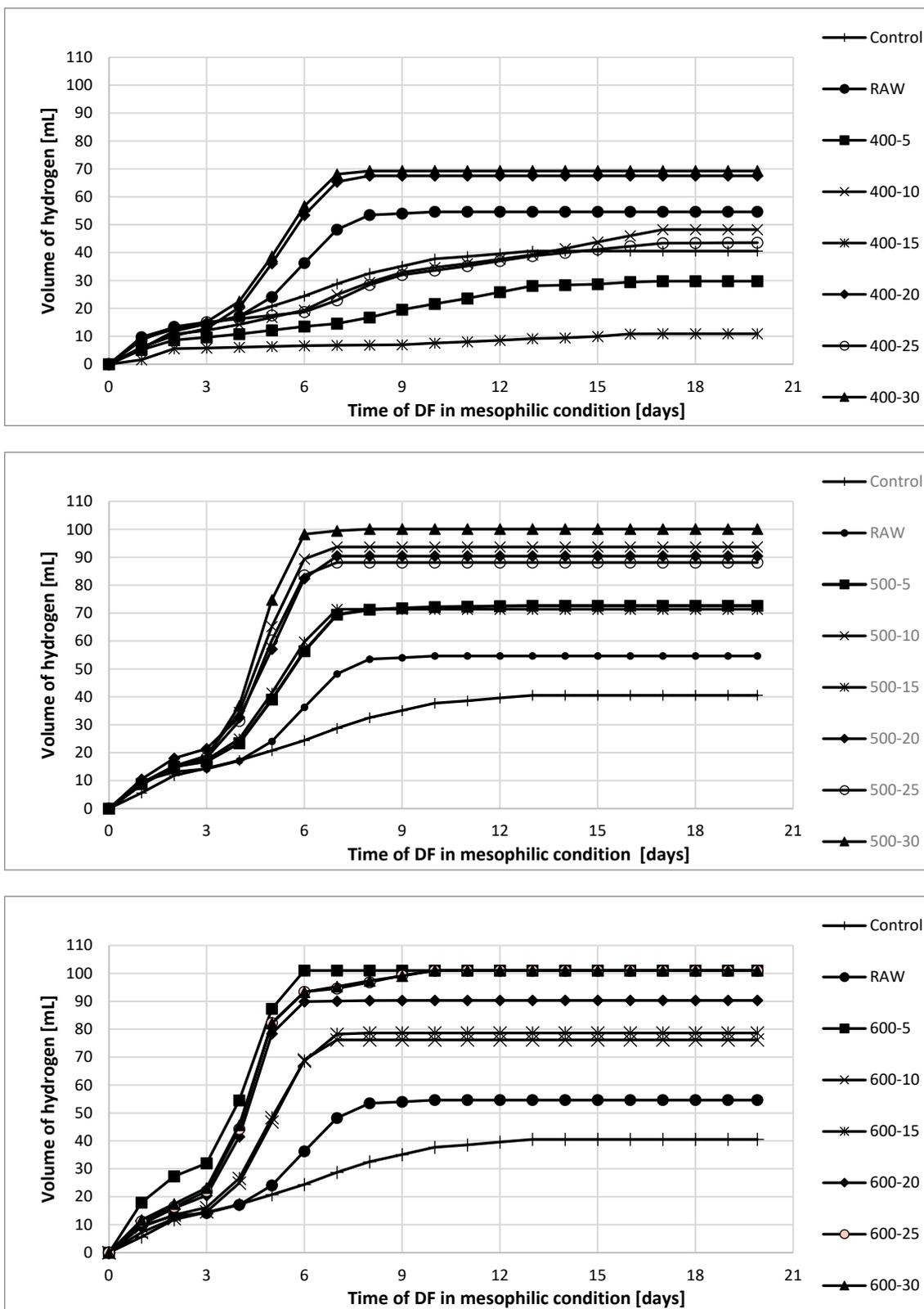


Figure 5. Accumulated hydrogen production using biochar samples under mesophilic conditions (mL H₂/g glucose/day).

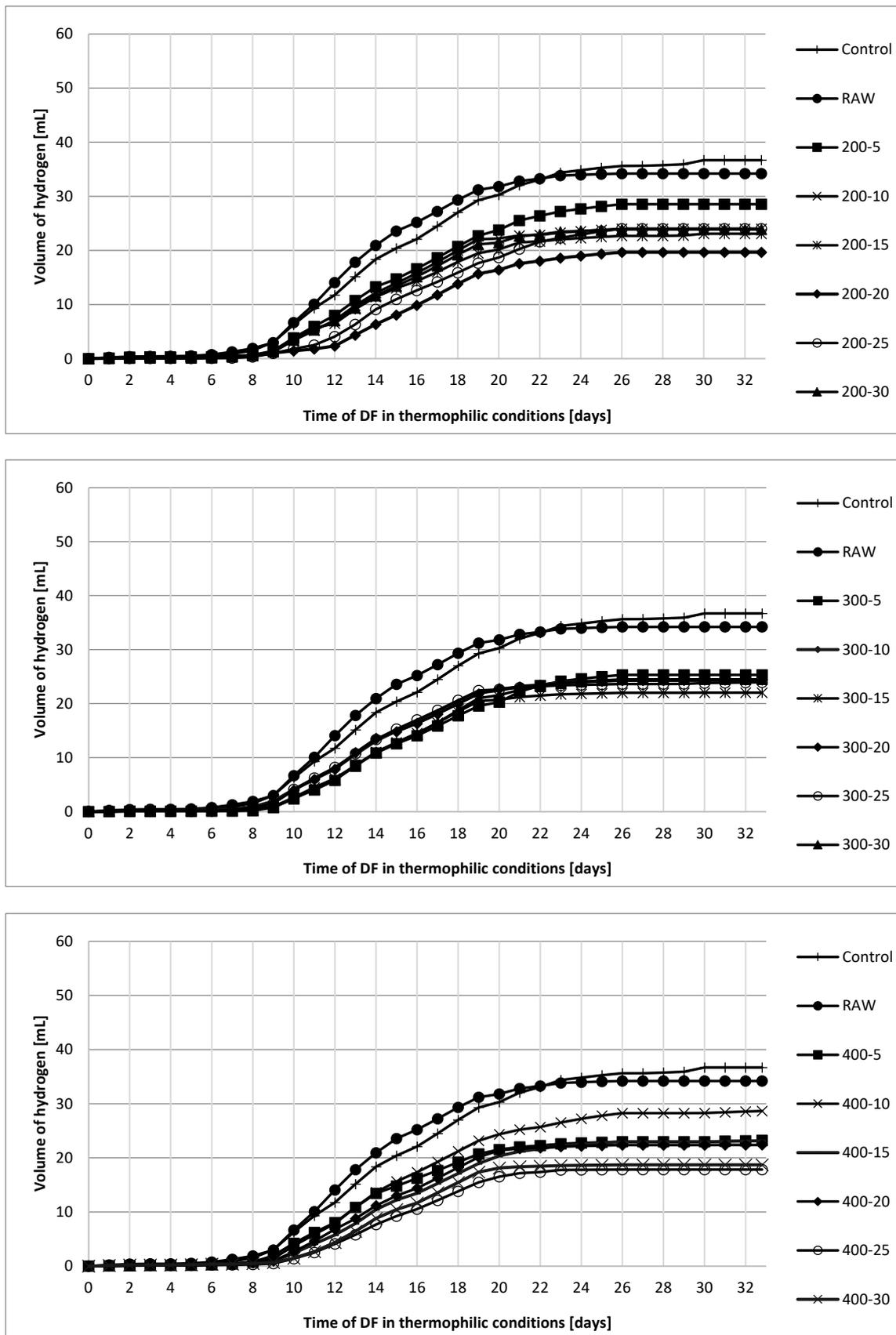


Figure 6. Cont.

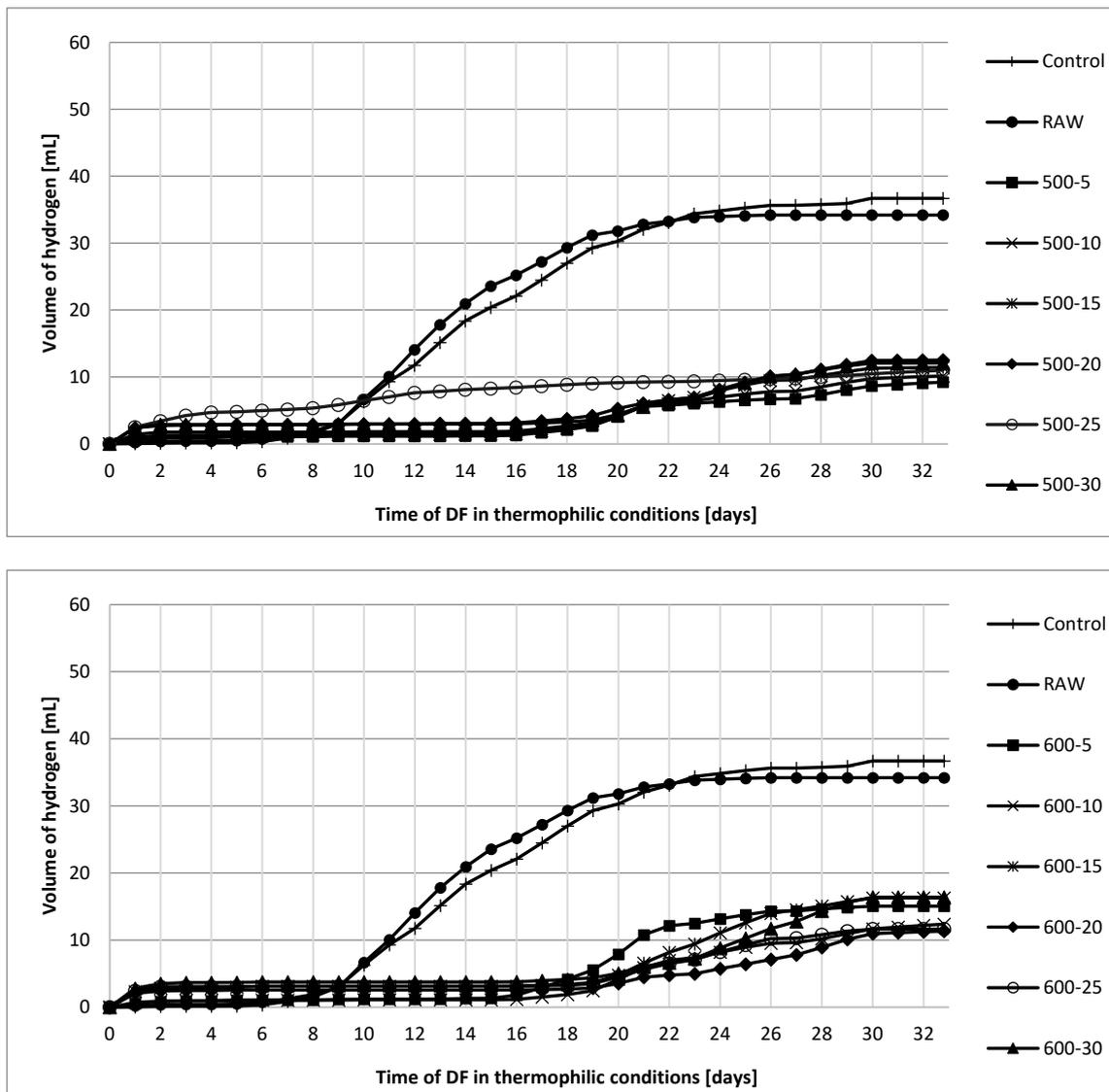


Figure 6. Accumulated hydrogen production using biochar samples under thermophilic conditions ($\text{mL H}_2/\text{g glucose/day}$).

The hydrogen production time of the biochar samples was in the range of between 6 (for the sample 600-5) and 17 days (for the sample of 400-10) with mesophilic digestion. In the case of the tests conducted under thermophilic conditions, an extended hydrogen fermentation time was found. This could be due to the lack of adaptation of the thermophilic microflora to the hydrogen production process, despite the previous acid treatment. Table 3 presents the biogas and hydrogen efficiency from the biochar with the dark fermentation process under mesophilic and thermophilic conditions.

In the dark fermentation process carried out under mesophilic conditions, the highest efficiency of hydrogen and biogas production was found with the sample marked 600-30 I, which were $101.1 \text{ mL H}_2/\text{gODM glucose}$ and $335.4 \text{ mL/gODM glucose}$, respectively. The biohydrogen production and yield during DF is dependent on temperature of the fermentation process, among other factors. According to the commonly known van't Hoff equation, the rate of a chemical reaction increases as the temperature of the chemical process increases. Anaerobic digestion and dark fermentation are catalyzed by microorganisms that are prone to activity loss at temperatures that deviate from their optimum (e.g., more than $60 \text{ }^\circ\text{C}$ and less than $20 \text{ }^\circ\text{C}$). Most of the research on biohydrogen fermentation to date has been conducted at mesophilic temperatures ($30\text{--}40 \text{ }^\circ\text{C}$) [49–51]. The results of one study

showed that the rate of hydrogen production increases up to 40 °C, and the process is inhibited at 45 °C [52], which was also shown by the research in this publication. These results were made available in the published version by Du et al. [53].

Table 3. Biogas and hydrogen efficiency from biochar with dark fermentation process under mesophilic and thermophilic conditions.

Name of Sample	DF Mesophilic		DF Thermophilic	
	Cumulative Production of H ₂ (mL H ₂ /gODM Glucose)	Cumulative Production of Biogas (mL/gODM Glucose)	Cumulative Production of H ₂ (mL H ₂ /gODM Glucose)	Cumulative Production of Biogas (mL/gODM Glucose)
Control	40.6	271.0	36.7	136.0
RAW	54.6	210.1	34.2	130.8
200-5	8.5	37.9	28.5	125.4
200-10	13.5	55.9	24.0	111.5
200-15	13.8	54.8	23.1	99.4
200-20	7.1	47.6	19.7	103.3
200-25	14.3	64.1	24.0	106.2
200-30	26.8	100.4	23.9	103.6
300-5	8.6	41.0	25.3	104.2
300-10	21.7	82.4	24.3	109.0
300-15	32.4	106.2	22.0	101.6
300-20	19.7	88.5	24.4	110.4
300-25	34.6	113.9	23.9	99.2
300-30	18.3	75.7	24.5	111.9
400-5	29.7	108.0	23.2	111.7
400-10	48.2	147.4	28.7	123.2
400-15	10.9	50.1	23.2	108.6
400-20	67.6	230.6	22.4	108.8
400-25	43.6	128.8	17.8	95.3
400-30	69.3	252.1	18.7	101.7
500-5	72.6	248.5	9.2	80.8
500-10	93.7	290.0	10.1	85.6
500-15	71.4	256.9	11.4	91.2
500-20	90.4	287.5	12.5	94.5
500-25	88.1	282.2	11.0	162.3
500-30	100.1	304.3	12.1	100.2
600-5	101.0	303.0	15.1	101.4
600-10	76.2	253.3	12.4	90.4
600-15	78.7	269.9	16.3	120.7
600-20	90.3	279.9	11.3	90.6
600-25	101.0	334.8	11.7	94.6
600-30	101.1	335.4	16.4	112.9

In the literature, DF under thermophilic conditions has also been investigated. The results of earlier research show that elevated temperatures result in increased enzymatic activity (hydrogenases) and the inhibition of microorganisms responsible for lactic acid formation [54]. The conducted tests did not show an increase in the efficiency of hydrogen production with increasing the temperature. All of the produced biochar samples were characterized by a lower efficiency of biohydrogen production than the control sample. This could be due to the failure to develop thermophilic bacteria responsible for the production of biogas and biohydrogen.

Further development perspectives should include an analysis of machine learning or artificial neural networks to predict the efficiency of biohydrogen production using biochar. These models could effectively predict important parameters in industrial chemical processes without the need for costly, energy-intensive and complex experiments [55].

4. Conclusions

During the tests, 31 biochar samples were produced under varying temperature conditions and with varying holding times. The highest moisture and organic matter contents were recorded for the biochar produced at 200 °C and with a residence time of 5 min. The highest value of the active and adsorption surface was obtained for the biocarbon produced at a temperature of 200 °C and with a residence time of 5 min, i.e., 181.523 m²/g. The conducted research and analyses confirmed that an increase in the temperature and holding time during the production of biochar from fermentation pulp improves the dynamics of hydrogen production during the dark fermentation process under mesophilic conditions. During the conducted research, no increase in the biohydrogen production efficiency was observed for the thermophilic technology. The highest efficiency of hydrogen production was obtained for the biochar produced at 600 °C and with a 30 min residence time, i.e., 101.1 mL H₂/gVTS glucose. The research described in this article was basic in nature. The published results constitute the basis for further considerations on the influence of the parameters of the produced biochar on the DF process.

Author Contributions: Conceptualization, K.K.; methodology, K.K., J.D., K.Ś. and A.L.; software, K.K.; validation, K.K. and J.P.; formal analysis, W.C.; investigation, K.K. and K.M.; resources, K.K. and W.C.; data curation, K.K.; writing—original draft preparation, K.K.; writing—review and editing, K.K. and W.C.; visualization, K.K.; supervision, J.D. and K.M.; project administration and funding acquisition, K.K. All authors have read and agreed to the published version of the manuscript.

Funding: The work was created as a result of the research project no. 2018/29/N/NZ9/00179 financed by the National Science Centre, Poland.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

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

References

1. Czekala, W. Solid Fraction of Digestate from Biogas Plant as a Material for Pellets Production. *Energies* **2021**, *14*, 5034. [CrossRef]
2. Selvakumar, P.; Adane, A.A.; Zelalem, T.; Hunegnaw, B.M.; Karthik, V.; Kavitha, S.; Jayakumar, M.; Karmegam, N.; Govarthan, M.; Kim, W. Optimization of binary acids pretreatment of corncob biomass for enhanced recovery of cellulose to produce bioethanol. *Fuel* **2022**, *321*, 124060. [CrossRef]
3. Czekala, W.; Bartnikowska, S.; Dach, J.; Janczak, D.; Smurzyńska, A.; Kozłowski, K.; Bugała, A.; Lewicki, A.; Cieślik, M.; Typańska, D.; et al. The energy value and economic efficiency of solid biofuels produced from digestate and sawdust. *Energy* **2018**, *159*, 1118–1122. [CrossRef]
4. Guo, X.M.; Trably, E.; Latrille, E.; Carrère, H.; Steyer, J.P. Hydrogen production from agricultural waste by dark fermentation: A review. *Int. J. Hydrogen Energy* **2010**, *35*, 10660–10673. [CrossRef]
5. Rahman, S.N.A.; Masdar, M.S.; Rosli, M.I.; Majlan, E.H.; Husaini, T.; Kamarudin, S.K.; Daud, W.R.W. Overview biohydrogen technologies and application in fuel cell technology. *Renew. Sustain. Energy Rev.* **2016**, *66*, 137–162. [CrossRef]
6. Alves, H.J.; Bley Junior, C.; Niklevicz, R.R.; Frigo, E.P.; Frigo, M.S.; Coimbra-Araújo, C.H. Overview of hydrogen production technologies from biogas and the applications in fuel cells. *Int. J. Hydrogen Energy* **2013**, *38*, 5215–5225. [CrossRef]
7. Kotay, S.M.; Das, D. Biohydrogen as a renewable energy resource—Prospects and potentials. *Int. J. Hydrogen Energy* **2008**, *33*, 258–263.

8. Balat, M. Potential importance of hydrogen as a future solution to environmental and transportation problems. *Int. J. Hydrogen Energy* **2008**, *33*, 4013–4029. [[CrossRef](#)]
9. Hassen Sellami, M.; Loudiyi, K. Electrolytes behavior during hydrogen production by solar energy. *Renew. Sustain. Energy Rev.* **2017**, *70*, 1331–1335. [[CrossRef](#)]
10. Ghimire, A.; Frunzo, L.; Pirozzi, F.; Trably, E.; Escudie, R.; Lens, P.N.L.; Esposito, G. A review on dark fermentative biohydrogen production from organic biomass: Process parameters and use of by-products. *Appl. Energy* **2015**, *144*, 73–95. [[CrossRef](#)]
11. Kanwal, F.; Torriero, A.A.J. Biohydrogen—A Green Fuel for Sustainable Energy Solutions. *Energies* **2022**, *15*, 7783. [[CrossRef](#)]
12. Fagbohungebe, M.O.; Herbert, B.M.J.; Hurst, L.; Ibeto, C.N.; Li, H.; Usmani, S.Q.; Semple, K.T. The challenges of anaerobic digestion and the role of biochar in optimizing anaerobic digestion. *Waste Manag.* **2017**, *61*, 236–249. [[CrossRef](#)]
13. Yadav, K.; Jagadevan, S. Example, Influence of Process Parameters on Synthesis of Biochar by Pyrolysis of Biomass: An Alternative Source of Energy. In *Recent Advances in Pyrolysis*, 2nd ed.; Al-Haj Ibrahim, H., Ed.; IntechOpen: London, UK, 2020. [[CrossRef](#)]
14. Wu, G.; Tham, P.E.; Chew, K.W.; Munawaroh, H.S.H.; Tan, I.S.; Wan-Mohtar, W.A.A.Q.I.; Sriariyanun, M.; Show, P.L. Net zero emission in circular bioeconomy from microalgae biochar production: A renewed possibility. *Bioresour. Technol.* **2023**, *388*, 129748. [[CrossRef](#)] [[PubMed](#)]
15. Lewandowski, W.M.; Rymys, M.; Meler, P. Termicznno-chemiczna piroliza do biopaliw ciekłych i gazowych, jako metoda podnoszenia sprawności konwersji energii biomasy. *Nafta-Gaz* **2010**, *8*, 675–680.
16. Luo, C.; Lü, F.; Shao, L.; He, P. Application of eco-compatible biochar in anaerobic digestion to relieve acid stress and promote the selective colonization of functional microbes. *Water Res.* **2015**, *68*, 710–718. [[CrossRef](#)]
17. Mumme, J.; Srocke, F.; Heeg, K.; Werner, M. Use of biochars in anaerobic digestion. *Bioresour. Technol.* **2014**, *151*, 189–197. [[CrossRef](#)] [[PubMed](#)]
18. Sun, Z.; Wang, X.; Wu, Y.; Du, W.; Li, H.; Zhang, P. Effect of sludge biochar on methane production from anaerobic granular sludge. *J. Water Process Eng.* **2023**, *56*, 104410. [[CrossRef](#)]
19. Xu, S.; Bu, J.; Li, C.; Tiong, Y.W.; Sharma, P.; Liu, K.; Jin, C.; Ma, C.; Tong, Y.W. Biochar enhanced methane yield on anaerobic digestion of shell waste and the synergistic effects of anaerobic co-digestion of shell and food waste. *Fuel* **2024**, *357*, 129933. [[CrossRef](#)]
20. Czekala, W.; Jasiński, T.; Grzelak, M.; Witaszek, K.; Dach, J. Biogas Plant Operation: Digestate as the Valuable Product. *Energies* **2022**, *15*, 8275. [[CrossRef](#)]
21. Lehman, J.; Joseph, S. *Biochar for Environmental Management: Science and Technology*; Earthscan: London, UK, 2009.
22. Verheijen, F.G.A.; Jeffery, S.; Bastos, A.C.; van der Velde, M.; Diafas, I. *Biochar Application to Soils—A Critical Scientific Review of Effects on Soil Properties, Process and Functions*; EUR 24099 EN; European Commission: Brussels, Belgium, 2010.
23. Ahmad, M.; Rajapaksha, A.U.; Lim, J.E.; Zhang, M.; Bolan, D.M.; Vithanage, M.; Lee, S.S.; Ok, S.Y. Biochar as a sorbent for contaminant management in soil and water: A review. *Chemosphere* **2014**, *99*, 19–33. [[CrossRef](#)] [[PubMed](#)]
24. Mohan, D.; Sarswat, A.; Ok, S.Y.; Pittman, C.U., Jr. Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent—A critical review. *Bioresour. Technol.* **2014**, *160*, 191–202. [[CrossRef](#)] [[PubMed](#)]
25. Steiner, C.; Das, K.C.; Melear, N.; Lakly, D. Reducing nitrogen loss during poultry litter composting using biochar. *J. Environ. Qual.* **2010**, *39*, 1236–1242. [[CrossRef](#)]
26. Deublein, D.; Steinhauser, A. *Biogas from Waste and Renewable Resources*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2008.
27. Kaltschmitt, M.; Hartmann, H. *Energie aus Biomasse—Grundlagen, Techniken und Verfahren*; Springer: Berlin/Heidelberg, Germany; New York, NY, USA, 2001.
28. Angelidaki, I.; Ahring, B.K. Thermophilic anaerobic digestion of livestock waste: Effect of ammonia. *Appl. Microbiol. Biotechnol.* **1993**, *38*, 560–564. [[CrossRef](#)]
29. Hashimoto, A.G. Ammonia inhibition of methanogenesis from cattle wastes. *Agric. Wastes* **1986**, *17*, 241–261. [[CrossRef](#)]
30. Jarrell, K.F.; Saulnier, M.; Ley, A. Inhibition of methanogenesis in pure cultures by ammonia, fatty acids, and heavy metals, and protection against heavy metal toxicity by sewage sludge. *Can. J. Microbiol.* **1987**, *33*, 551–555. [[CrossRef](#)] [[PubMed](#)]
31. Parkin, G.F.; Lynch, N.A.; Kuo, W.; Van Keuren, E.L.; Bhattacharya, S.K. Interaction between sulfate reducers and methanogens fed acetate and propionate. *Res. J. Water Pollut. Control Fed.* **1990**, *62*, 780–788.
32. Nie, W.; He, S.; Lin, Y.; Cheng, J.J.; Yang, C. Functional biochar in enhanced anaerobic digestion: Synthesis, performances, and mechanisms. *Sci. Total Environ.* **2024**, *906*, 167681. [[CrossRef](#)]
33. Sanchez-Monedero, M.A.; Cayuela, M.L.; Roig, A.; Jindo, K.; Mondini, C.; Bolan, N. Role of biochar as an additive in organic waste composting. *Bioresour. Technol.* **2018**, *247*, 1155–1164. [[CrossRef](#)]
34. Pan, J.; Ma, J.; Zhai, L.; Luo, T.; Mei, Z.; Liu, H. Achievements of biochar application for enhanced anaerobic digestion: A review. *Bioresour. Technol.* **2019**, *292*, 122058. [[CrossRef](#)]
35. Świechowski, K.; Hnat, M.; Stępień, P.; Stegenta-Dąbrowska, S.; Kugler, S.; Koziel, J.A.; Białowiec, A. Waste to Energy: Solid Fuel Production from Biogas Plant Digestate and Sewage Sludge by Torrefaction-Process Kinetics, Fuel Properties, and Energy Balance. *Energies* **2020**, *13*, 3161. [[CrossRef](#)]
36. *Polish Standard PN-EN 14346:2011; Waste Characteristics. Calculation of Dry Mass on the Basis of Dry Residue or Water Content.* Polski Komitet Normalizacji: Miar i Jakości, Poland, 2011.

37. Polish Standard PN-EN 15169:2011; Waste Characteristics. Content of Organic Matter. Polski Komitet Normalizacji: Miar i Jakości, Poland, 2011.
38. Polish Standard PN-Z-15008-04:1993; Municipal Solid Waste. Combustible and Non-Combustible Content. Polski Komitet Normalizacji: Miar i Jakości, Poland, 1993.
39. Cieřlik, M.; Dach, J.; Lewicki, A.; Smurzyńska, A.; Janczak, D.; Pawlicka-Kaczorowska, J.; Boniecki, P.; Cyplik, P. Methane fermentation of the maize straw silage under meso- and thermophilic conditions. *Energy* **2016**, *115*, 1495–1502. [[CrossRef](#)]
40. Dach, J.; Boniecki, P.; Przybył, J.; Janczak, D.; Lewicki, A.; Czekala, W.; Witaszek, K.; Rodriguez Carmona, P.C.; Cieřlik, M. Energetic efficiency analysis of the agricultural biogas plant in 250 kW(e) experimental installation. *Energy* **2014**, *69*, 34–38. [[CrossRef](#)]
41. Kozłowski, K.; Dach, J.; Lewicki, A.; Malińska, K.; Paulino do Carmo, I.E.; Czekala, W. Potential of biogas production from animal manure in Poland. *Arch. Environ. Prot.* **2019**, *45*, 99–108. [[CrossRef](#)]
42. Ajeng, A.A.; Abdullah, R.; Ling, T.C.; Ismail, S.; Lau, B.F.; Ong, H.C.; Chew, K.W.; Show, P.L.; Chang, J.S. Bioformulation of biochar as a potential inoculant carrier for sustainable agriculture. *Environ. Technol. Innov.* **2020**, *20*, 101168. [[CrossRef](#)]
43. Gan, Y.Y.; Ong, H.C.; Chen, W.H.; Sheen, H.K.; Chang, J.S.; Chong, C.T.; Ling, T.C. Microwave-assisted wet torrefaction of microalgae under various acids for coproduction of biochar and sugar. *J. Clean. Prod.* **2020**, *253*, 119944. [[CrossRef](#)]
44. Bagreev, A.; Badosz, T.J.; Locke, D.C. Pore structure and surface chemistry of adsorbents obtained by pyrolysis of sewage sludge-derived fertilizer. *Carbon* **2001**, *39*, 1971–1979. [[CrossRef](#)]
45. Pelaez-Samaniego, M.R.; Smith, M.W.; Zhao, Q.; Garcia-Perez, T.; Frear, C.; GarciaPerez, M. Charcoal from anaerobically digested dairy fiber for removal of hydrogen sulfide within biogas. *Waste Manag.* **2018**, *76*, 374–382. [[CrossRef](#)] [[PubMed](#)]
46. Xu, X.; Cao, X.; Zhao, L.; Sun, T. Comparison of sewage sludge- and pig manure-derived biochars for hydrogen sulfide removal. *Chemosphere* **2014**, *111*, 296–303. [[CrossRef](#)] [[PubMed](#)]
47. Bu, J.; Wei, H.L.; Wang, Y.T.; Cheng, J.R.; Zhu, M.J. Biochar boosts dark fermentative H₂ production from sugarcane bagasse by selective enrichment/colonization of functional bacteria and enhancing extracellular electron transfer. *Water Res.* **2021**, *202*, 117440. [[CrossRef](#)]
48. Abukari, A.; Kaba, J.S.; Dawoe, E.; Abunyewa, A.A. A comprehensive review of the effects of biochar on soil physicochemical properties and crop productivity. *Waste Dispos. Sustain. Energy* **2022**, *4*, 343–359. [[CrossRef](#)]
49. Dong, L.; Zhenhong, Y.; Yongming, S.; Xiaoying, K.; Yu, Z. Hydrogen production characteristics of the organic fraction of municipal solid wastes by anaerobic mixed culture fermentation. *Int. J. Hydrogen Energy* **2009**, *34*, 812–820. [[CrossRef](#)]
50. Wang, X.; Zhao, Y. A bench scale study of fermentative hydrogen and methane production from food waste in integrated two-stage process. *Int. J. Hydrogen Energy* **2009**, *34*, 245–254. [[CrossRef](#)]
51. Zong, W.; Yu, R.; Zhang, P.; Fan, M.; Zhou, Z. Efficient hydrogen gas production from cassava and food waste by a two-step process of dark fermentation and photo-fermentation. *Biomass Bioenergy* **2009**, *33*, 1458–1463. [[CrossRef](#)]
52. Kim, J.K.; Nhat, L.; Chun, Y.N.; Kim, S.W. Hydrogen production conditions from food waste by dark fermentation with *Clostridium beijerinckii* KCTC 1785. *Biotechnol. Bioprocess* **2008**, *13*, 499–504. [[CrossRef](#)]
53. Du, J.; Xu, P.P.; Ren, H.Y.; Cao, G.L.; Xie, G.J.; Ren, N.Q.; Liu, B.F. Improved sequential production of hydrogen and caproate by addition of biochar prepared from cornstalk residues. *Bioresour. Technol.* **2023**, *387*, 129702. [[CrossRef](#)] [[PubMed](#)]
54. Valdez-Vazquez, I.; Rios-Leal, E.; Esparza-García, F.; Cecchi, F.; Poggi-Varaldo, H. Semi-continuous solid substrate anaerobic reactors for H₂ production from organic waste: Mesophilic versus thermophilic regime. *Int. J. Hydrogen Energy* **2005**, *30*, 1383–1391. [[CrossRef](#)]
55. Hai, A.; Bharath, G.; Patah, M.F.A.; Daud, W.M.A.W.; Rambabu, K.; Show, P.L.; Banat, F. Machine learning models for the prediction of total yield and specific surface area of biochar derived from agricultural biomass by pyrolysis. *Environ. Technol. Innov.* **2023**, *30*, 103071. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.