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Hyperthermophilic Treatment of Grass and Leaves to Produce Hydrogen, Methane and VFA-Rich Digestate: Preliminary Results

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Abstract: In this study, the feasibility of hydrogen and methane production from grass and leaves via hyperthermophilic anaerobic digestion was investigated. The hyperthermophilic treatment of grass at 70 °C resulted in the highest concentrations of volatile fatty acids (TVFA) and reducing sugars in the supernatant of over 21 and 6.5 g/L reported on day 3 and 4 of the experiment. In contrast, hydrolysis and acidification of leaves performed slower and with lower efficiency, as the peak concentrations of TVFA and reducing sugars were observed at the end of the process. However, the highest cumulative hydrogen and methane yields of 69.64 mLH_2/gVS and 38.63 $mLCH_4$/gVS were reported for leaves digested at 70 °C, whereas the corresponding maximum productions observed for grass were 50 mLH_2/gVS and 1.98 $mLCH_4$/gVS, respectively. A temperature increase to 80 °C hampered hydrogen and methane production and also resulted in lower yields of volatile fatty acids, reducing sugars and ammonia as compared to the corresponding values reported for 70 °C.

Keywords: hydrogen; methane; hyperthermophilic treatment; grass; leaves

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1. Introduction

Waste is considered as an attribute of modern civilization. Global demand and energy consumption are increasing at a rate of 2–3% per year. In the European Union member states, the share of electricity generated from renewable sources in 2017 more than doubled compared to 2005 [1]. Renewable energy sources include biofuels produced from biomass and organic waste, of which biomethane and biohydrogen are of great importance [2]. Grass and leaves represent biomass, which is cheap, available and quickly regenerates. Huge amounts of these wastes originate from grassy areas, parks, municipal forests as well as individual households [3]. According to the Central Statistical Office, urban and housing estate greenery in Poland accounts for approximately 84,000 ha. With an average production of 5 tons of dry matter per hectare, this gives approximately 420,000 tons of dry matter obtained every year for processing [1]. Nowadays, in Poland, grass and leaves waste are most often composted, however, the composting process requires a long processing time (a few months), and the final product is often difficult to sell. An alternative approach could be the use of grass and leaves for the production of biogas (methane and hydrogen) via anaerobic digestion [4]. These materials are composed of lignocellulosic structures, in which cellulose and hemicelluloses are surrounded by a lignin coating. Simple sugars (pentoses and hexoses) within the lignocellulose structure are not easy to liberate because the lignin coating is highly resistant to environmental factors [5,6]. Therefore, a proper pretreatment needs to be applied to efficiently produce biogas from lignocellulosic biomass by anaerobic digestion. From an economic point of view, the use of hyperthermophilic pretreatment (70–80 °C) on grass and leaves seems to be a reasonable approach, in contrast to the application of expensive enzymes or chemical compounds [7]. In contrast to heat treatment typically performed at 120–140 °C, the application of the temperature below the boiling point of water avoids pressurizing and generating undegradable compounds [8,9]. Hyperthermophilic pretreatment has been successfully applied for increasing methane yield in the subsequent mesophilic or thermophilic digestion of municipal sewage sludge [10–12], the mixture of sewage sludge with kitchen waste [13,14], fat, oil and grease [15], agriculture waste [16], and the mixture of sewage sludge with ground grass [17]. Literature data also suggest that greater hydrogen yields from biomass could be achieved by dark fermentation operated under hyperthermophilic or thermophilic conditions, compared to mesophilic process [18,19]. In contrast, the authors in [20] reported no hydrogen production from sewage sludge at 70 °C, whereas the maximum H_2 yield was observed at 55 °C. In another study [21], a hyperthermophilic bacteria *Caldicellulosiruptor bescii* was found to efficiently utilize biosolids and produce hydrogen with simultaneous secretion of acetic acid as the main substrate for methane production in the subsequent stage. It was also documented that hyperthermophilic bacteria *Thermotoga maritima* could enhance hydrogen production from fruit–vegetable and fish wastes [22].

Hence, the aim of this research was to assess if hyperthermophilic anaerobic treatment could efficiently hydrolyze grass and leaves, and whether it is accompanied by the production of hydrogen or methane. Two temperatures of 70 and 80 °C were applied, and the experiments were performed under two experimental protocols, with and without inoculation of substrates. To the best of the authors' knowledge, there are no reports which evaluate hyperthermophilic digestion of grass and leaves treated with no addition of other substrate types.

2. Materials and Methods

2.1. Feedstock and Inoculum

The experiments were performed using the following substrates: grass from house garden and leaves from fruit trees (walnut, apple and cherry), which were harvested in October 2019. After harvesting, the raw moist grass was shredded in a grinder (FIMAR TS-32D400V) to obtain the particles of approximately 3–5 mm, and then portioned and stored at −18 °C prior to use. The leaves after harvesting were dried at room temperature for 7 days. Then, the dried leaves were mixed with a kitchen blender and the milled dried material was stored in a closed container to avoid moisture. Anaerobic sludge from the anaerobic mesophilic digester was provided by the Municipal Wastewater Treatment Plant in Lodz, Poland, and served as inoculum for the experiments. The characteristics of the substrates and inoculum are summarized in Table 1.

2.2. Experimental Design

2.2.1. Batch Thermal Treatment—Experiments in Flasks

The first series of experiments was conducted in Erlenmeyer flasks, each with a total capacity of 0.5 dm^3 and a working volume of 0.3 dm^3. The flasks were filled with the mixtures of substrates and water in a few dilutions. Specifically, grass was mixed with water in the mass ratios of 1:1, 1:2, 1:3 and 1:4, whereas leaves with water in the ratios of 1:2, 1:3, 1:4. No inoculum was added to the mixtures. The mouths of the Erlenmeyer flasks were covered with aluminum foil to minimize water evaporation, and the flasks were then placed in a thermostat for incubation at two temperatures of 70 and 80 °C and incubated for 10 days. The samples of supernatant were collected every day of incubation and analyzed for soluble COD (sCOD), TVFA, concentration of reducing sugars, ammonium nitrogen (NH_4-N) and pH. Each experimental run was performed in duplicate and the results were then presented as means.

Table 1. Characteristics of substrates used for the experiments.

Material	Indicator								
	Total Solids (g/kg)	**Volatile Solids (g/kg)**	**Volatile Solids (%TS)**	**Carbon (%TS)**	**Nitrogen (%TS)**	**Phosphorus (%TS)**	**Hydrogen (%TS)**	**Sulfur (%TS)**	**C/N**
Grass	127.68 ± 10.51	104.18 ± 8.79	81.59 ± 3.80	59.8 ± 3.22	2.63 ± 0.12	0.98 ± 0.06	5.31 ± 0.45	0.89 ± 0.02	22.74
Leaves	928.76 ± 1.70	815.55 ± 3.78	87.81 ± 0.28	58.4 ± 1.95	2.91 ± 0.20	0.24 ± 0.01	5.33 ± 0.36	0.36 ± 0.01	20.07
Inoculum	26.29 ± 0.61	21.84 ± 0.20	83.07 ± 1.34	64.6 ± 2.30	6.43 ± 0.28	1.23 ± 0.06	5.82 ± 0.30	0.58 ± 0.03	10.05

2.2.2. Anaerobic Digestion Batch Tests

The second series of experiments was carried out using an installation as shown in Figure 1. The digestion process was performed in glass bottles, each with a total and working volume of 1 and 0.7 dm^3, respectively. The bottles were placed in a thermostat to maintain constant temperatures of 70 and 80 °C. The headspace of each bottle was connected to a 1 dm^3 gas collecting tank to enable anaerobic conditions and measure daily biogas production by a water displacement method. The bottles were filled with inoculum and substrates in various proportions as depicted in Table 2. Before closing the bottles and starting the experiments, the headspace of each bottle was rinsed with nitrogen gas for 3 min to ensure anaerobic conditions. The bottles were then incubated in two temperature variants at 70 and 80 °C, and they were manually shaken once a day. Each experiment was continued to the point at which only residual or no biogas production was measured. The individual runs were performed in duplicate, the results of which are expressed as averages.

2.3. Analytical Methods

Total and volatile solids, and pH were analyzed based on Standard Methods for the Examination of Water and Wastewater [23]. Soluble chemical oxygen demand (sCOD), ammonium nitrogen and total volatile fatty acids were determined using a DR3900 spectrophotometer and HACH-Lange tests no. LCK 514, 8038, and LCK365, respectively. The tests were conducted according to the manufacturer's instructions. The total concentration of reducing sugars were measured by the 3,5-dinitrosalicylic acid (DNS) method using glucose as the standard [24]. Elemental analysis (C, N, H, P, S) was performed with a Flash Elemental Analyzer (Thermo Finnigan, Italy), following the manufacturer's instructions. Biogas composition was analyzed using a portable gas analyzer GA-21plus (Madur, Poland). The analyses of individual samples were performed in triplicate. The calculation of the average values were performed in Microsoft Excel 2010.

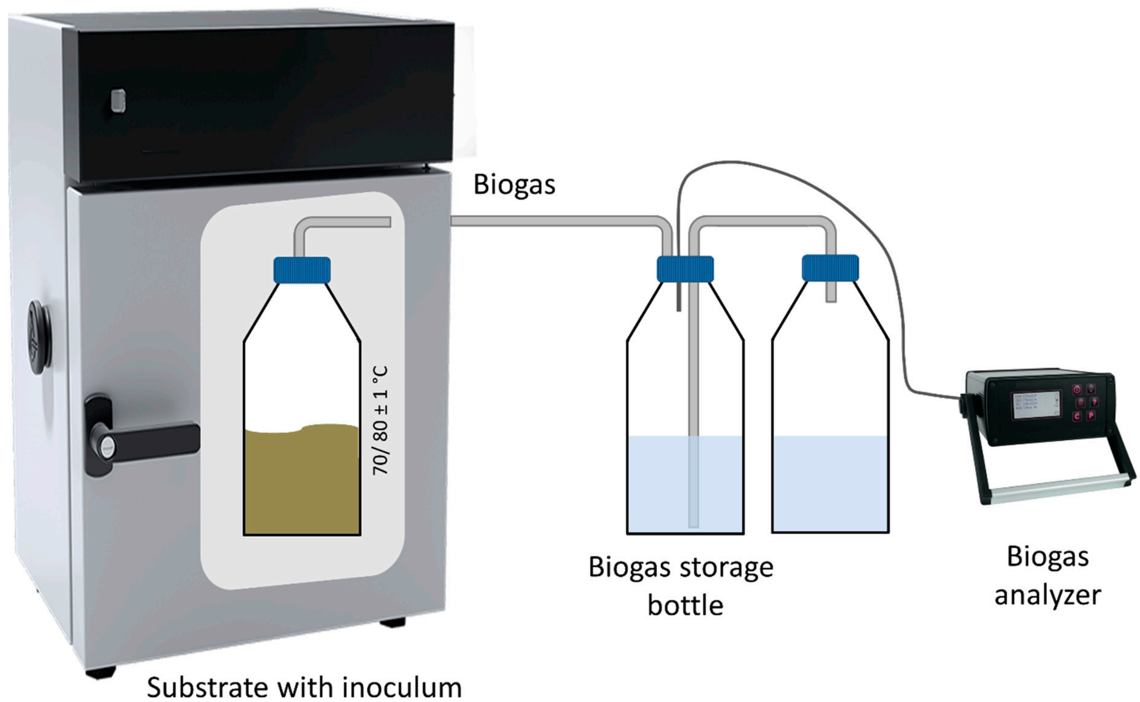

Figure 1. Laboratory setup for biogas production experiments.

Table 2. Operating parameters and performances of the experiments with biogas production.

		Grass				**Leaves**			
	I/S	**2:1**	**1:1**	**1:2**	**1:4**	**2:1**	**1:1**	**1:2**	**1:4**
Mass of inoculum added (g)		500	500	500	500	500	500	500	500
Initial substrate TS content (gTS)		6.69	13.38	26.77	53.53	6.22	12.44	24.87	49.74
Initial substrate vs. content (gVS)		5.46	10.92	21.84	43.68	5.46	10.92	21.84	43.68
Duration time (d)		14	14	14	14	14	14	14	14
Cumulative hydrogen yield (mL/gVS)	70 °C	50.09 ± 6.33	2.09 ± 0.27	2.47 ± 2.45	6.23 ± 1.91	69.64 ± 10.50	42.85 ± 4.68	17.52 ± 3.67	11.66 ± 2.98
	80 °C	0.00 ± 0	0.00 ± 0	0.00 ± 0	0.00 ± 0	0.00 ± 0	0.00 ± 0	0.00 ± 0	0.00 ± 0
Cumulative methane yield (mL/gVS)	70 °C	1.98 ± 0.47	1.81 ± 0.07	0.17 ± 0.08	0.08 ± 0.04	38.63 ± 8.42	25.85 ± 4.84	1.03 ± 0.03	0.38 ± 0.01
	80 °C	1.53 ± 0.29	0.76 ± 0.28	0.25 ± 0.004	0.17 ± 0.03	4.09 ± 1.07	0.58 ± 0.06	0.35 ± 0.07	1.57 ± 0.17
Final pH	70 °C	6.51 ± 0.05	6.12 ± 0.7	6.05 ± 0.015	5.76 ± 0.025	7.26 ± 0.08	7.27 ± 0.19	5.07 ± 0.055	4.73 ± 0.07
	80 °C	6.97 ± 0.15	6.52 ± 0.17	5.79 ± 0.15	5.95 ± 0.03	7.16 ± 0.06	6.61 ± 0.01	5.775 ± 0.075	5.155 ± 0.055

I/S—inoculum-to substrate ratio (gVS of inoculum/gVS of substrate).

3. Results and Discussion

3.1. Batch Thermal Treatment—Experiments in Flasks

The experiments in flasks were carried out in order to assess the dynamics of hyperthermophilic treatment based on metabolic products released to the supernatant, including TVFA, reducing sugars, and ammonium nitrogen. As illustrated in Figures 2 and 3, the temperature and dilution rate considerably impacted the variations of measured indicators. The hyperthermophilic treatment of grass at 70 °C gave the highest volatile fatty acids production of over 21 g/L reported on day 4 of the run. Interestingly, the TVFA concentrations dropped in the following days, and then, increased again up to 20 g/L on day 8 of this run. The changes of sCOD showed a similar trend, with two peaks observed on days 2 and 8. The sCOD value reached 15 g/L, which may indicate that hyperthermophilic conditions induced both enzymatic and chemical hydrolysis as discussed by the authors in [25]. Regarding reducing sugars, a rapid increase of up to 6.5 g/L was reported within three days of the run followed by a drop to less than 3 g/L in the next day. Contrary to them, ammonium nitrogen revealed an almost linear increase along with the experimental period and had the greatest impact on pH. The lowest pH was reported for the dilution rate of 1:1, which is not surprising since the greatest TVFA concentrations were measured for that dilution. Nevertheless, the pH value at the end of the experiments reached 6.5–7.4 (depending on the dilution rate). It seems that volatile fatty acids were effectively neutralized by ammonium nitrogen, the concentration of which reached 500 mg/L at the end of the experiment at 70 °C (Figure 2). As reported in the literature, the free NH_4 produced from nitrogen sources combines with the TVFA produced, and establishes a buffer system, which effectively controls the pH value in the digester [26,27].

Since ammonium nitrogen is basically a product of protein degradation, the results indicate that proteins are degraded slower than carbohydrates. Moreover, carbohydrates tend to suppress the synthesis of exopeptidases, which is a group of enzymes that facilitate protein degradation [9].

An increase in the temperature to 80 °C resulted in an extension of lag-phase. Within three days of the experiment, there was almost no change in all measured indicators. Considering sCOD and TVFA, their concentrations slowly increased up to around 15 and 20 g/L, respectively on day 9, however, a considerable increase was only observed for the dilution 1:1. Interestingly, there was no change of reducing sugars, whereas ammonia reached a peak of 350 mg/L on day 8. A lower ammonia did not fully stabilize pH, which dropped to 5.3–5.8 at the end of these trails. However, the pH value along the experiments at 80 °C was within the optimal range for anaerobic hydrolysis and acidogenesis as described by the authors in [19].

In contrast to the findings for grass, the results of the experiments performed with leaves are ambiguous. Irrespective of the temperature applied, sCOD and TVFA showed an increasing trend as the experiments proceeded with the peaks reported on day 10, which is illustrated in Figure 3. Likewise, for grass, the greatest release of sCOD and TVFA was also reported for the dilution 1:1. On the other hand, volatile fatty acid production did not fully reflect the sCOD levels. The highest TVFA of 18 g/L was reported at the end of the experiment performed at 80 °C and corresponded to 13.8 g/L of sCOD. However, the greatest TVFA concentration reported for 70 °C was 13.6 g/L, whereas the corresponding highest sCOD value was 15.5 g/L. Furthermore, the concentrations of reducing sugars and ammonium nitrogen were also higher at 80 °C, especially at the end of these runs. Generally, the ammonia content did not exceed 300 mg/L, and therefore, could not buffer volatile fatty acids produced in the course of the hyperthermophilic process. As a result, pH dropped to approximately 4.5 at the end of the runs.

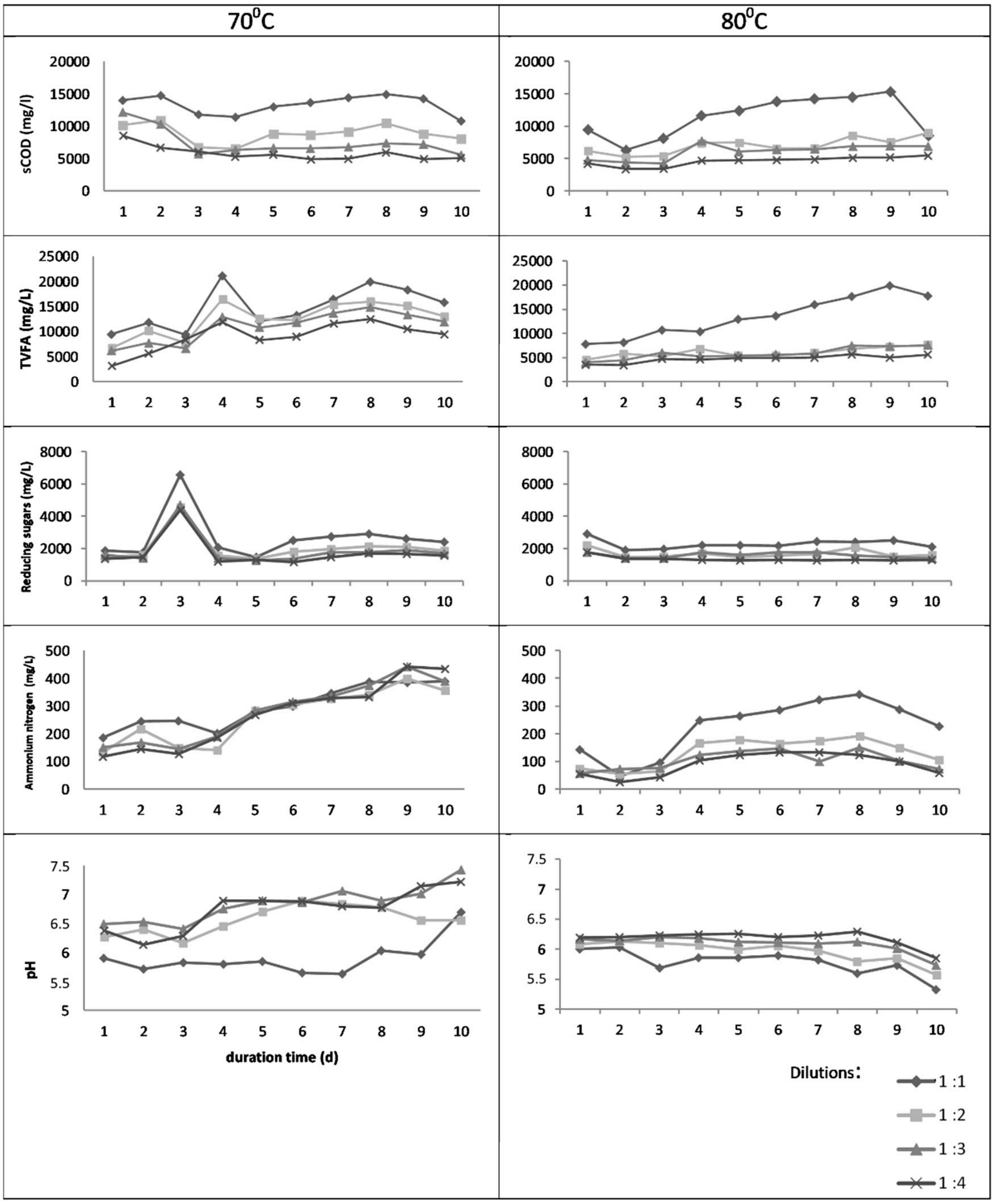

Figure 2. Evolution of sCOD, TVFA, reducing sugars, ammonium nitrogen and pH during hyperthermophilic treatment of grass.

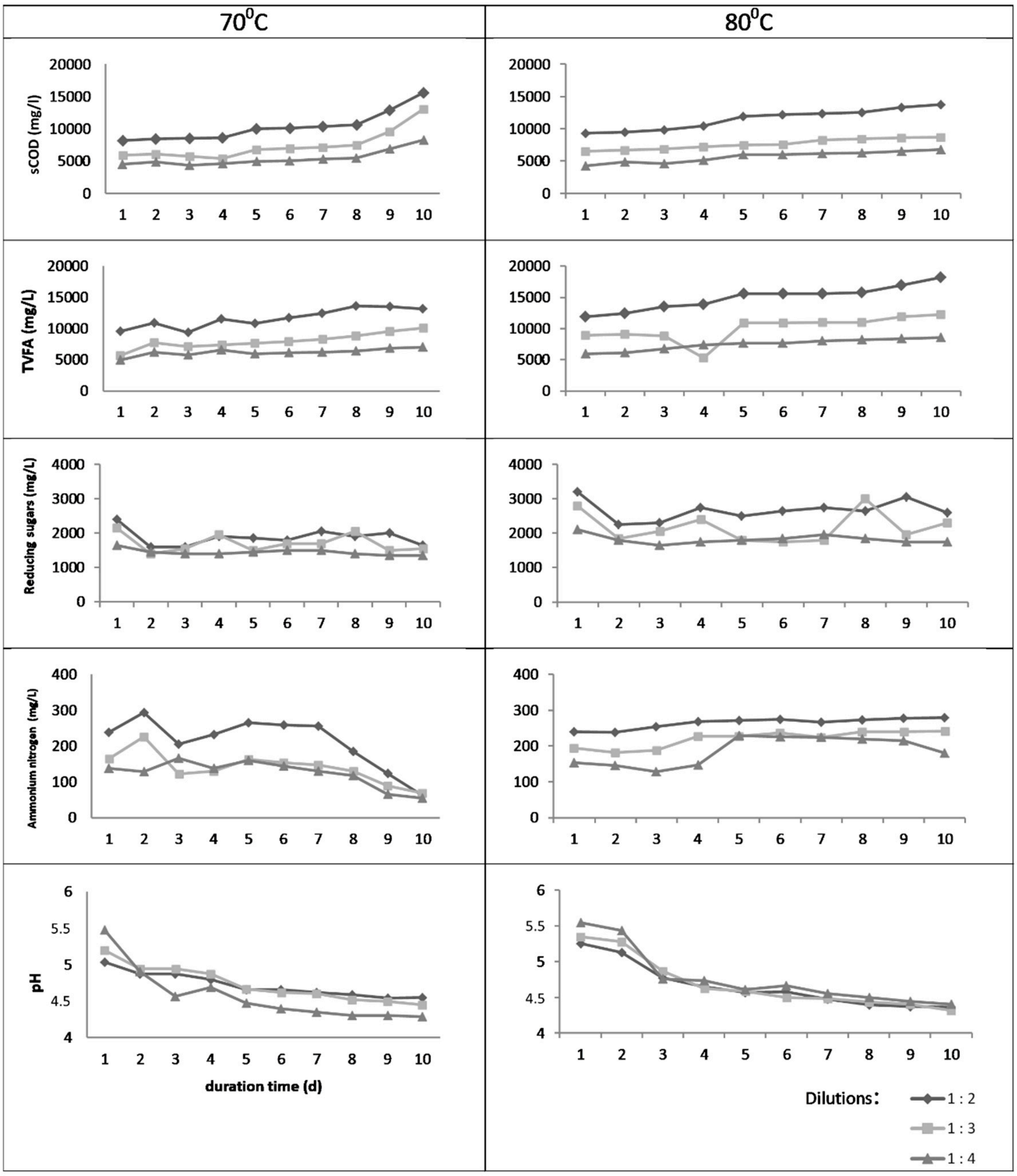

Figure 3. Evolution of sCOD, TVFA, reducing sugars, ammonium nitrogen and pH during hyperthermophilic treatment of leaves.

3.2. Anaerobic Digestion Batch Tests

The anaerobic digestion tests were performed to assess the yields of hydrogen and methane from grass and leaves at various inoculum-to-substrate ratios, and data of these experiments are shown in Table 2 and Figures 4 and 5. The cumulative hydrogen and methane yields were plotted only for the runs performed at 70 °C, because at 80 °C, no hydrogen and only residual methane production were reported. In addition, the experimental runs performed with inoculum alone as well as with grass and leaves only diluted with water did not give any biogas production. As shown in Table 2, leaves yielded much more hydrogen and especially methane than grass, with the maximum productions of 69.64 mLH_2/gVS and 38.63 $mLCH_4/gVS$ reported for the greatest inoculum-to-substrate (I/S) ratio of 2:1. At the same I/S ratio, the hydrogen production from grass reached 50 mLH_2/gVS, whereas the yield of methane was only residual. Methane production observed in the experiments with leaves is surprising because hyperthermophilic conditions effectively inhibit methanogenesis mainly due to a rapid drop in pH [25]. However, the reported pH at I/S ratios of 2:1 and 1:1 was above 7, which could explain this finding. Moreover, high I/S ratio could favor methane production. It is widely known that inoculum provides necessary nutrients and organic substances for microorganisms involved in biogas production as well as buffers volatile fatty acids produced via anaerobic digestion. Greater I/S ratio has been reported to improve methane yields and provide stable digestion performance [28,29]. On the other hand, higher I/S ratio may hamper hydrogen production because organic substances from the inoculum are much more involved in biomass formation rather than H_2 production [30,31]. Yet, in our study, the greatest hydrogen production was observed at I/S ratio of 2:1 and lower inoculum addition to the substrate resulted in deterioration of dark fermentation efficiency. This is especially visible in the experiments with grass, in which hydrogen production drastically dropped at I/S of 1:1 and lower. Moreover, pH of the digestate was generally within the range of 5–7, at which both the activity of hydrogenases and development of microorganisms should be favored [32]. However, decreased hydrogen yields observed at lower I/S ratios, especially for grass, can be linked to the formation of byproducts, which might inhibit hydrogen production, including furfural, levulinic acid or phenolic compounds [2,33]. Regarding the experiments performed at 80 °C, no hydrogen yield and residual methane production were observed for both grass and leaves. It seems that this temperature inactivates most microorganisms. This is in agreement with the findings of [17,34], who reported neither methane nor hydrogen production in a hyperthermophilic reactor operated at 80 °C with various substrates.

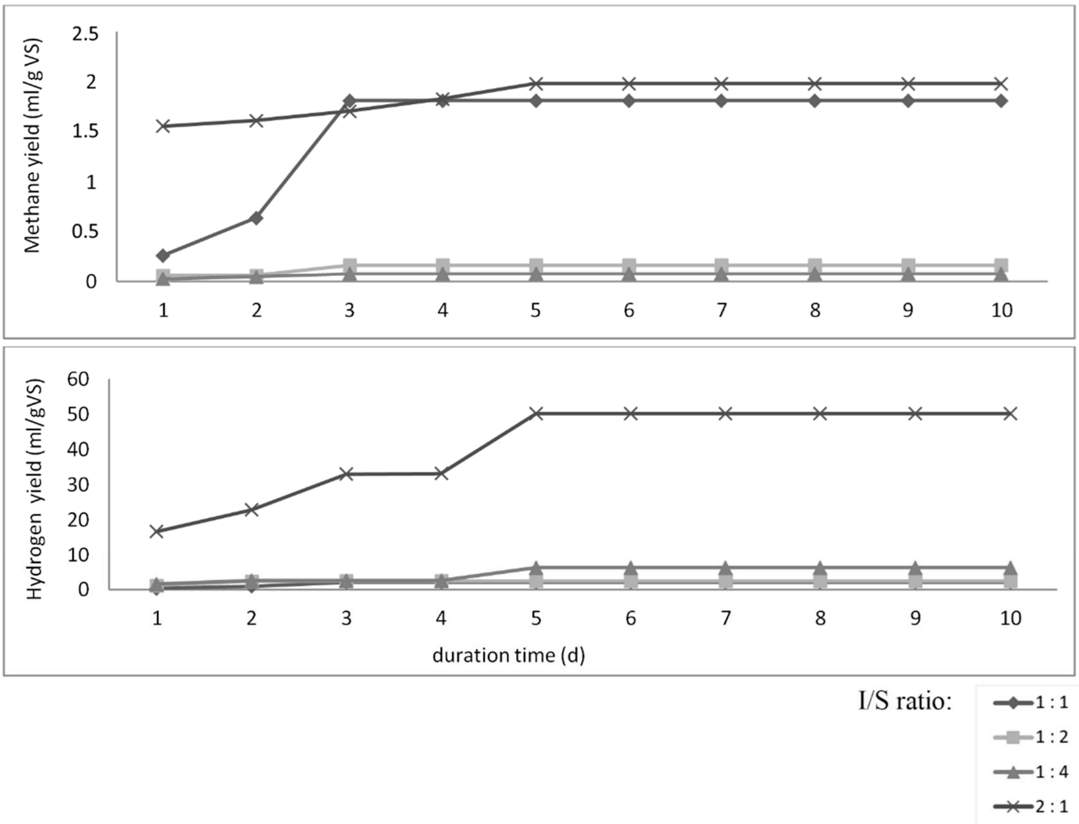

Figure 4. Cumulative methane and hydrogen productions during hyperthermophilic treatment of grass at 70 °C.

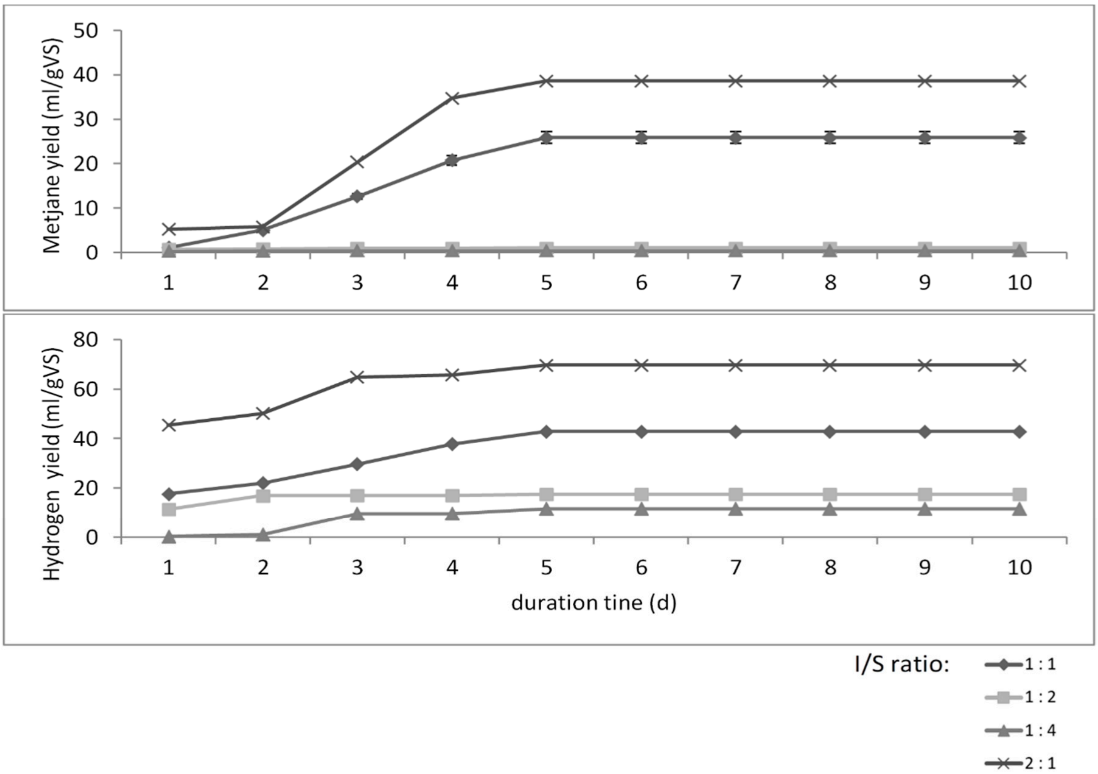

Figure 5. Cumulative hydrogen and methane productions during hyperthermophilic treatment of leaves at 70 °C.

4. Conclusions

This study demonstrated that grass and leaves can be successfully treated via hyperthermophilic digestion to produce significant amounts of hydrogen and the digestate potentially used for further biological processing. The maximum hydrogen production from grass and leaves of 50 and nearly 70 mLH_2/gVS, respectively, were reported at 70 °C, whereas higher temperature strongly inhibited the digestion process. Interestingly, small amounts of methane of up to 38.63 $mLCH_4$/gVS were also yielded from leaves at the temperature of 70 °C. Finally, greater TVFA, reducing sugars and ammonia yielded from grass combined with a faster hydrolysis rate may suggest that pretreated grass will be a better substrate for methane and hydrogen production in the subsequent anaerobic digestion process performed in mesophilic or thermophilic conditions.

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