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Effect of Temperature and Organic Load on the Performance of Anaerobic Bioreactors Treating Grasses

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Abstract: The organic residues generated in grasslands can be treated by adopting anaerobic digestion technology. This technology can enhance the efforts for sustainable waste management around the world. In the northern Netherlands, there is a vast amount of ditch clippings and canal grasses that can be used as a renewable source of energy; however, optimal bioenergy production from grasses is still under research and this study aims to evaluate biogas production from grassy residues at the local level in the context of a sustainable waste management scheme. Batch tests were facilitated to investigate the impact of temperature and organic load on the anaerobic digestion performance of grass mixtures (ditch clippings and canal grasses). The results showed that high temperature favors the degradation of high lignocellulosic materials like grasses. Specifically, bioreactors at 55 °C with an organic load of 30 g volatile solids (VS) L^{-1} reached 360.4 mL g $VS_{substrate}{}^{-1}$. Moreover, reactors with low organic loads resulted in a lower methane yield. The kinetics study also showed good fitting of the predicted and experimental values.

Keywords: anaerobic treatment; methane; grasses; kinetic model

1. Introduction

Sustainable energy production is an urgent necessity due to the depletion of fossil fuels, the continuing increase in the world population, food security, and the augmentation in environmental problems [1–3]. Bioenergy from agricultural and farming waste plays a major role in research efforts [4–7]. Different treatment processes are implemented to treat organic waste, with the anaerobic digestion (AD) technology having among others economic merit in large-scale utilization [8–11]. The biochemical process of AD that converts organic waste into useful products is regarded as another choice for bioenergy production [12–16]. Biogas is an energy carrier and its composition consists of 66% CH_4, 33% CO_2, 0.5% N_2, 0.1% O_2, and 103 mg H_2S (L $biogas^{-1}$) [17]. Based on the application, the biogas may probably undergo post-treatment (upgrading) to reach the natural gas properties [18]. The multiple utilization of biogas for heat and electricity generation or vehicle fuel (upgrade biogas) can support the drive for its application [19–21]. It is also obvious that the AD antagonism will be affected by the use of other energy sources (e.g., wind, nuclear, shale oil/gas) [22–26].

Yet however, AD applicability can be recognized from both socioeconomic and ecological perspectives. Sustainable engineering has paved the way for AD technology to be widely used in the EU and, thus, biogas is a crucial factor for the transition to the bioeconomy [27–30]. The subsidization by the administration of each country has played a key role in the inexorable augmentation in the number of biogas plants around the globe. To date, it is essential to sustainably ameliorate rural areas' life by materials recovery and diminished energy consumption [31,32]. The use of highly

lignocellulosic waste streams may be a restriction for the applicability of AD technology because of their recalcitrance [33–35].

Many researchers agree to establish a competent bioreactor performance correlated to process conditions and microbiome dynamics. Nevertheless, the problems during the operation of the waste-treating bioreactors have made inquiring about the co-digestion process a matter of great concern. The co-digestion process has been formerly pointed out as an alternative to treat two or more substrates [36–38]. The carbon to nitrogen (C:N) ratio (ideal ratio ranges from 20 to 30) is an important player for the efficient simultaneous treatment of alternative substrates. Additionally, previous studies review the importance of temperature and organic load on the biogas production [39]. The farm-scale biogas plant increases across Europe and there is great potential for using grassy wastes to produce biomethane [40]. Ditch clippings and canal grasses can be collected in vast amounts and can be used for energy recovery. Optimal bioenergy production from grasses is still under research. The interest of using grasses as feedstock in biogas plants is due to its high yield of biogas produced. However, the use of grasses for biogas production in Europe still occurs at a moderate level compared to other waste resources [41].

This experimental study aims to appraise the AD performance from grasses mixtures (ditch clippings and canal grasses) under different process conditions. Specifically, the experimental study attempted to determine the influence of temperature and organic load (OL) in the AD efficiency in terms of methane yield. Ditch clippings and canal grasses were chosen as feedstocks for the experimental tests. This report particularly aims to (1) determine the methane yield of the grass mixtures, (2) examine the impact of temperature and organic load on the AD efficiency, and (3) predict methane production using the first order and cone models.

2. Materials and Methods

2.1. Inoculum and Substrates

The inoculum used in this study was obtained from a long-term operating anaerobic digester from the wastewater treatment plant of Garmervolde in Groningen, the Netherlands. The inoculum was stored at 4 °C to maintain freshness and microbial activity. It was reactivated at 37 °C for three days before use. The ditch clippings (DC) and canal grasses (CG) were collected from the suburban area in Groningen city. All substrates were stored in the freezer prior to the digestion tests. The characteristics of the anaerobic inoculum, canal grasses, and ditch clippings are summarized in Table 1.

Table 1. Physico–chemical characteristics of the inoculum and substrates used in the batch tests. DC: ditch clippings; CG: canal grasses.

Parameter	Unit	Inoculum	DC	CG
pH	-	7.23 (0.21)	n.d.	n.d.
VS	g VS/kg biomass	31.9 (1.1)	153.9 (11.5)	244.5 (8.5)
TS	g TS/kg biomass	77.5 (3.6)	165.2 (9.7)	268.7 (9.9)
C	% (based on TS)	n.d.	42.6 (0.1)	43.0 (0.1)
O	% (based on TS)	n.d.	47.1 (0.1)	46.7 (0.2)
H	% (based on TS)	n.d.	6.0 (0.0)	6.1 (0.1)
N	% (based on TS)	n.d.	3.9 (0.1)	4.0 (0.00)
S	% (based on TS)	n.d.	0.5 (0.2)	0.3 (0.1)
Total carbohydrates	% *w*/*w*	n.d.	34.6 (4.5)	51.3 (2.3)
Protein	% *w*/*w*	n.d.	24.3 (0.3)	24.7 (0.0)
Lignin	% *w*/*w*	n.d.	31.9	11.5
Ash	% *w*/*w*	n.d.	9.2 (0.8)	12.5 (1.6)

2.2. Batch Tests

Batch tests were facilitated to determine the influence of temperature and organic load on the methane yield. Glass reactors with a working volume of 400 mL (500 mL total volume) were used in the experimental study. The inoculum-to-substrate ratio was set at two based on the preceding study [42]. Table 2 provides the process conditions applied in the test system. Reactors were filled with inoculum, grass mixture, and distilled water based on a predetermined ratio. All reactors (triplicate) were flushed with N_2 for 5 min, sealed with butyl rubbers, and placed in a rotating incubator (150 rpm).

Table 2. Process conditions applied in the batch tests.

Reactors	Temperature (°C)	Organic Load (g $VS_{substrate}$ L^{-1}	I/S Ratio	Replicates
R1	25	10	2	3
R2	25	20	2	3
R3	25	30	2	3
R4	35	10	2	3
R5	35	20	2	3
R6	35	30	2	3
R7	45	10	2	3
R8	45	20	2	3
R9	45	30	2	3
R10	55	10	2	3
R11	55	20	2	3
R12	55	30	2	3

Blank reactors containing only inoculum were used to correct the methane production of the batch reactors containing the grass mixtures. Methane yields from the control were subtracted from the data obtained in the experiments with DC and CG. Biogas production and methane content were measured daily.

2.3. Analytical Methods

For the determination of total solids (TS), volatile solids (VS), and ash, the standard methods of APHA were applied. A pH meter (HI991001, Hanna Instruments, Woonsocket, RI, USA) was used to determine the pH of the bioreactors at the beginning and end of the experimental tests. The Nordmann titration method was applied to determine the total alkalinity based on a previous study [42]. A micro gas chromatographer (GC) was used to quantify the methane content of the biogas. The gas chromatographer has a single channel 2-stream selector system (Thermo Fisher Scientific Inc, USA) equipped with a chromatographic column (PLOT-U). Helium was used as carrier gas at a total flow of 10 mL min^{-1}. The calibration gas used in GC device consisted of 50% (*v*/*v*) CH_4, 20% (*v*/*v*) CO_2, and 30% (*v*/*v*) N_2. The daily methane volume (mL g VSsubstrate-1 day-1) was determined using the water displacement method.

The protocol described by Hames et al. [43] was used to estimate the protein content based on the nitrogen content (*w*/*w*% DM). According to this protocol, the protein content (*w*/*w*%) for biomass samples is calculated according to the equation:

$$Protein = N_C \times N_F \quad (1)$$

where N_C is the nitrogen content (*w*/*w*%) and N_F the nitrogen factor, which is 6.25 for all types of biomass excluding wheat grains (NF = 5.70). The initial biomass oven-dry-weight corrected for the carbohydrate, ash, and protein contents, was assigned to the lignin content of the samples. Air-dried grass samples were used for elemental analysis with an elemental analyzer (Vario EL/microcube, Elementar, Langenselbode, Germany).

2.4. Kinetics Models

Two models (first-order and cone) were applied for the kinetic study using Microsoft Office Excel (Microsoft Office 2010). The two models determined the hydrolysis rates during the degradation of organic matter and the equations used are [42]:

$$M(\mathrm{t}) = \mathrm{M_O} \times \left(1 - \mathrm{e}^{(-\mathrm{Kt})}\right) \tag{2}$$

$$M(\mathrm{t}) = \frac{\mathrm{M_O}}{1 + (\mathrm{Kt})^{-\mathrm{n}}} \tag{3}$$

where M(t) is the cumulative methane yield at digestion time t days (mL methane g $VS_{substrate}^{-1}$), M_O is the maximum methane potential of the substrate (mL methane g $VS_{substrate}^{-1}$), n is the shape factor, K is the methane production rate constant (d^{-1}), and t is the time (days). To validate the models, the statistical indicators root mean square error (RMSE) and correlation coefficient (R^2) were calculated from the formulas [44]:

$$\mathrm{RMSE} = \left(\frac{1}{\mathrm{n}}\sum_{\mathrm{i}=1}^{\mathrm{n}} \mathrm{d_i}^2\right)^{\frac{1}{2}} \tag{4}$$

where d_i is the deviation between the i^{th} measured and the predicted values and n is the number of data points; and

$$\mathrm{R}^2 = \left[\frac{\mathrm{n}\left(\sum_{\mathrm{i}=1}^{\mathrm{n}} \mathrm{X}_i\mathrm{Y}_i\right) - \left(\sum_{\mathrm{i}=1}^{\mathrm{n}} \mathrm{X_i}\right)\left(\sum_{\mathrm{i}=1}^{\mathrm{n}} \mathrm{Y_i}\right)}{\sqrt{\left[\mathrm{n}\sum_{\mathrm{i}=1}^{\mathrm{n}} \mathrm{X}_i^2 - \left(\sum_{\mathrm{i}=1}^{n} \mathrm{X_i}\right)^2\right]\left[\mathrm{n}\sum_{\mathrm{i}=1}^{\mathrm{n}} \mathrm{Y}_i^2 - \left(\sum_{\mathrm{i}=1}^{\mathrm{n}} \mathrm{Y}_i\right)^2\right]}}\right]^2 \tag{5}$$

where X_i is the i^{th} predicted value.

2.5. Statistical Analysis

Differences in the methane yields obtained in co-digestion of DC and CG at different OLRs and temperatures were assessed by using one-way analysis of variances (ANOVA) in Microsoft Office Excel (Microsoft, USA). Statistical significance of the data was determined using Student's *t*-test with a threshold *p*-value of 0.05.

3. Results and Discussion

3.1. Daily Methane Production

During the anaerobic digestion at 25 °C the individual substrates, R1, R2, and R3 began to produce ≥10 mL g VSsubstrate^{-1} day^{-1} on the 4th, 2nd, and 2nd day, respectively (Figure 1). Rapid methane production began in the reactors at 25 °C even though process speed did not show any clear dependence on the OL. The highest daily biogas production rates for R1, R2, and R3 were 11.1, 12.3, and 12.8 mL g $VS_{substrate}^{-1}$ on 4th, 5th, and 6th day, respectively. Methane production of R2 with 20 g VS L^{-1} (25 days) was terminated faster than those in R1 and R3 (34 and 30 days, respectively). The overall performance was at low ebb due to fast hydrolysis and the subsequent VFA (volatile fatty acids) acidification that inhibits the methanogenic activities. R4, R5, and R6 at mesophilic conditions (35 °C) showed a similar pattern of daily methane production.

Methane remained in the range of 10–15 mL g $VS_{substrate}^{-1}$ day^{-1} for 4, 7, and 7 days, respectively, and afterwards declined to a lower level until the methane production dropped to zero on day 35, 34 and 34, respectively. R4 and R5 reached the maximum daily methane production rate of 13.5 and 14.2 mL g $VS_{substrate}^{-1}$ on days 4 and 5, respectively, whereas R6 with OL of 20 g VS L^{-1} reached 16 mL g $VS_{substrate}^{-1}$ on day 4. In contrast, the maximum daily biogas derived from the reactors (R7→9) at 45 °C was 19.5, 20.8, and 18.6 mL g $VS_{substrate}^{-1}$ on days 4, 6, and 4, respectively.

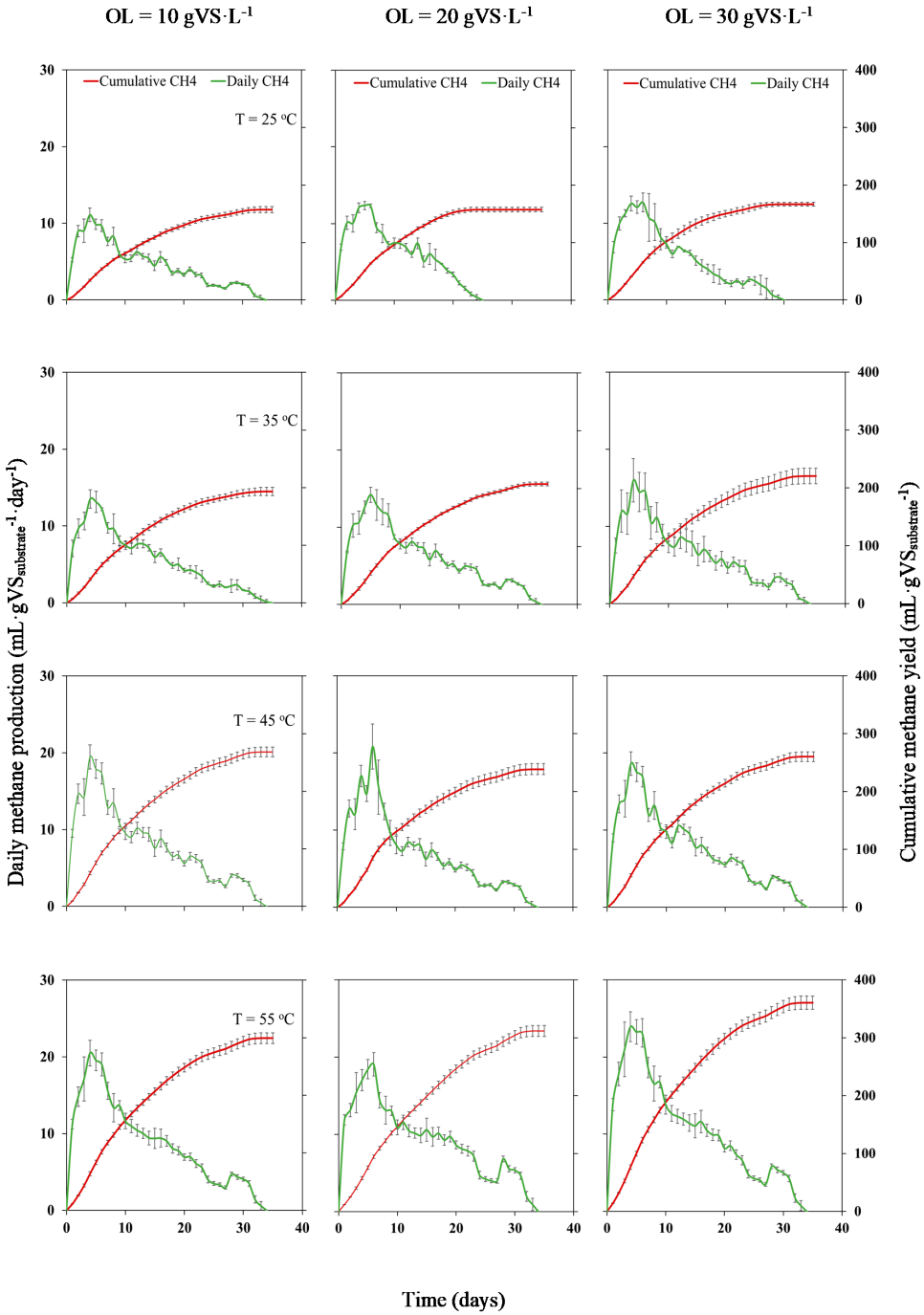

Figure 1. Daily and cumulative biogas production for all the reactors.

Reactors with 30 g VS L^{-1} (R10, R11, and R12) began rapidly to produce a high amount of biogas reaching 20.5, 19.1, and 23.9 mL g $VS_{substrate}^{-1}$ on days 4, 6 and 4, respectively. The methane produced daily remained for the first 15 days in the range of 10–21 mL g $VS_{substrate}^{-1}$ day^{-1} and afterwards gradually declined to a lower level until the biogas production dropped to zero on day 35.

3.2. Cumulative Methane Production

Figure 1 describes the cumulative methane yield for all the reactors. Concerning the digestion at 25 °C, the reactor with OL of 30 g VS L^{-1} reached a methane yield of 166.7 mL g $VS_{substrate}^{-1}$. It was slightly higher (5.7%) than the methane yield produced in treatments with OL of 10 and 20 g VS L^{-1} (157.3 and 157.9 mL g $VS_{substrate}^{-1}$, respectively). Concerning the digestion at 35 °C, methane yield

from the bioreactors was in correspondence with organic load reaching a methane yield of 193.3, 208.2, and 220.4 mL g $VS_{substrate}^{-1}$ for 10, 20, and 30 g VS L^{-1}, respectively (Table 3). Nevertheless, the utilization of grass residues as the sole substrate may increase the VFA concentration due to the high lignocellulose content, and which perturbates the bioreactors' stability. Low methane yields derived from highly lignocellulosic feedstocks have been formerly cited by Chiumenti et al. [45].

Table 3. Results of the kinetic study using the first order and cone model.

Reactor	Measured (mL g $VS_{substrate}^{-1}$)	K (day^{-1})	R^2	RMSE	Predicted (mL g $VS_{substrate}^{-1}$)
R1	157.3	0.0803	0.9989	8.16	154.7
R2	157.9	0.1018	0.9945	10.77	156.7
R3	166.7	0.1116	0.995	9.76	163.6
R4	193.3	0.0817	0.9983	10.62	193.2
R5	208.2	0.0867	0.9962	12.19	198.2
R6	220.4	0.0859	0.9966	11.66	209.5
R7	268.4	0.0900	0.9918	12.26	255.5
R8	238.6	0.1826	0.9951	12.90	228.4
R9	260.5	0.0801	0.9974	13.65	249.2
R10	299.1	0.0815	0.999	15.25	281.8
R11	311.7	0.0694	0.9985	20.43	284.2
R12	360.4	0.0811	0.999	18.34	339.3

Treatments in thermophilic conditions (at 55 °C) showed a higher discrepancy in the methane yields. Treatment with the highest OL (30 g VS L^{-1}) reached a methane yield of 360.4 mL g $VS_{substrate}^{-1}$ approximately 20.4 and 15.6% higher than the methane yield recovered from R10 and R11 (299.1 and 311.7 mL g $VS_{substrate}^{-1}$, respectively). The results from the experiments connoted that thermophilic tests resulted in higher methane yields and specifically R12 was 2.2-, 1.6- and 1.4-fold higher than those reactors (R3, R6, and R9) treating the grass mixture at 25 °C, 35 °C and 45 °C, respectively. A previous study examined the simultaneous digestion of clipped urban grasses collected from abandoned areas with sewage sludge and reported an average methane yield of 150 mL g $VS_{substrate}^{-1}$ [46]. They mentioned that the low methane yield is likely ascribed to the high fiber concentration contained in this type of grass. However, the material was derived from un-maintained areas, which presumably contained relatively high fiber concentrations.

A previous study reported high methane yield (approximately 70%) in the biogas produced from grass in a two-stage AD process [47]. Authors stated that this is due to the chemical composition of grass. Nizami et al. determined the methane yield from grass silage and reported that methane content can vary depending on the AD technique. Specifically, upflow anaerobic sludge blanket reactors resulted in 71% methane content in biogas, whereas, small scale methane potential systems resulted in 52% methane [48]

Additionally, temperature perturbation may influence the process performance and subsequently the biogas production. Ghatak and Mahanta investigated the biogas production in lignocellulosic biomasses (bamboo dust and saw dust) at 35 °C, 45 °C and 55 °C temperatures and they observed that biogas production increases with temperature [49]. However, Ramaraj and Unpaprom examined the biogas production from duckweed at 35 °C and 50 °C and they reported that thermophilic bioreactors produced less biogas and lower methane concentration [50]. Ahn et al. [51] evaluate the dry digestion of switchgrass (Panicum capillare L.) with swine manure resulting in 337 mL g $VS_{substrate}^{-1}$. Lehtomäki et al. [52] also investigated the methane potential of grass in a batch leach bed and reported a range of 141–0.204 mL g $VS_{substrate}^{-1}$. In a previous study, a positive effect on methane yield was observed from the co-digestion of organic wastes in high organic load [53]. An alternative to ameliorate the methane yield is a bioreactor intake with animal slurry, a fact that may influence the digesters' stability. Świątek et al. [54] reported the addition of chicken manure increases the methane yield. Moreover, an extensive determination of a bioreactor's microbiome is recommended as the high heterogeneity of microbial species can insinuate the discrepancies in methane yields derived using different inoculums [55,56].

Industrial and research efforts to overcome the technological constraints for lignocellulosic waste treatment have been previously reported [57]. Nonetheless, there are still issues that have to be

examined like the value of the end-products, different waste sources, or the biology of bioreactors [58–60]. Considering the above results, a techno–economic assessment of the thermophilic anaerobic digestion of grasses in continuous mode would be interesting to evaluate the process in the context of viability and profitability in a pilot or full-scale application [61–63].

3.3. pH, Alkalinity and Volatile Solids Removal

PH variation is given in Figure 2 for all the glass bottles at the starting and ending point. The ending pH values ranged from 6.91 to 7.32, indicating a suitable environment for the decomposition of the feedstocks. Reactors at 25 °C and 35 °C resulted in a final pH lower than the starting pH in comparison to the reactors in higher temperatures. A pH range of 6.8–7.4 is highly favorable for the efficient degradation of the material. Nevertheless, lower pH values are indicated to increase the hydrolysis efficiency due to the enhanced bacteria activity in the pH range of 5.5–6.5.

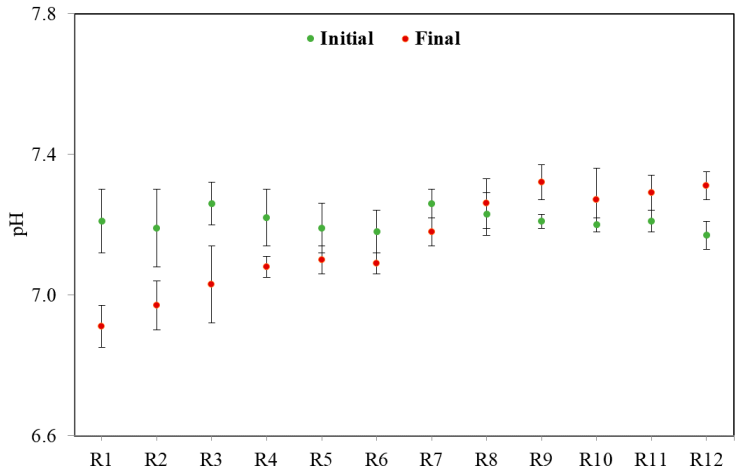

Figure 2. pH for all the reactors.

Microbes are 'nifty' concerning pH tolerance and exposition to low pH may cause process perturbations that can lead the reactor to a 'sour' situation. Low pH languishes the activity and growth of methanogens resulting in a deficit of methane. This is due to the sensitivity of methanogens in acidic environments. Methanogenic species identification is a crucial factor for the stable behavior of anaerobic digesters due to the high sensitivity of methanogens in pH variation. A previous study ascertains that increasing the concentration of VFAs impedes the growth of methanogens, consequently lessening the methane content in biogas.

Alkalinity is also a parameter to assess the stability of the bioreactors. The total alkalinity of all the treatments is displayed shown in Figure 3. The ISR was set to two to provide sufficient alkalinity as it is regarded as an optimal value for the anaerobic digestion of lignocellulosic material. No additional buffers were added in the bioreactors as it was entirely provided by the inoculum. The inoculum being provided to the bioreactors as a nutrients supply is the essential condition for the microbial growth as mentioned by previous studies [64,65]. The preceding study also examined the impact of source-based inoculums in the digestion of lignocellulosic waste [66] and the authors elucidated that the source of the inoculum is critical for the degradation efficiency.

The determination of VS removal is also an indicator used to verify the soundness of the experimental tests and the correspondence of VS removal with the methane produced is expected. They ascertained the significant impact of macronutrients (i.e., nitrogen) on the microbial activity. The functional correlation of cumulative methane and VS reduction is shown is Figure 4. The plot displays the curved regression equation ($y = -0.0002x^2 + 0.1881x + 6.5975$, $R^2 = 0.9567$) and, as expected, methane yield shows an incremental tendency as the VS removal increases.

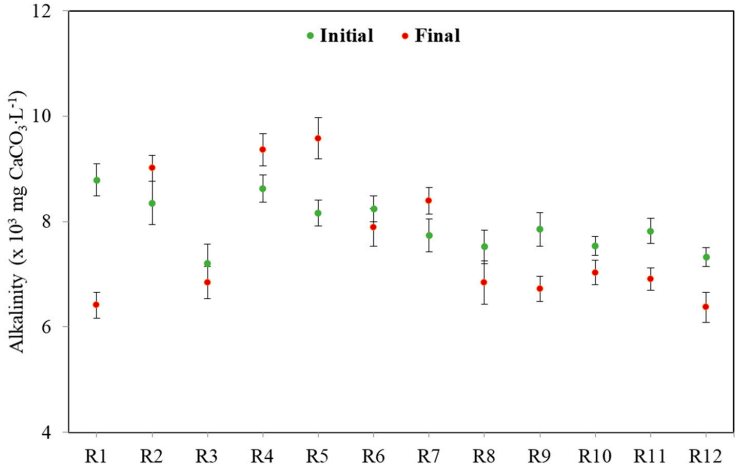

Figure 3. Alkalinity for all the reactors.

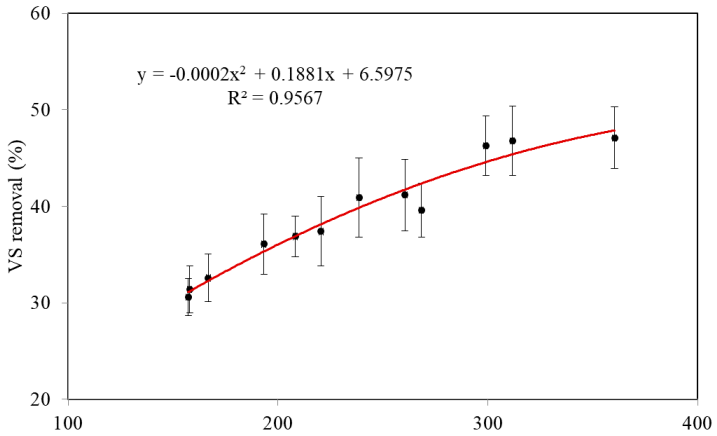

Figure 4. Correlation of biogas produced per g volatile solids (VS) and percentage of VS removal for all the experiments.

3.4. Kinetics Results

The results from the kinetic analysis using the first order and cone models are given in Tables 3 and 4. To appraise the models' soundness, the predicted methane values were plotted against the experimental values. Figure 5 demonstrates the picture of the kinetics indicating a good congruence of the models with the experimental study with a difference of less than 5% for the bioreactors. Lignin and its derivatives were present, and herein lies the observed low hydrolysis efficiency.

Table 4. Results of the kinetic study using the cone model.

Reactor	Measured (mL g $VS_{substrate}^{-1}$)	K (day^{-1})	n	R^2	RMSE	Predicted (mL g $VS_{substrate}^{-1}$)
R1	157.3	0.1112	2.04	0.9783	6.90	148.0
R2	157.9	0.1356	2.38	0.9775	7.22	154.1
R3	166.7	0.1329	2.18	0.9857	5.91	161.0
R4	193.3	0.1123	2.11	0.9805	8.11	183.1
R5	208.2	0.1085	2.10	0.9812	8.63	196.3
R6	220.4	0.1109	2.04	0.9785	9.63	207.3
R7	268.4	0.1113	2.03	0.9783	11.76	252.5
R8	238.6	0.1157	2.03	0.9825	9.37	225.4
R9	260.5	0.1105	2.05	0.9791	11.25	245.2
R10	299.1	0.1109	2.04	0.9792	12.84	281.4
R11	311.7	0.0988	2.10	0.9733	15.44	290.2
R12	360.4	0.1107	2.04	0.9789	15.59	339.0

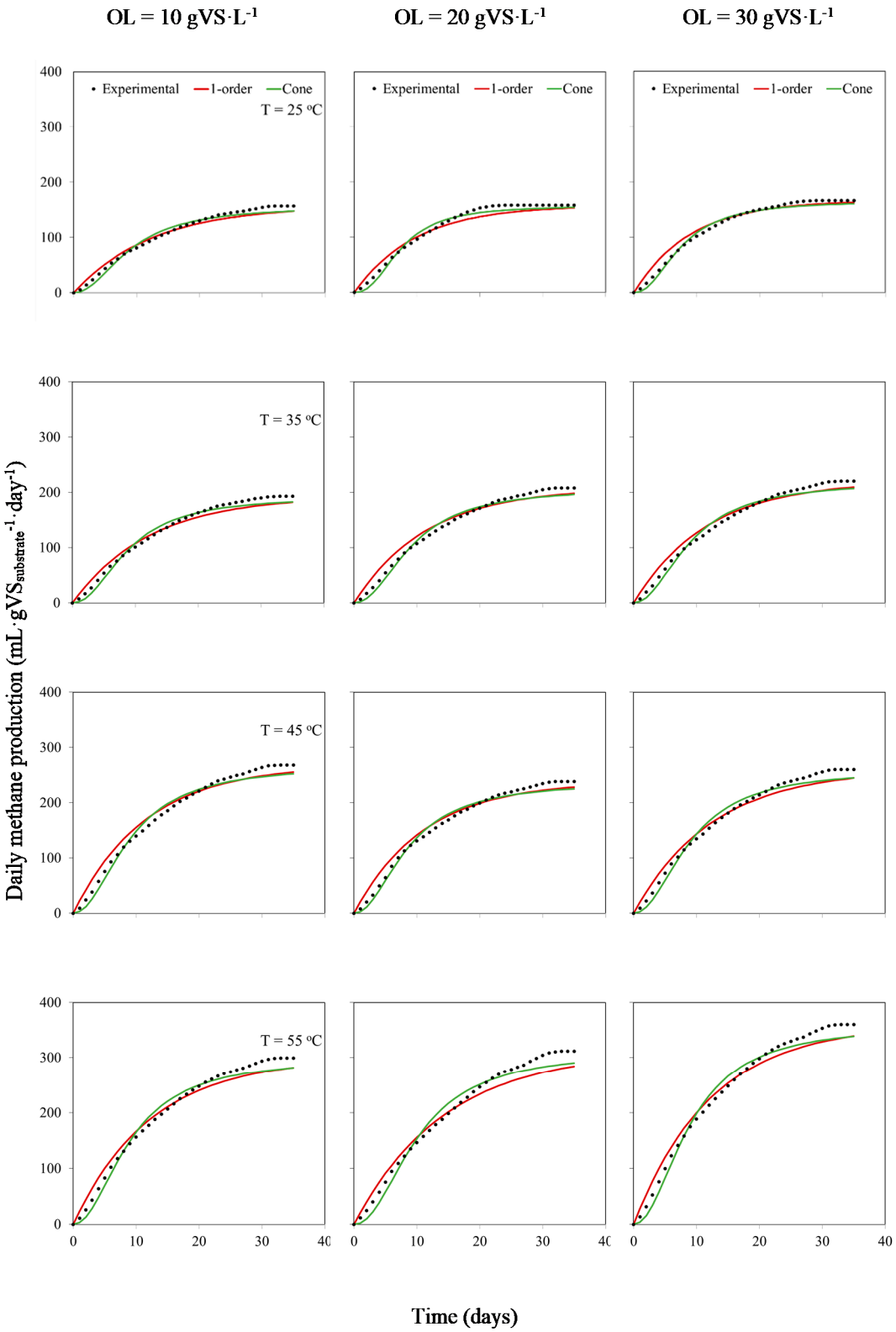

Figure 5. Plot of measured and predicted methane yields for all the reactors.

R8 at 45 °C with 20 g VS L^{-1} showed the highest hydrolysis rate 0.1826 (R2 = 0.9651) using the first-order model. In the cone model, treatments (R2 and R3) at 25 °C with 20 and 30 g VS L^{-1} showed the highest hydrolysis rates 0.1356 (R^2 = 0.9775) and 0.1329 (R^2 = 0.9857), respectively. However, the difference with the other reactors is not significant, indicating that there is no clear independence

between methane yield and hydrolysis rates. Indeed, R12 showed lower hydrolysis rates, but it resulted in the highest methane yield reaching 360.4 mL g $VS_{substrate}^{-1}$.

4. Conclusions

This study examined the effect of organic load and temperature on the methane yield from the anaerobic treatment of ditch clippings and canal grasses. The positive impact of elevated temperature on methane yield was revealed from the experimental tests. Specifically, bioreactors at 55 °C with an organic load of 30 g VS L^{-1} reached 360.4 mL g $VS_{substrate}^{-1}$. Moreover, reactors with low organic loads resulted in lower methane yields. Additionally, the high organic load did not hinder the degradation of the grass, a fact that may overcome the impediments of high-rate digestion. Furthermore, from the kinetics analysis, both models were found to have a good fit with the experimental data. It is concluded that grass residues have the potential for bioenergy and their use as a substrate is recommended for farm-scale biogas production.

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