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Enhanced Biogas Production from Rice Husks and Okra Stalks by Co-digestion with Ostrich Dung and Cow Manure

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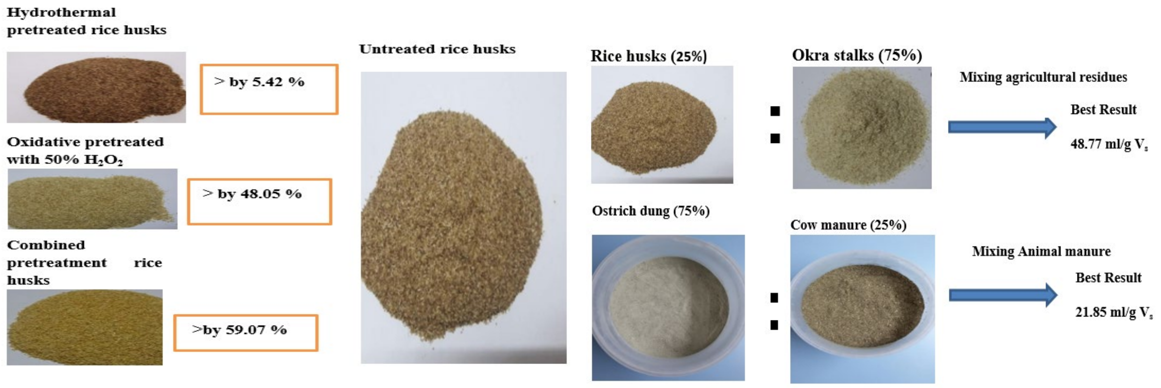

ABSTRACT: In this study, two sides were studied. First one included single and combined pretreatment which were performed to treat milled rice husks compared to increase the production of biogas untreated rice husks. The other side included investigate the effect of two types of mixing: mixing rice husks and okra stalks using three mixing ratios inoculated with ostrich dung compared to rice husks and okra stalks inoculated with ostrich dung separately and mixing ostrich dung and cow manure using three mixing ratios with rice husks compared to using rice husks inoculated with ostrich dung and cow manure separately. The ten reactors, which were carried out in this study, were in batch mode. Single pretreatment included hydrothermal and oxidative pretreatment with 50% (v/v) H_2O_2, while combined pretreatment consisted of both hydrothermal and H_2O_2 pretreatment. The cow manure was used as inoculum in this anaerobic co-digestion process. The results clarified that the increase of biogas productions were by 5.42%, 48.05%, and 59.07% for hydrothermal, H_2O_2, and combined pretreatment of hydrothermal and H_2O_2, respectively. For first mixing, 25:75 ratio (rice husks: okra stalks) was better than other ratios in the production of biogas (48.77 ml/g VS). In the second case of mixing, 75:25 ratio (ostrich dung: cow manure) was better than other ratios in the production of biogas (21.85 ml/g VS). Kinetic study was applied using modified Gompertz model, and there was well agreement between the predicted and measured values of the all pretreatments with correlation coefficient > 0.95. The pretreatment samples of rice husks and the mixing of materials improve the production of biogas and methane.

الملخص: فِي هٰذِهِ الدِراسَةِ تَمَّ طَرْحُ جانِبَيْنِ، الاول هُوَ تَطْبِيقُ المُعالَجَةِ الفَرْدِيَّةِ وَالمَرْكَبَةِ لِمَطْحُونِ قُشُورِ الرُزِّ وَمُقارَنَةُ نَتائِجِ الغازِ الحَيَوِيِّ فِي حالَةِ النَمُوذَجِ غَيْرِ المُعالِجِ . الجانِبُ الثانِي هُوَ دِراسَةُ تَأْثِيرِ المَزْجِ بِحالَتِيَّةٍ، حالَةُ مَزْجِ قُشُورِ الرُزِّ وَسِيقانِ البامِيَةِ مَعَ فَضَلاتِ النَعامِ بِثَلاثِ نِسَبٍ وَمُقارَنَةِ النَتائِجِ مَعَ مَزْجِ قُشُورِ الرُزِّ وَسِيقانِ البامِيَةِ مَعَ فَضَلاتِ النَعامِ بِشَكْلٍ مُنْفَصِلٍ. الحالَةُ الأُخْرَى هِيَ مَزْجُ فَضَلاتِ النَعامِ وَفَضَلاتُ البَقَرِ مَعَ مَسْحُوقِ قُشُورِ الرُزِّ بِثَلاثِ نِسَبٍ وَمُقارَنَةِ نَتائِجِ الغازِ الحَيَوِيِّ مَعَ نَتائِجِ مَزْجِ قُشُورِ الرُزِّ مَعَ فَضَلاتِ النَعامِ وَفَضَلاتِ البَقَرِ بِشَكْلٍ مُنْفَصِلٍ. تَمَّ اِسْتِخْدامُ عَشَرَةِ مُفاعَلاتٍ فِي هٰذِهِ الدِراسَةِ بِاِسْتِخْدامِ تِقْنِيَّةِ التَغْذِيَةِ المُسْبَقَةِ لِلمُفاعِلِ. المُعالَجَةُ الفَرْدِيَّةُ تَضَمَّنَت مُعالَجَةَ مَسْحُوقِ قُشُورِ الرُزِّ بِبُخارِ الماءِ تَحْتَ دَرَجَةِ حَرارَةٍ وَمُعالَجَتِها كِيمْيائِيّاً بِمادَّةِ بِيرُوكْسِيد الهيدروجين المُؤْكْسَدَةِ بِتَرْكِيزِ50% (v/v) وَمُعالَجَةٌ مَرْكَبَةٍ مِن البُخارِ وَبِيرُوكْسِيد الهيدروجين مَعاً. تَمَّ اِسْتِخْدامُ فَضَلاتِ البَقَرِ كَمَصْدَرٍ لِلبكتريا. النَتائِجُ أَوْضَحَت بِأَنَّ الزِيادَةَ فِي إِنْتاجِ الغازِ الحَيَوِيِّ بِالنِسَبِ التالِيَةِ %5.42,%48.05 وَ %59.07 لِلمُعالَجَةِ بِالبُخارِ وَالمُعالَجَةِ الكِيمْيائِيَّةِ وَالمُعالَجَةِ المُرَكَّبَةِ عَلَى التَوالِي. بِالنِسْبَةِ لِلحالَةِ الأُولَى مِن المَزْجِ فَإِنَّ نِسْبَةَ 25:75 هِيَ أَفْضَلُ نِسْبَةٍ فِي تَحْسِينِ كَمِّيَّةِ الغازِ الحَيَوِيِّ المُنْتِجَةِ ,امّا الحالَةُ الثانِيَةُ لِلمَزْجِ فَإِنَّ نِسْبَةَ 75:25 هِيَ الاِفْضَلُ . تَمَّ اِسْتِخْدامُ النَمُوذَجِ الرِياضِيِّ modified Gompertz model وَكانَ هُناكَ تُطابِقُ بَيْنَ النَتائِجِ المُخْتَبَرِيَّةِ وَالنَتائِجِ المُقاسَةِ بِمَعامِلِ اِرْتِباطٍ> 0.95 . نَماذِجُ مَسْحُوقِ قُشُورِ الرُزِّ أَظْهَرَت تَحَسُّناً فِي كَمِّيَّةِ الغازِ الحَيَوِيِّ وَغازِ المِيثانِ المُنْتِجَةِ مُقارَنَةً بِالنَمُوذَجِ غَيْرِ المُعالِجِ, وَكَذٰلِكَ مَزَجَ المَوادَّ أَسْهَمَ فِي تَحْسِينِ الإِنْتاجِ.

Keywords: co-digestion, cow manure, hydrothermal, rice husks, okra stalks, ostrich dung.

الكلمات المفتاحية: الهضم المشترك ، فضلات البقر ، المعالجة بالبخار، قشور الرز، سيقان البامية ، فضلات النعام .

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1. INTRODUCTION

In recent years, climate change and the high request for energy because of the exhaustion of fossil fuels have led to attention toward the production of bioenergy (Ushani et al., 2017). Biogas is considered one of the most effective and efficient options and environmentally friendly for sustainable energy among different alternative sources (Miah *et al.,* 2016). Among different technologies of energy conversion, anaerobic digestion (AD) has high efficiency in the domain of biogas production (Girolamo *et al.,* 2013). In AD, which is included four major stages (hydrolysis, acitogensis, acidogensis, and methanogensis), the organic matter degrades into a simple structure. The composition of biogas generated through the AD process contains methane (CH_4) by 60-65%, carbon dioxide (CO_2) by 30-35%, and 5% of other gases like hydrogen (H_2), nitrogen (N_2), hydrogen sulphide (H_2S) and ammonia (NH3) (Kannah *et al.,* 2017). Co-digestion is a simple approach of AD by mixing wastes together by different ratios, and it is preferred over AD for many advantages, like the adjustment of moisture content (Jassim,2019). Lignocellulosic biomass, for example, energy crops and agricultural residues, is classified as a renewable source of organics. Large amounts of lignocellulosic residues from municipal, forestry, agricultural, and others. These biomasses include majorly three contents: cellulose, hemicellulose, and lignin. (Zheng *et al.,* 2014). Lignocellulosic materials show high resistance to degradation by microorganisms, and untreated lignocellulosic biomass results in low methane yields (Saboor *et al.,* 2017). Thus, it is a very important step to pretreat the lignocellulosic biomass before the anaerobic process. In Iraq, rice is mainly grown under irrigation during the summer season. It is the second most important staple food crop and the third major cereal crop. Rice covers an annual average area of about 48,065 hectograms per hectare, and its production is estimated at about 181,320 tons (FOASTAT, 2016). Since rice husks (RH) are agriculture residues that have limited applications. Therefore, the most used application of rice husks can be in the production of biogas. Okra residues, especially okra stalks (OS), are lignocellulose biomass (Ullah *et al.,* 2018). They are typically disposed of as wastes or burned in the field, causing environmental pollution. In contrast, the cellulose and hemicellulose contents of okra stalks make it a potential feedstock for biomethane production. During hydrothermal pre-treatment, the structure of biomass is penetrated by water, and that leads to expanding the susceptible and attainable surface area of cellulose by taking off hemicellulose and part of lignin (Hesami *et al.,* 2015). Hydrogen peroxide (H_2O_2) is recognized for its characteristics in the removal of lignin and oxidative behaviour. H_2O_2 is strongly oxidizing. In the liquid state, hydroxyl radical (OH●) is produced by H_2O_2, and this radical is more powerful than H_2O_2 itself because of its capability to disrupt the tissues that link hemicellulose and lignin (Hassan *et al.,* 2016). Cow manure (CM) is considered good for AD due to its properties, such as its high buffering capacity, high nutrient content, steady availability, and no toxic particles like heavy metals (Zieliński *et al.,* 2017). For that reason, the co-digestion of cow manure with substrates such as fruit & vegetable waste, food waste, and lignocellulosic biomass enhances biogas production (Saboor *et al.,* 2017). Moreover, ostrich dung (OD) is also used as a bacterium source in AD. In this study, the target was to estimate the influence of single pre-treatment (hydrothermal and oxidant pre-treatment), combined pre-treatment (hydrothermal and 50% H_2O_2-pretreatment), mixing two different types of agro-lignocellulosic wastes (RH and OS with different mixing ratios) with one type of animal manures, and mixing two different types of animal manures (CO and OD with different mixing ratios) with one type of agro-lignocellulosic wastes on the productivity of biogas. The research survey was conducted in Al-Musayib City (Hilla), Iraq, in 2018.

2- MATERIALS AND METHODS

2.1 Collection and Preparation of Feedstock

Fresh RH and OS were collected from rice and okra farms located in the city of AL-Musayib (Babylon, Iraq). The first step was cleaning RH and OS and screened to obtain a size of (0.3-0.6) according to the previous research (Ismail and Noori, 2018). The chemical composition of RH and OS are described in Table 1. in order to get rid of unwanted particulates and sands. The second step includes air drying of RH for three days and OS for two weeks. Then, the samples were grinded

2.1.1 Collection and Preparation of Inoculum

In this study, CM and OD were used as a source of bacteria. Fresh CM and OD were collected from cattle and ostrich farms. They had passed through several steps, starting with drying, crushing, and finally, storage in clean containers until they were used. In order to reduce unwanted gases and to simplify the degradation process of CM and OD, these inoculums were incubated for one week at 37 °C (Saboor *et al.,* 2017; BundÓ *et al.,* 2017). 300 ml of distilled water and 20 g of CM were mixed together, while 35 g of OD were mixed with 300 ml of distilled water to provide the slurry inoculums. The chemical composition of CM and OD are described in Table 1.

2.2 Pre-treatment Methods

In order to increase the ability of lignocellulosic material to degrade during the AD process, three pre-treatments were applied to RH in this study. First, hydrothermal pre-treatment (RH-3) was used to treat 30 g of crushed RH. The process was done in an electrically heated stainless-steel autoclave (Model: DAIHAN LABTECH, Korea). The conditions inside the autoclave, which include temperature, pressure, and pre-treatment time were 121 °C, 15 psi, and 15 min, respectively. Pre-treatment time was selected depending on the recorded study (Alzate *et al.,* 2012), while the selection of temperature and pressure were modified. The pre-treatment was done by mixing 300 ml of distilled water with 30 g of crushed RH in a glass bottle in a volume of 500 ml. Then, this mixture was put inside the autoclave under the above conditions. After that, the biomass was filtered, washed with distilled water many times to obtain pH=7, dried at room temperature, and stored to be used. The procedure was according to the previous works (Passos and Ferrer, 2015; Hesami *et al.,* 2015). The second pre-treatment was the oxidation using 50% H_2O_2 (w/w) (99% purity, provided by BDH-England) (RH-9). The selection of this chemical agent and its optimum concentration level was adopted by the study (Ismail and Noori, 2018). then the 50% H_2O_2 was added to 30 g of the grounded RH. The resulting slurry was air-dried before it was used. Finally, the combined pre-treatment (RH-11), which consisted of hydrothermal pre-treatment followed by 50% H2O2 pre-treatment and was done with the same procedures above.

Table 1.The composition of CM, OD, RH, and OS.

Variables	CM	OD	RH	OS
VS (%)	64.16	32.52	82.08	56.24
TS (%)	93.2	94.4	94.2	92.1
VS/TS	0.69	0.34	0.88	0.61
Lignin content (%)	-	-	20	22
Hemicellulose content (%)	-	-	25	23
Cellulose content (%)	-	-	33	26
Nitrogen content (%)	1.99	2.33	0.52	0.48
Carbon content (%)	37.21	18.86	48.58	32.62
C/N	18.7	8.09	93.42	67.96

2.3 Experimental Setup

Ten batch reactors were carried out to examine the production of biogas. Thermostatic water bath was the place where the reactors were put. The thermostatic water bath was set at 50 °C as cited by Deepanraj et al. (2014). Each reactor was a Pyrex bottle with a volume of 500 ml. Inside each reactor, 300 ml of CM slurry was mixed with 30 g of milled RH and that presented by the ratio 1:10. Selection of ratio was according to the previous study (Ismail and Noori, 2018). Each reactor was plugged by rubber stopper which has two holes. Through these two holes, glass tubes were connected with 5 mm diameter for each to submerge inside the reactor. Rubber tubes were connected from the other end of the glass tubes. Rubber tubes were connected to valves to block the generated biogas from escape, according to previous research (Ismail & Noori., 2018). Purging of Nitrogen was very important step to obtain the anaerobic conditions. It is very important to adjust pH inside each reactor and the required value was 7, and HCl concentrated was used if necessary (Sambusiti et al., 2013). During the AD period, it is very important to shake the reactors to be sure that the substrate and the microorganisms were contacted. Figure 1 represent the experimental set up system, as the same it's described in the experimental work in the study by Jassim and Khalil (2021).

Figure 1. Experimental setup system.

2.4 Analytical Methods

Lignocellulosic properties, total solids (TS), volatile solids (VS), total nitrogen (TN), and total carbon (TC) were analyzed in this study for all the samples of RH and OS. Ismail and Noori (2018) were dependent on calculating TS, VS, TC and TN. According to the reported study (Van Soest et al., 1991), which stated Van Soest's method, the characteristics of lignocellulosic RH were estimated using an extractor of raw fibres (England). The generated biogas was measured daily by the manometer method according to the previous work adopted by Ismail and Noori (2018).

2.5 Statistical Analysis and Kinetic Study of Data

The characteristics of RH were analyzed using (IBM SPSS 24.0.1FP2) depending on the methods described by Ismail and Noori (2018). Microsoft Excel was used in statistical analysis of data. The modified Gompertz model was applied in the present study to estimate the potential production of biogas. This model was described as good empirical non-linear regression to different substrates (Talha *et al.,* 2018).

$$G(t) = G0.\exp\{-\exp[\frac{Rmax.e}{G0}(\lambda - t) + 1]\} \quad (1)$$

where, R_{max} is the maximum production rate of methane, ml/ g.VS; G0 is the potential production of methane, ml/ g.VS; G (t) is cumulated methane yield, ml/g. VS; t is retention time, day; λ is the duration of lag phase, day; and exp is exp (1) = 2.7183 (Talha et al., 2018; Ismail and Noori, 2018).

3- RESULTS AND DISCUSSION

3.1 Impact of Pre-treatments on the Properties of RH

The pre-treatments were often performed so that the lignocellulosic structure decays easily and leads to reduce cellulose crystallinity and enhanced hydrolysis. The results of the composition of lignocellulosic RH are clarified in Table 2.

From these results, it is very noticeable that all the pre-treatments, which were applied to RH, led to changes in the components of this substrate compared with the control sample (RH-1). Cellulose content was at its maximum value in (RH-1). In the case of RH-3 pre-treatment, the parameters like TS, VS, C/N, hemicellulose, and lignin were decreased by 1.59%, 3.29%, 3.2%, 13.04%, and 17%, respectively, in comparing to RH-1. These results were in good acceptance with the reported study (Passos and Ferrer, 2015). In the case of RH-9 pre-treatment, all the properties decreased by 1.06%, 6.075%, 27.25%, 23%, and 22% for TS, VS, C/N, reduction in hemicellulose, and lignin removal, respectively, as compared to the control sample RH-1. The results recorded by Hassan et al. (2016) have in excellent agreement with these results. Finally, the changes caused by RH-11 pre-treatment included a reduction in TS, VS, C/N, lignin, and hemicellulose by 0.11%, 3.03%, 3.2%, 24%, and 27.5%, respectively, compared to RH-1.

3.2 Impact of the Pre-treatments on the Production of Biogas

To increase the probability of enhancement of biogas production, the pre-treatments RH-3, RH-9, and RH-11 were dependent. Fig. 2 and 3, together with Table 3, describe the results of the cumulated production of biogas and methane, respectively.

Table 2. Impact of pre-treatments on the properties of RH.

Variable	Cellulose content (%)	Hemicellulose Content (%)	Lignin content (%)	TS (%)	VS (%)	C/N
RH-1(control)	33	25	20	94.2	82.08	93.42
RH-3	32.8	21.74	16.6	92.7	79.38	90.43
RH-9	32.2	19.25	15.6	93.2	77.1	68.01
RH-11	31.3	19	14.5	94.1	79.59	90.27

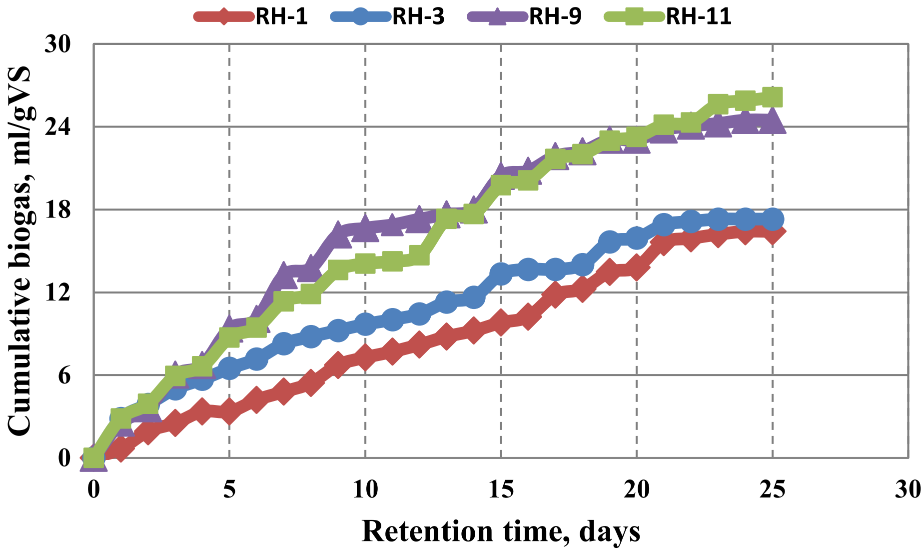

Figure 2. The cumulated production of biogas during the retention time for untreated and pretreated samples of RH inoculated with CM.

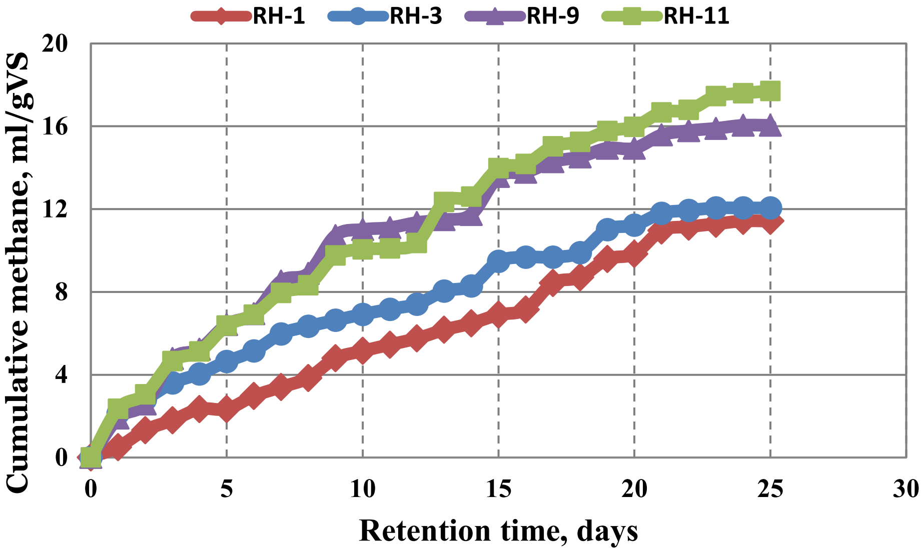

Figure 3. The cumulated production of methane during the retention time for untreated and pretreated samples of RH inoculated with CM

For RH-3 pre-treatment, it is obvious that the rates of biogas production and methane contents were 17.31 ml/gVS (i.e., Biogas increment was equal to 5.42% and 12.06 ml/g VS (methane increment was equal to 5.51%), respectively which were higher than that (16.42 and 11.43 ml/gVS for the production of biogas and methane, respectively) from RH-1. The improvement in RH-3 pre-treatment was because of the fact that during the hydrothermal pre-treatment, the structure of biomass is penetrated by water, and that leads to expanding the susceptible and attainable surface area of cellulose by taking off hemicellulose and part of lignin (Hesami *et al.*, 2015). Many previous studies showed agreement with these results (Girolamo *et al.*, 2013; Li *et al.*, 2015; Passos and Ferrer, 2015). For RH-9 pre-treatment, it is noticeable that the rates of biogas production and methane content were 24.48 ml/gVS (i.e., Biogas increment was equal to 48.05 %) and 16.05 ml/g VS (methane increment was equal to 40.42%), respectively, which were higher than that from RH-1. These results were due to the fact that Hydrogen peroxide (H2O2) is recognized for its characteristics in the removal of lignin and oxidative behaviour.H_2O_2 is strongly oxidizing. In a liquid state, hydroxyl radical (OH•) is produced by H2O2, and this radical is more powerful than H_2O_2 itself because of its capability to disrupt the tissues that link hemicellulose and lignin (Hassan *et al.*, 2016). The results of RH-9 pre-treatments showed clear consistency (Ismail and Noori, 2018). For RH-11 pre-treatment, it is obvious that the rates of biogas production and methane content in biogas were 26.12 ml/gVS (i.e., Biogas increment was equal to 59.07 %) and 17.71 ml/g VS (methane increment was equal to 54.94 %), respectively, which were higher than that from the sample of control RH-1. This increase was due to the fact that the combined pre-treatment was better than the single pre-treatment in the removal of recalcitrant polymers of lignocellulosic biomass. As RH-11 was combined with two pre-treatments which were RH-3 and RH-9, that made the effect of RH-11 was stronger than the effect of each one of them in the improvement of biogas and methane yields. The presented results stated well acceptable with previous work reported by Hassan *et al.* (2016).

Table 3. Impact of different pre-treatments on the cumulated production of biogas and methane.

Sample	Digester NO.	Max. Biogas production (ml/gVS)	Max. Methane yield (ml/gVS)
RH-1	1	16.42	11.43
RH-3	2	17.31	12.06
RH-9	3	24.48	16.05
RH-11	4	26.12	17.71

3.3 Effect of Mixing of Substrates and Inoculums on Biogas Production

As described previously, mixing substrates and mixing inoculums were studied as two different conditions of mixing with three mixing ratios for each one (as stated in Table 4) to examine their effect on biogas production and methane yield.

i) A: 50% RH+50 % OS: inoculated with OD.
ii) B: 75 % RH+ 25 % OS: inoculated with OD.
iii) C: 25% RH+75 % OS: inoculated with OD.
iv) D: RH inoculated with 50% OD+ 50% CM.
v) E: RH inoculated with 75% OD+ 25% CM.
vi) F: RH inoculated with 25% OD+ 75% CM.

3.4 Effect of Mixing Two Different Types of Substrates with One Type of Inoculums

Fig. 4 and 5 summarize the effect of mixing RH and OS with three different mixing ratios inoculated with OD (A, B, and C) on the profiles of cumulative biogas production and methane yield, respectively.

The three mixing ratios, A, B, and C, produced biogas volumes of (33.27, 29.82, and 48.77) ml/gVS, respectively. Moreover, methane yield resulted in (14.37, 12.84, and 21.81) ml/gVS for A, B, and C, respectively. The highest increases in biogas production were observed at C, which was 66.34% and 71.6% compared to RH-1 and OS-1, respectively, when they were separately inoculated with ostrich dung. On the other hand, 17.89% and 121.65% were the biggest improvements in methane yield, which were observed at C, compared to RH-1 and OS-1, respectively. This led to that C > A > B in the enhancement of both biogas production and methane yield compared to the controls RH-1 and OS-1. These results were due to the low values of C/N ratios (74.71) for C as compared to A and B (81.2 and 87.43, respectively, as stated in Table 4), Bagudo *et al.* (2008) proposed that biogas production potentials are inversely proportional to the C/N ratio. In other words, the higher the C/N ratio, the lower the biogas production. According to the C/N ratios of the mixtures, it was found that C < A < B.

Table 4. The characteristics of the mixtures A, B, C, D, E, and F.

Parameter	A	B	C	D	E	F
TS(%)	93.15	93.68	92.63	93.8	94.1	93.5
VS(%)	70	76.88	63.12	48.34	40.43	56.25
VS/TS	0.75	0.82	0.68	0.52	0.430	0.6
Carbon content (%)	40.6	44.59	36.61	28.04	23.45	32.64
Nitrogen content (%)	0.5	0.51	0.49	2.16	2.25	2.05
C/N	81.2	87.43	74.71	12.98	10.42	15.92

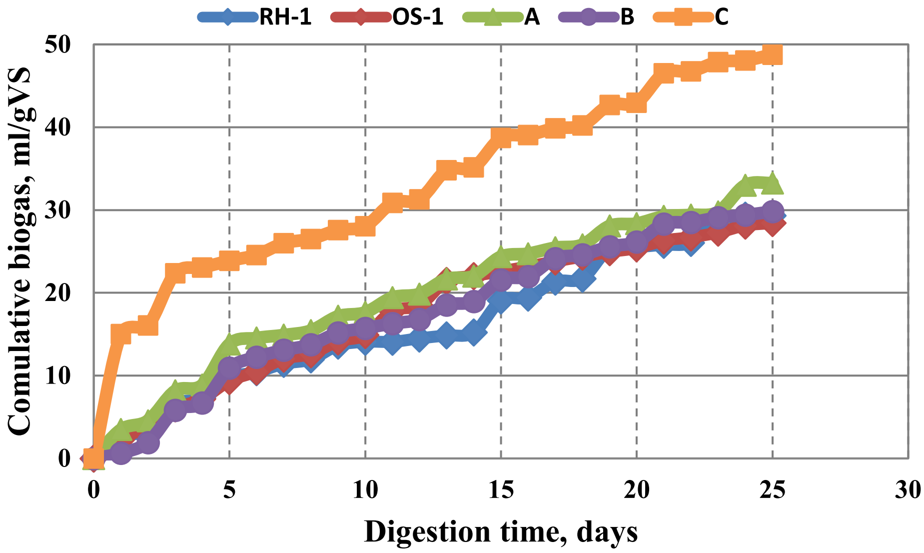

Figure 4. Effect of mixing RH and OS with three different mixing ratios inoculated with OD (A, B, and C) on the biogas production compared to RH-1 and OS-1.

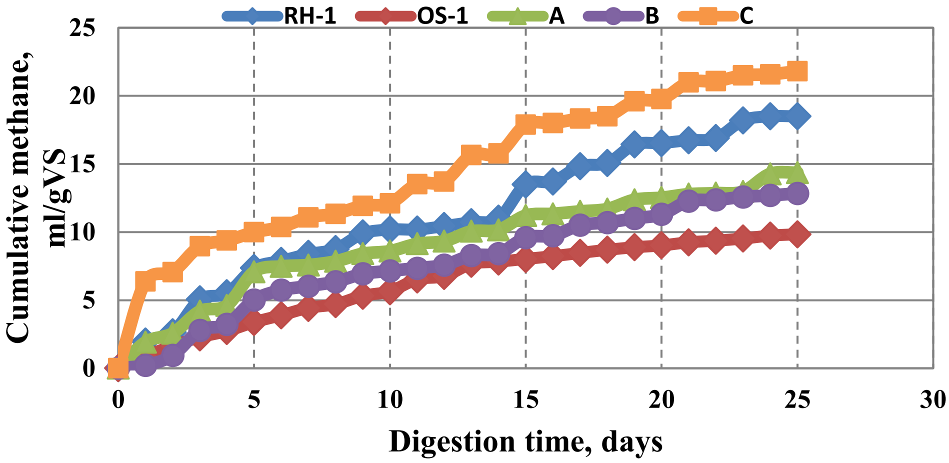

Figure 5. Effect of mixing RH and OS with three different mixing ratios inoculated with OD (A, B, and C) on methane yield compared to RH-1 and OS-1.

3.5 Effect of Mixing Two Different Types of Inoculums with One Type of Substrates

Fig. 6 and 7 summarize the effect of mixing OD and CM with three different mixing ratios co-digested with RH (D, E and F) on the profiles of biogas production and methane yield, respectively.

The three mixing ratios, D, E, and F, produced biogas volumes of (32.86, 35.46, 31.19) ml/gVS, respectively. Moreover, methane yield resulted in (19.61, 21.85, 18.82) ml/gVS for D, E, and F, respectively. The highest increases in biogas production were observed at E, which were 20.94% and 115.96% compared to ostrich dung and cow manure, respectively, when they were separately used to inoculate rice husks. On the other hand, 18.11% and 91.16% were the biggest improvements in methane yield, which were observed at E. This led to that E > D > F in the enhancement of both biogas production and methane yield compared to the control samples. These results were due to the values of C/N ratios where E (C/N=10.42) < D (C/N=12.98) < F (C/N=15.92), as listed in Table 4.

Biogas production potentials are inversely proportional to the C/N ratio, which means the higher the C/N ratio, the lower the biogas production, as reported by Bagudo *et al.* (2008).

3.6 Modified Gompertz Model for Kinetic Study

For all untreated and pretreated samples of RH, a modified Gompertz model was performed to check the correspondence between the predicted and estimated values of methane yield. The results are clarified in Fig. 8 and Table 5. The results showed that the lag phase values (λ) were in the range (0.68-1.67) day. The compatibility between the predicted and estimated values was > 0.95. Thus there was an excellent fit between the predicted and estimated values of methane yield.

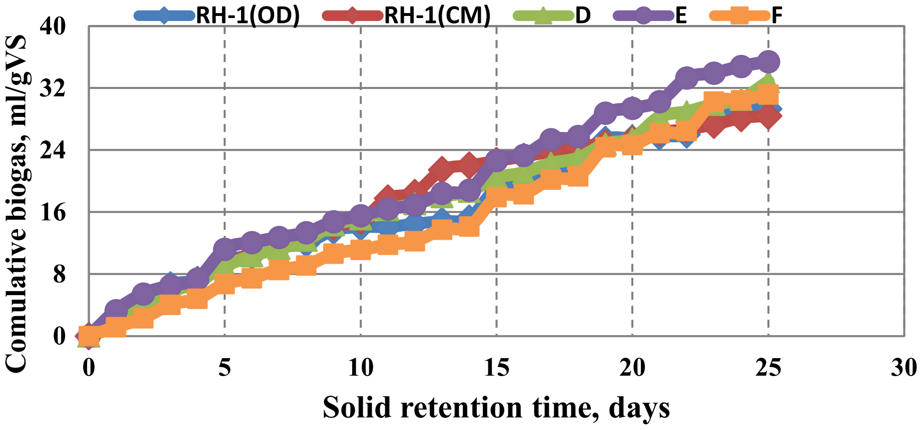

Figure 6. Effect of mixing OD and CM with three different mixing ratios co-digested with RH (D, E, and F) on the biogas production compared to RH-1OD and RH-1CM.

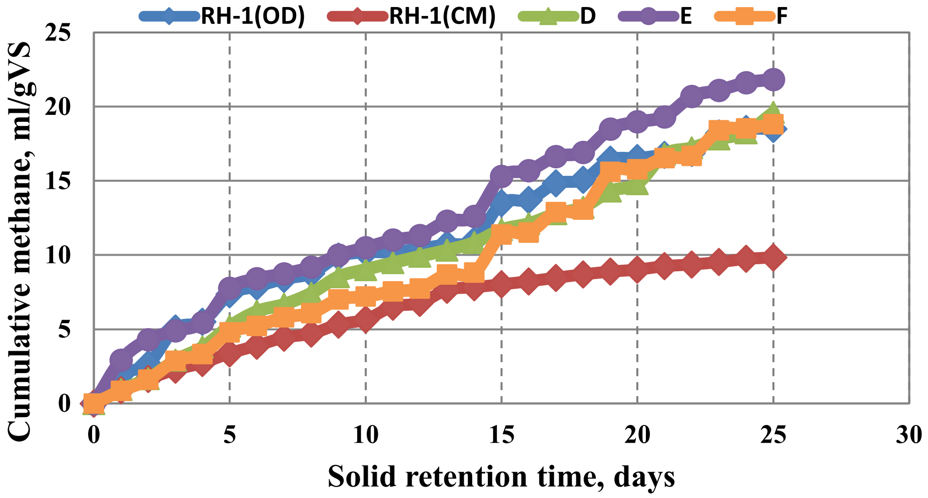

Figure 7. Effect of mixing OD and CM with three different mixing ratios co-digested with RH (D, E, and F) on methane yield compared to RH-1OD and RH-1CM.

Table 5. Results from performing modified Gompertz model.

Digester No.	Symbol	G(t) exp. (ml methane/g VS)	Gompertz model parameters				R2
			λ (day)	Rmax. (ml methane/g VS)	G0 (ml methane/g VS)	G(t) model (ml methane/g VS)	
1	RH-1CM	11..43	1.67	0.62	11.43	10.47	0.966
2	RH-3	12.06	1.62	0.65	12.06	11.02	0.967
3	RH-9	16.05	0.72	1.04	16.05	15.46	0.984
4	RH-11	17.71	0.68	1.00	17.71	16.57	0.982
5	RH-1OD	18.5	1.25	0.93	18.5	16.63	0.96
6	OS-1	9.84	0.02	0.63	9.84	9.49	0.99
7	A	14.37	2.28	0.74	14.37	12.82	0.96
8	B	12.84	0.26	0.72	12.84	12.07	0.968
9	C	21.81	3.44	1.07	21.81	19.52	0.95
10	D	19.61	0.21	.0.89	19.61	17.22	0.97
11	E	19.61	0.21	.0.89	19.61	17.22	0.97
12	F	18.82	1.72	0.93	18.82	16.72	0.96

4- CONCLUSION

Hydrothermal pre-treatment, oxidative pre-treatment with 50% H_2O_2, and hydrothermal pre-treatment assisted with 50% H_2O_2 pre-treatment were used in this study to increase the production of biogas and methane. In comparison to the untreated RH, the combined pre-treatment was the highest in the productivity of biogas and methane by 26.12 and 17.71 ml/g. VS, respectively, with the enhancement of 59.07% and 54.94%. Thus, this combined pre-treatment was better than each one of the sole pre-treatments. Moreover, it was proved that the 25:75 ratio (rice husks: okra stalks) was better than other ratios in the production of biogas (48.77 ml/g VS), and the 75:25 ratio (ostrich dung: cow manure) was better than other ratios in the production of biogas (21.85 ml/g VS).

AUTHOR CONTRIBUTIONS

H. Jassim (M.Sc Student, Department of Environmental) performed the literature review, and experimental design, analyzed and interpreted the data, and prepared the manuscript text and manuscript edition. A. Khalil (supervisor) compiled the manuscript preparation.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding this article.

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REFERENCES

Alzate, M. E., Muñoz, R., Rogalla, F., Fdz-Polanco, F., & Pérez-Elvira, S. I. (2012). Biochemical Methane Potential of Microalgae: Influence of Substrate to Inoculum Ratio, Biomass Concentration and Pre-treatment. Bioresource Technology, 123, 488-494.

Baguio, B. U., Garba, B., Dangoggo, S. M., & Hassan, L. G. (2008). Comparative Study of Biogas Production from Locally Soured Substrate Materials. International Journal of Agricultural and Biological Engineering, 16.(2)

Bundó, M. S., Eskicioglu, M. G., Carrère, H., & Ferrer, I. (2017). Anaerobic Co-Digestion of Microalgal Biomass and Wheat Straw with and without Thermo Alkaline Pre-treatment. Bioresource Technology. Retrieved from https://hal.archives-ouvertes.fr/hal-01605999/document

Deepanraj, B., Velmurugan, S., Jayaraj., Simon. (2014, IVth), Enhancement of biogas production by pretreatment. International Conference on Advance in Energy Research Indian Institue of Technology,Bombay, India.

FAOSTAT. (2016). Food and Agriculture Organization Statistics. Retrieved from http://www.fao.org/faostat/en/#data/QC/Crops.

Girolamo, G. D., Grigatti, M., & Angelidaki, I. (2013). Effects Of Hydrothermal Pre-Treatment on Giant Reed (Arundo Donax) Methane Yield. Bioresource Technology, 147, 152-159.

Hassan, M., Ding, W., Bi, J., Mehryar, E., Talha, Z. A. A., & Huang, H. (2016). Methane Enhancement through Oxidative Cleavage and Alkali Solubilization Pre-treatments for Corn Stover with Anaerobic Activated Sludge. Bioresource Technology, 200, 405-412.

Hesami, S. A., Zilouei, H., Karimi, K., & Asadinezhad, A. (2015). Enhanced Biogas Production from Sunflower Stalks Using Hydrothermal and Organsolv Pre-treatment. Industrial Crops and Products, 76, 449-455.

Ismail, Z. Z., & Noori, N. A. (2018). Anaerobic Co-Digestion of Giant Reed for Biogas Recovery. Engineering Journal, 24(3), 68-83.

Jassim, H. (2019). Enhanced Biogas production from Rice Husks and Okra Stalks by Co-Digestion with Ostrich Dung and Cow Manure. (Master's Thesis). University of Babylon, Iraq.

Jassim, H., & Khalil, A. H. (2021). Improving Biogas Production Using Different Pre-treatment of Rice Husk Inoculated with Ostrich Dung. The Journal of Engineering Research, 18(1), 1-11.

Kannah, R. U., Kavitha, S., & Banu, J. R. (2017). BMP a Tool for Assessing Energy Efficient Biogas Production. Advances in Biotechnology & Microbiology, 5.(2)

Li, C., Zhang,G., Zhang,Z., Ma,D., Wang,L.,Xu,G.(2015).Hydrothermal pretreatment for biogas production from anaerobic digestion of antibiotic mycelial residue. Chemical Engineering Journal 279,530-537.

Miah, M. R., Md, A. K., Rahman, L., Akanda, M. R., Pulak, A., & Rouf, M. A. (2016). Production of Biogas from Poultry Litter Mixed with the Co-Substrate Cow Dung. Journal of Taibah University Science, 10, 497-504.

Passos, F., & Ferrer, L. (2015). Influence of Hydrothermal Pre-treatment on Microalgal Biomass Anaerobic Digestion and Bioenergy Production. Water Research, 68, 364-373

Saboor, A., Khan, S., Shah, A. A., Hasan, F., Khan, Haji, & Badshah, M. (2017). Enhancement of Biomethane Production from Cattle Manure with Codigestion of Dilute Acid Pretreated Lignocellulose. International Journal of Green Energy, 14, 632-637.

Sambusiti, C., Monlau,F., Ficara,E., Carrere,H., Malpei,F.(2013). A comparison of different pretreatments to increase methane production from two agricultural substrates. Applied Energy 104, 62-70.

Talha, Z., Hamid, A., Guo, D., Hassan, M., Mehryar, E., Okinda1, C., & Ding, W. (2018). Ultrasound Assisted Alkaline Pre-treatment of Sugarcane Filter Mud for Performance Enhancement in Biogas Production. International Journal of Agricultural and Biological Engineering, 11(1), 226-231.

Ullah,S., Kilpeainen,P.,Ilvesniemi,H.,Pakkanen,H., Alen,R. (2018).Chemival characterization of Okra Stalk (Abelmoschus Esculentus)as a potential raw material for biorefiery utilization. Cellulose Chemistry and Technology 4(6), 657-659.

Ushani, U., Banu, J. R., Tamilarasan, K., Kavitha, S., & Yeom, I. T. (2017). Surfactant Coupled Sonic Pre-treatment of Waste Activated Sludge for Energetically Positive Biogas Generation. Bioresource Technology, 241, 710-719.

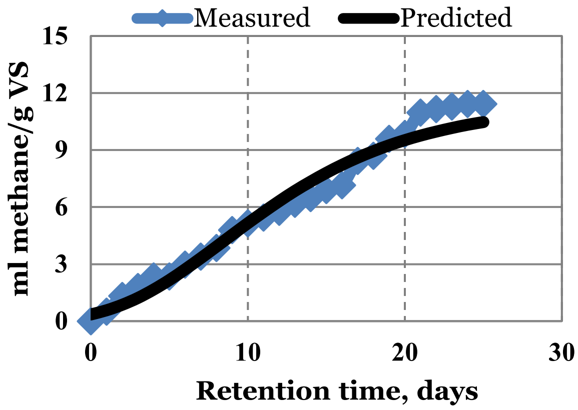
Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Symposium: Carbohydrate Methodology, Metabolism, and Nutritional Implications in Dairy Cattle. Journal of Dairy Science, 74.(10)

Zheng, Y., Zhao, J., Xu, F., & Li, Y. (2014). Pre-treatment of Lignocellulosic Biomass for Enhanced Biogas Production. Progress in

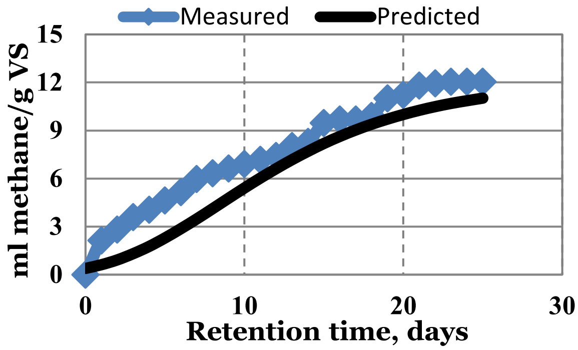
Energy and Combustion Science, 42, 35–53.

Zieliński, M., Dębowski, M., Kisielewska, M., Nowicka, A., Rokicka, M., & Szwarc, M. (2017). Comparison of Ultrasonic and Hydrothermal Cavitation Pre-treatments of Cattle Manure Mixed with Straw Wheat on Fermentative Biogas Production. Waste Biomass Valorization. Retrieved from https://link.springer.com/article/10.1007/s12649-017-9977.

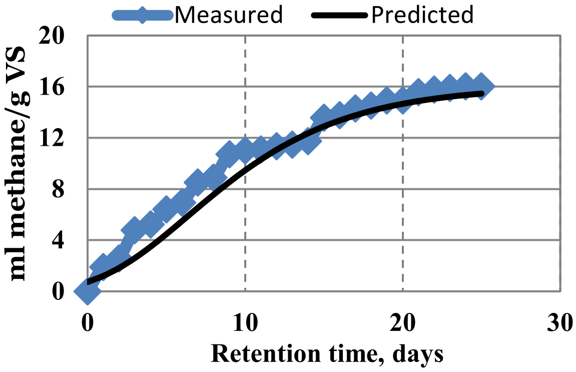
APPENDIX 1



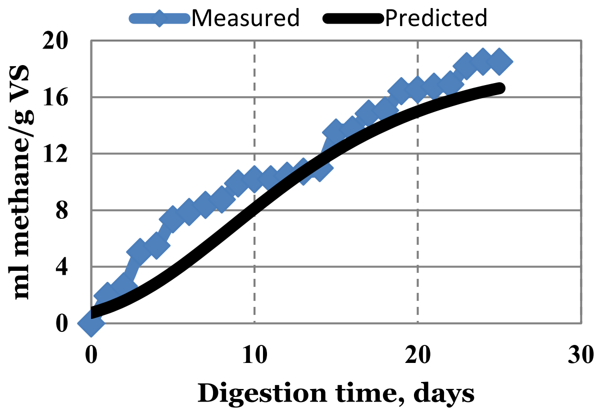
a: predicted and measured results for RH-1 Cow pretreatment (R2 = 0.966).



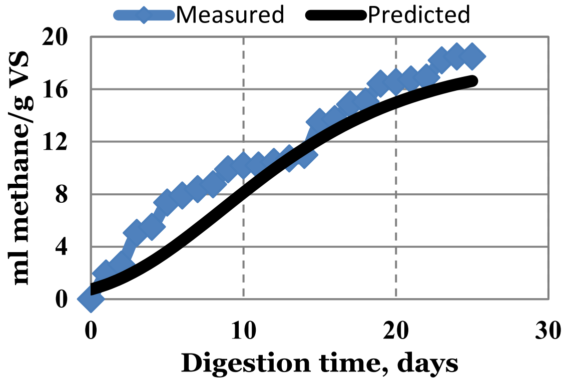
b: predicted and measured results for RH-3 pretreatment (R2 = 0.967).



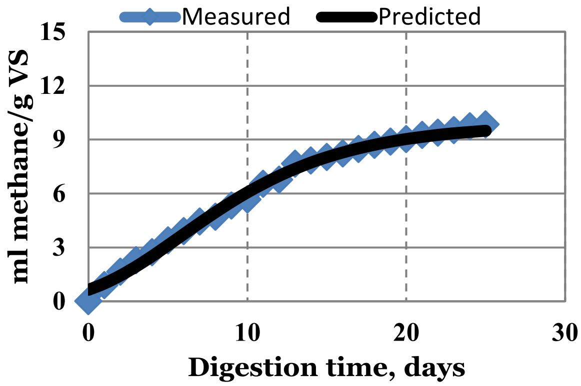
c: predicted and measured results for RH-9 pretreatment (R2 = 0.984).



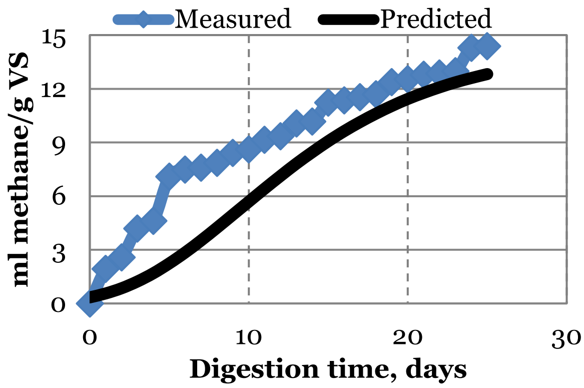
d: predicted and measured results for RH-11 pretreatment (R2 = 0.982).



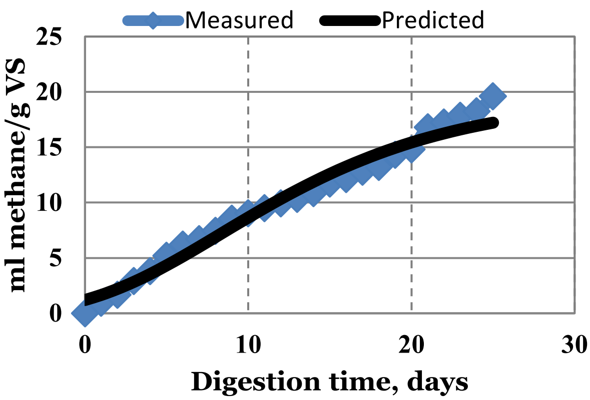
e: predicted and measured results for RH-1Ostrich pretreatment (R2 = 0.959).



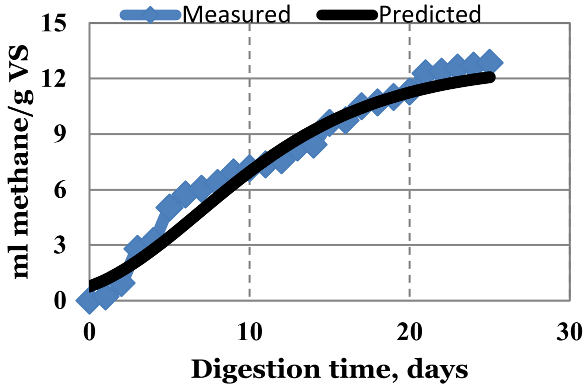
f: predicted and measured results for OS-1 (R2 =0.994).



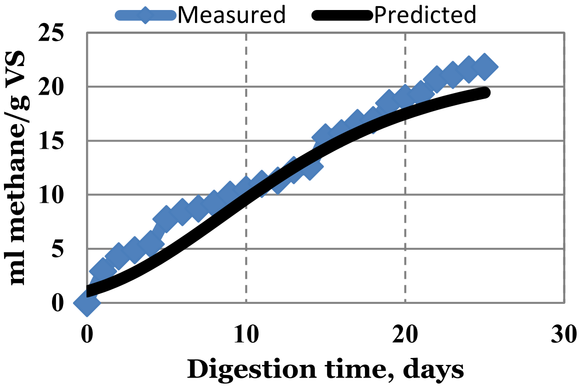
g: predicted and measured results for A (R2=0.963).



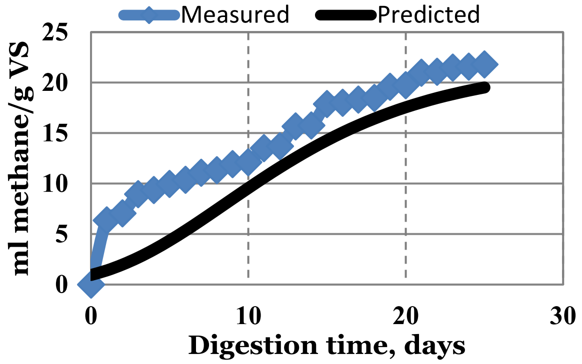
j: predicted and measured results for D (R2=0.970).



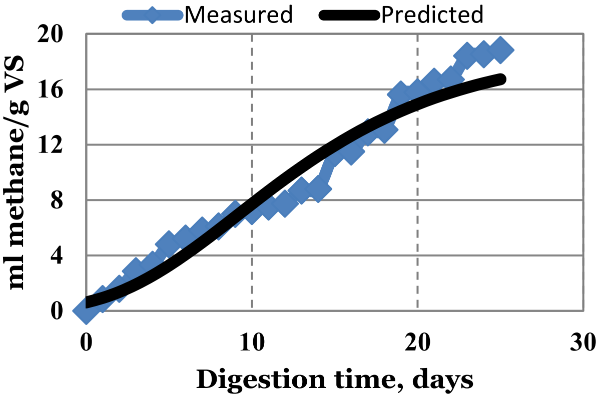
h: predicted and measured results for B (R2=0.968).



k: predicted and measured results for E (R2=0.962).



i: predicted and measured results for C (R2=0.950).



l: predicted and measured results for F (R2=0.956).

Figure 8. The predicted and measured results for methane yield to all the untreated, pretreated, and mixed samples.