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ENHANCED BIOGAS GENERATION FROM LIGNOCELLULOSIC BIOMASS WITH THE IMPLEMENTATION OF A CHEMICAL PRE-TREATMENT PROCEDURE

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ABSTRACT

The present study evaluated the impact of chemical pre-treatment on biogas and methane generation from corn and wheat stalks. Three types of animal manure were used as a source of bacteria (cow, cattle and ostrich manure). Chemical treatment using sodium hydroxide led to an increase in biogas and methane generation from wheat and corn stalks, where the increase for wheat stalks pretreated with cow, cattle and ostrich manure was (468.28; 469.08%), (242.51; 230.39%), (317.90; 295.77%) respectively compared to the untreated sample. As for corn stalks, the increase for biogas and methane with the same wastes above was (345.28; 444.59%), (230.90; 291.20%), (277.99; 297.011%) respectively compared to the untreated sample. With a correlation coefficient of more than 96, the findings also demonstrated that the values that were measured and the values that were derived by using the modified Gompertz model were in accord with one another.

KEYWORDS

Animal waste; biogas generation; Corn stalks; Wheat stalks; Chemical Pretreatment.

1. INTRODUCTION

Discourse, particularly regarding the diversification and growth of the bioenergy matrix. The necessity for biofuels is becoming increasingly evident as be replacement for big scale utilization of oil reserves. Biogas constitutes a renewable energy source derived from the anaerobic digestion of various biomasses, as reported by Venturin et al. (2018). Biomass constitutes a significant alternative energy source and offers a means to mitigate environmental issues, including pollution and the exhaustion of natural resource, as mentioned by Koryś et al. (2019). Biomass primarily denotes any organic material originating from the agricultural or forestry sectors that is consistently available (sustainable), as cited by Ahmed et al. (2012). Anaerobic digestion is a longstanding and well-established biological mechanism initially employed to handle a diverse array of substrates. In the absence of oxygen microbial organisms facilitate anaerobic digestion, a process that involves the decomposition and stabilization of organic matters, resulting in the production of biogas, primarily composed of CO_2 (25-45%) and CH_4 (55-75%). The ecology of anaerobic digestion is complex, involving multiple bacterial species and as many as nine stages of organic matter conversion. Nonetheless, four primary stages may be identified: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Sambusiti, 2012). Wheat straw is a type of lignocellulosic biomass derived from agriculture. Wheat straw can be transformed into bioenergy via multiple biological processes, as documented by Dongyan et al. (2014). Corn generates a substantial quantity of wastes, sometimes referred to as stover, primarily consisting of stalks, leaves, cobs, and husks. The corn stalk is a plentiful, renewable, cost-effective, and broadly available resource, containing a high cellulose content of 35–50%, as reported by Menardo et al. (2015). The usage of corn stalks for biogas production helps mitigate the environmental issues associated with their incineration, as cited by Dong et al. (2018). Pretreatment plays a crucial role in altering the complex chemical structure of many lignocellulosic materials to enhance the subsequent hydrolysis process, resulting in increased digestibility of the treated material, as reported by Venturin et al. (2018). Chemical pre-treatment involves the application of chemicals, including acids, bases, and ionic liquids, to change the chemical and physical properties of lignocellulosic biomass. Alkaline pre-treatment employs bases, including NaOH, $Ca(OH)_2$, KOH, and $NH_3.H_2O$, to extract lignin, hemicellulose, and/or cellulose, supplying biomass of the lignocellulosic more degradable to microbes and enzymes. Sodium hydroxide is the predominant base employed in alkaline pretreatment and has been thoroughly investigated to enhance biogas production from lignocellulosic biomass (Zheng et al., 2014). Current study aims to investigate the potential of enhancing the production of biogas and methane yield through chemical pretreatment of wheat stalks and corn stalks as a lignocellulosic source co-digested with four types of manure.

2. MATERIALS AND METHODS

2.1. Substrate and Inoculum

In current study, two types of substrates were used: wheat and corn stalks, which were collected from a local farm in Hillah city (Babil, Iraq). These wastes underwent several processes before use, where they were cleaned to get rid of dust and unwanted particles, air-dried, and Subsequently, it was milled using a grinder and passed through a 1 mm sieve, conforming to Hassan et al. (2016). Chemical properties of the wheat stalks and corn stalks shown in Table 1. Three types of animal manures were used as a source of bacteria in this study: cow, cattle, and ostrich manure. Before use, they were air-dried, cleaned of coarse fibers and then ground. To increase the decomposition, they were placed in an incubator at 37°C for one week, as per the procedure implemented by Saboor et al. (2017). To create a slurry, it is subsequently introduced into the digesters as a supplementary material to enhance bacterial activity and optimize the anaerobic bio-digestion process, according adopted by Ismail and Talib (2014). A clarification of the chemical characteristics of cow manure, cattle manure, ostrich dung and is provided in Table 1 and samples of air dried and milled of substrates are showed in Fig.1.

Fig. 1. Samples of air dried and milled: (a) corn stalks and (b) wheat stalks.

Table 1. Chemical properties of the substrate and inoculums.

Variables	Wheat stalks	Corn stalks	Cow manure	Cattle manure	Ostrich dung
TS(%)	93.02	92.46	92.5	91.3	97.5
VS(%)	85.74	89.08	78.08	76.23	37.43
VS/TS	0.92	0.96	0.84	0.83	0.38
Carbon content (%)	49.72	51.66	45.28	44.21	21.70
Nitrogen content (%)	0.91	1.3	2	0.75	2.40
C/N	54.63	39.73	22.8	58.95	9.04

2.2. Chemical Pretreatment

A pre-treatment process is required to weaken the lignocellulosic structure, increase the surface area available for enzyme attack in order to improve the biodegradability and remove the lignin, as reported by Dell'Omo and Spena, (2020). Alkaline Pretreatment with NaOH was applied. A 4% (w/v) solution was prepared by dissolving 8 g of alkaline (NaOH) granules in 200 ml of distilled water. Subsequently, the prepared solution was introduced to 30 g of milled substrate, and the mixture was continuously stirred by hand using a glass rode. the resulting slurry was heated to 105 degrees Celsius in an oven according to the procedure outlined by Noori (2017). Then, the pretreated substrate was repeatedly washed with distilled water to neutralize the pH before it entered the next stage, as the procedure adopted by Wicaksono et al. (2017).

2.3. Experimental Setup for Biogas Generation System

In a sequential system, three digesters were executed. The digesters contained wheat stalks and corn stalks in the untreated (WS-1) and (CS-1) cases, as well as in two pretreatment cases: WS-4 and CS-4 (alkaline Pretreatment with NaOH). The experimental setup system, depicted in Fig. 2, is identical to the arrangement of the lab-scale system as described in the experimental work of Jassim and Khalil (2021) with a few modifications to the dimensions. The digester is primarily composed of 500 ml Pyrex borosilicate glass cylinders that are heat-resistant and have been prepared for anaerobic digestion. The contents were preserved in a 1:10 ratio in each digestion, according to previous research Ismail and Talib (2014). This percentage is equivalent to 30 grams of solid debris, which is equivalent to 300 ml of inoculum slurry. The rubber stoppers with two openings for purging nitrogen gas to the digester and gas exit is the other hole of the digester was connected to a rubber tube that was connected to a valve to inhibit the release of the generated gas, in accordance with relevant prior research conducted by Pax et al. (2020). Ultimately, the pH of the digesting solution was calibrated at 6.8–7.2 to establish an appropriate anaerobic environment, and any residual air was eliminated using nitrogen purging. Extra care was taken to prevent oxygen from entering the digester, according to practical method adopted by Li et al. (2015).

Fig. 2. Experimental setup for Biogas production system

2.4. Chemical composition analysis

An analysis was conducted on total solids (TS), volatile solids (VS), and carbon/nitrogen ratio of WS and CS before and after pretreatment. The sample has evaporated and dried in a laboratory oven at (105°C) for one hour to determine the TS, following the procedure used by Orhorhoro et al. (2017). After determining the total solids, the sample was then burned at a temperature of 550-600°C for a duration of 4 hours. The Kjeldahl method is used to measure the amount of nitrogen present, as mentioned in the study by Ismail and Noori (2018).

2.5. Kinetic Model

The growth rate of methanogenic bacteria in the digester is directly related to the biogas production rate under batch conditions. The following is equation (1) the modified Gompertz model can be used to predict the rate of biogas generation (Jasim and Ismail, 2022):

$$P_{(t)} = P_o . \exp\left[- \exp\left[\frac{Rmax * 2.7183}{P_o}(\lambda - t) + 1\right]\right] \quad (1)$$

- R_{max}: Max. methane generation rate, ml/g VS. day;
- t: time, day;
- $P_{(t)}$: The total biogas production over a digestion period (mL/g VS);
- P_0 = The potential for the substrate to produce biogas (mL/g VS); and
- λ= Lag phase (day), day.

3. RESULTS AND DISCUSSION

3.1. Evaluation of the effect of chemical pretreatment on the production of biogas

This study used chemical pretreatments (4% NaOH pretreatment (WS-4) and (CS-4)) to improve biogas generation and methane yield. In 4% NaOH pretreatment, three types of inoculums that were used with the pretreated and untreated wheat stalks (WS) and corn stalks (CS). Fig.3 and 4 along with Table 2, illustrate the outcomes of the cumulative generation of biogas and methane, respectively. It is clear that the wheat stalks pretreated with 4% NaOH (WS-4a, WS-4c, and WS-4d) that co-digested with cow manure, cattle manure, and ostrich dung, respectively, increased both biogas production and methane yield by yielding 84.39 and 52.071 ml/g VS;54.94 and 29.636 ml/g VS;105.219 and 64.59 ml/g VS, respectively as compared with control tests (when untreated wheat stalks co-digested with cow manure, cattle manure, and ostrich dung, respectively) WS-1a, WS-1c, and WS-1d (14.85 and 9.15 ml/g VS;16.043 and 8.973 ml/g VS ; 25.1783 and 16.323 ml/g VS for biogas production and methane yield, respectively). It was noticed that the maximum amount of biogas produced and methane yield when inoculated pretreated wheat stalks with 4% NaOH with ostrich dung (WS-4d test) were 105.219& 64.59 ml/g VS, respectively, as compared with the control WS-1d test

(25.178 and 16.32 ml/g VS for biogas generation and methane yield, respectively), corresponding to an increase biogas production and methane yield of 317.9 and 295.77%, respectively, as compared with the control WS-1d test. The amount of biogas produced and methane yield of control CS-1a, CS-1c, and CS-1d tests (when untreated corn stalks co-digested with cow manure, cattle manure, and ostrich dung, respectively) were 19.19 and 9.72 ml/g VS; 17.401 and 8.116 ml/g VS and 32.778 and 22.289 ml/g VS. When 4% NaOH pretreatment corn stalks co-digested with cow manure, cattle manure, and ostrich dung (CS-4a, CS-4c, and CS-4d, respectively), the values of biogas and methane generation were equal to 85.49 and 52.89 ml/g VS; 57.58 and 31.75 ml/g VS;123.90 and 88.49 ml/g VS, respectively. It was clear from the above results for two substrates (wheat stalks and corn stalks), the amount of biogas production and methane yield for 4% NaOH pretreated of the corn stalks that co-digested ostrich dung (123.9 and 88.49 ml/g VS, respectively) was higher than when 4% NaOH pretreated wheat stalks that co-digested ostrich dung (105.219 and 64.59 ml/g VS). These results were likely due to the (C/N) ratios. The (C/N) ratio of ostrich dung (9.04) was lower than that of 4% NaOH-pretreated wheat stalks (WS-4), which was 45.72, and the C/N ratio of 4% NaOH-pretreated cornstalks (CS-4) was 23.13. According to pervious study by Zhang et al. (2013) showed that the biogas production was substantially increased at all carbon-to-nitrogen (C/N) ratios when goat manure (GM) was mixed with crop residues (corn stalks (CS) or rice straw (RS)). The maximum biogas yields were achieved from various co-substrates when GM/CS (30:70), GM/CS (70:30), GM/RS (30:70), and GM/RS (50:50) were used. Nevertheless, the (C/N) ratios of WS, CS, and RS were substantially higher (91.17, 88.13, and 92.91, respectively) than those of GM. This result suggested that CRs increased methane generation when co-digested with GM under the optimal C/N ratio. The results of this part had provided significant agreement with the previous studies. Chandra et al. (2012) demonstrated that the substrate treated with NaOH resulted in an 87.5% increase in biogas generation and a 111.6% increase in methane generation compared to the untreated wheat straw substrate. Rani et al., (2022) showed that a complex lignin structure of wheat straw is disrupted by alkaline pretreatment, which increases the content of celluloses and hemicelluloses by promoting merization for breakdown into sugars and subsequently to CH_4 and CO_2. Orlando and Borja., (2020) found that NaOH separates the hydrogen bonds in cellulose and hemicellulose, destroys the ester bonds between lignin and xylan, and leads to deprotonation of phenolic groups. Jassim (2019) reported that the amounts of biogas production and methane yield from rice husks treated with 3%NaOH inoculated with ostrich dung, which were 47.16 and 32.91 ml/g VS, respectively, exceeded their amounts from the control test (29.32 and 18.5 (ml/g VS) for biogas production and methane yield, respectively). Moreover, hemicellulose and lignin reduction for rice husks treated with 3%NaOH were 24 and 31%, respectively.

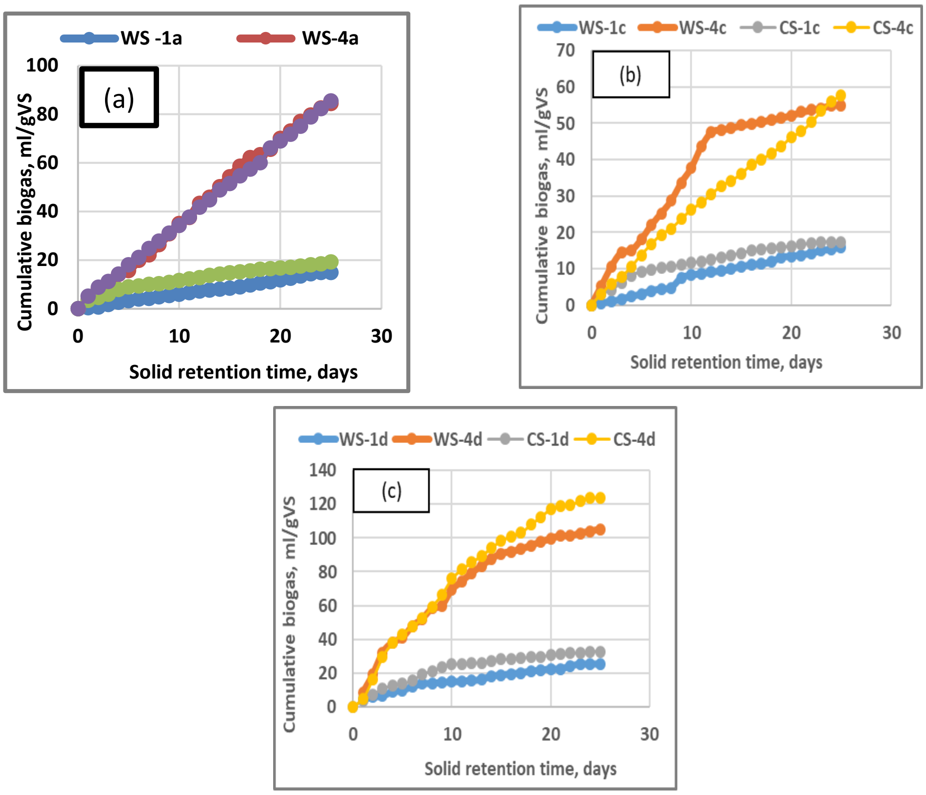


Fig. 3. Impact of 4% NaOH pre-treatment for WS and CS on the generation of biogas that co-digested with (a) cow manure, (b) cattle manure, and (c) ostrich dung.

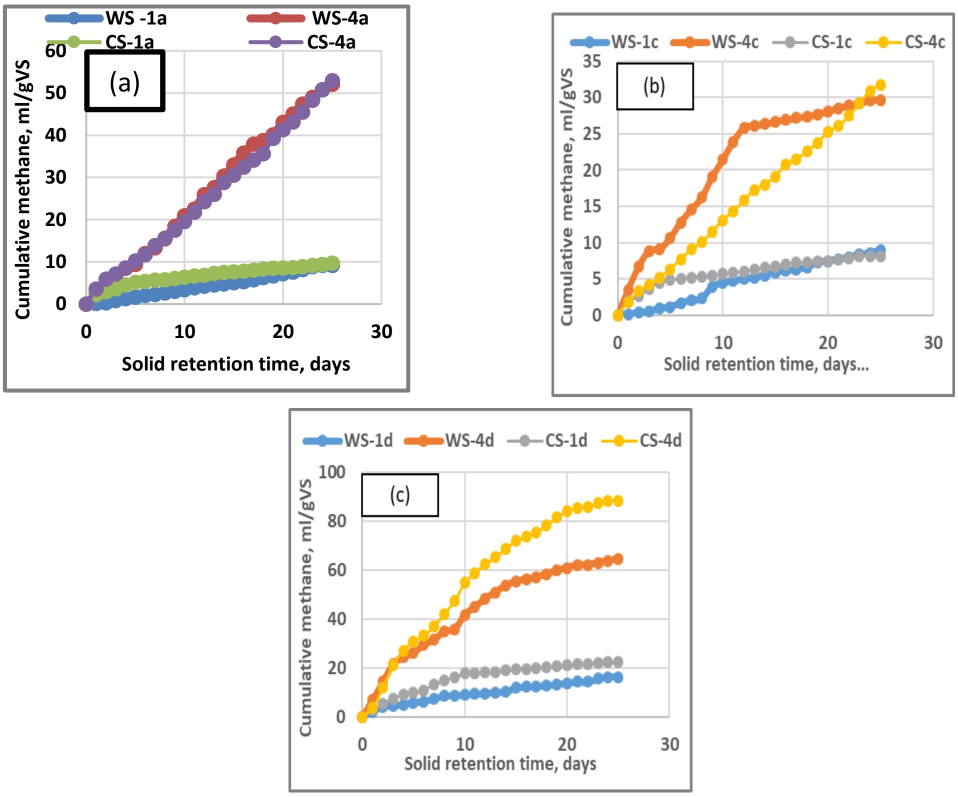


Fig. 4. Impact of 4% NaOH pre-treatment for WS and CS on the amount of methane that co-digested with (a) cow manure, (b) cattle manure, and (c) ostrich dung.

Table 2. Effect of 4% NaOH for (WS-4& CS-4) pre-treatment on biogas generation and methane yield.

Pre-treatment	Maximum Biogas generation (ml/g VS)	Maximum Methane yield (ml/g VS)	Biogas increment %	Methane increment %
WS-1a	14.85	9.15	-	-
WS-4a	84.39	52.071	468.28	469.08
CS-1a	19.199	9.72	-	-
CS-4a	85.49	52.89	345.28	444.59
WS-1c	16.04	8.97	-	-
WS-4c	54.94	29.636	242.51	230.39
CS-1c	17.401	8.116	-	-
CS-4c	57.58	31.75	230.90	291.20
WS-1d	25.178	16.32	-	-
WS-4d	105.219	64.59	317.90	295.77
CS-1d	32.778	22.289	-	-
CS-4d	123.90	88.49	277.99	297.011

a: Cow manure, c: Cattle, d: Ostrich, WS: Wheat Stalks, and CS: Corn Stalks

3.2. The Impact of 4% NaOH Pretreatment on The Structure of WS and CS

Fig. 5 (a, b, c and d) shows the scanning electron microscopy (SEM) of untreated and pretreated wheat stalks and corn stalks. As shown in Fig.5 (a and c), the surface of untreated wheat straw and corn stalks is not flat, and different sizes and shapes of particles adhere to them, some of which appear white. But in Fig. 5 (b and d) it appeared to be a warped surface with a large hole and deep. The results obtained in this point consistence with the previous study by Krishania et al. (2013) demonstrated that scanning electron microscopy (SEM) pictures reveal physical structural changes in wheat straw after NaOH treatment (T2). The distinct separation of the fibers is evident, a consequence of the destruction of the bonds among lignin, hemicellulose, and cellulose. Thus, the application of alkali pretreatment (T2) and Ca $(OH)_2$ and Na_2CO_3 pretreatment (T4) resulted in swelling of lignocellulosic wheat straw biomass. This swelling resulted in an augmented internal surface area, diminished crystallinity, disjunction of structural connections between lignin and carbohydrates, and destruction of the lignin architecture.

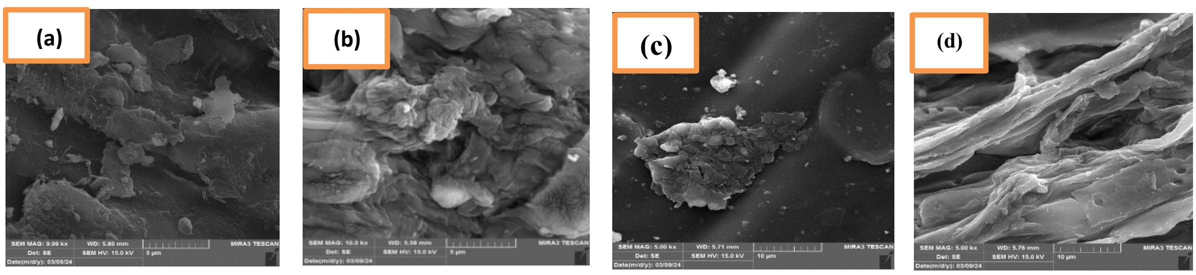


Fig. 5 SEM images of (a) untreated & (b) 4% NaOH pretreated for wheat stalks; (c) untreated & (d) 4% NaOH pretreated for corn stalks.

3.3. Impact of 4% NaOH Pretreatment on The Characteristics of WS and CS

The FTIR spectra were measured within the range of 4000-500 cm^{-1} in this study of untreated (wheat stalk sand corn stalks), and that treated with 4% NaOH as shown in Fig. 6. The spectral band at (3415 cm^{-1}) and (3456 cm^{-1}) were changed from a higher intensity for untreated wheat stalks and corn stalks to a lower intensity when treated with 4% NaOH. In additional, the spectral band in the region of 1046 cm^{-1} and 1400 cm^{-1} were transmitted from highest peak for untreated wheat stalks and corn stalks to the lowest peak for treated wheat stalks and corn stalks with 4% NaOH. This can be explained by referring to study by Rani et al. (2022) showed the impact of alkaline pretreatment (KOH) on the substrate, specifically lignocellulosic biomass or wheat straw, is that it comprises a complex structure of lignin, cellulose, and hemicellulose. Alkaline pre-treatment destructs the complicated lignin structure of wheat straw and enhances the amount of celluloses and hemicelluloses by depolymerization for degradation to sugars and then to CH_4 and CO_2. The absorption band in the FTIR data of samples is observed at (3385 cm^{-1}), corresponding to the O–H stretching in the hydrogen bond of cellulose, which diminishes as the concentrations of alkaline pre-treatment of wheat straw change. The absorption peaks at (1035 cm^{-1}) lowered with increasing pretreatment concentrations, attributable to aromatic C–C stretching from lignin and aliphatic C–H stretching in methyl and phenolic –OH, along with syringyl units of lignin.

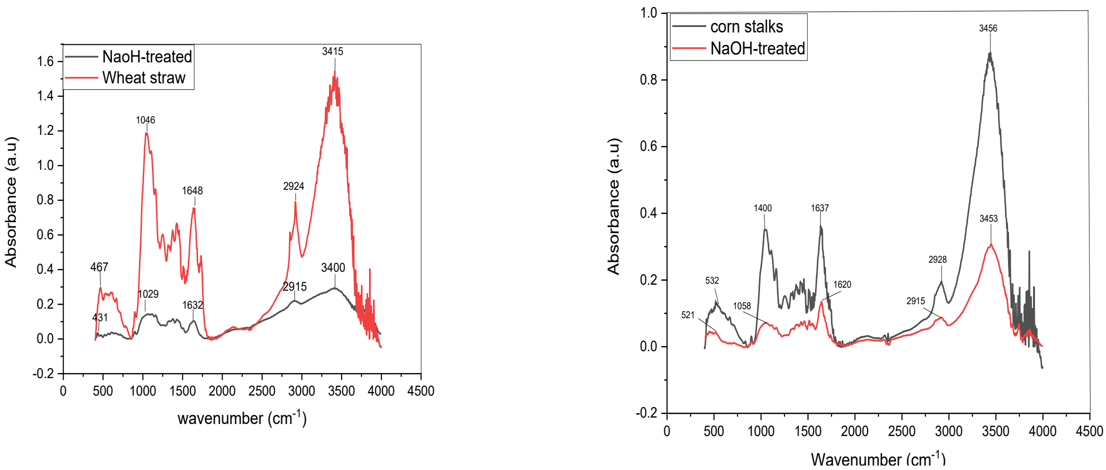


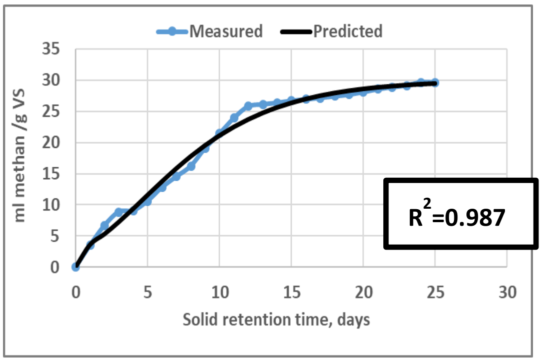
Fig. 6. FTIR of untreated and 4% NaOH pretreated for: (a) wheat stalks and (b) corn stalks.

3.4. Kinetic study

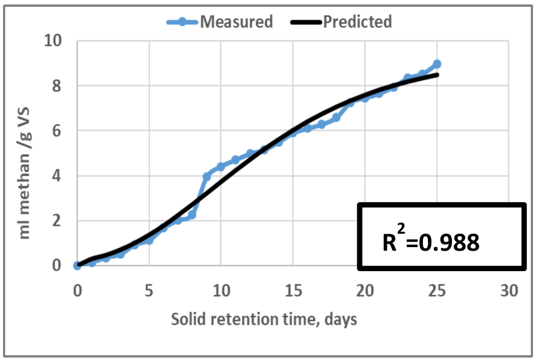
Fig.7 illustrate the extent of consistency between the measured and projected values for digesters concerning both untreated and pretreated (WS and CS) samples. Furthermore, Table3 displays the results and kinetic constants derived from the (Modified Gompertz Model). The correlation between the measured and projected values of biogas recovery was consistent with previously reported investigations. Jassim and Khalil (2023) achieved concordance between the measured and anticipated values derived from the (modified Gompertz model) for the co-digestion of inoculated rice husk.

Table 3. The subsequent outcomes of the modified Gompertz model produced:

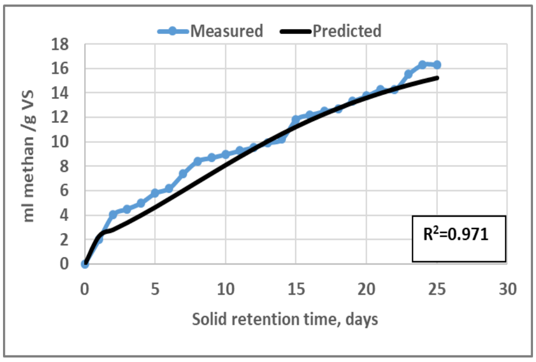
Digester No.	$G_{(t)}$ exp. (ml methane/g VS)	Gompertz model parameters				R^2
		λ (day)	R_{max}. (ml methane /g VS)	G_0 (ml methane /g VS)	$G_{(t)}$ model(ml methane /g VS	
1	8.97	2.640	0.506	8.97	8.47	0.987
2	29.64	0.241	2.209	29.64	29.52	0.988
3	16.32	1.622	0.628	16.32	15.20	0.971
4	64.60	1.792	3.712	64.60	64.43	0.991
5	9.72	5.910	0.419	9.72	9.26	0.981
6	52.90	1.777	2.290	52.90	52.10	0.998



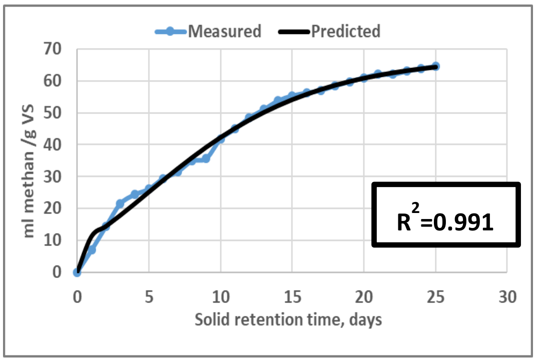
a. Methane yield from digester No. 1 (WS-1c) predictions and measurements



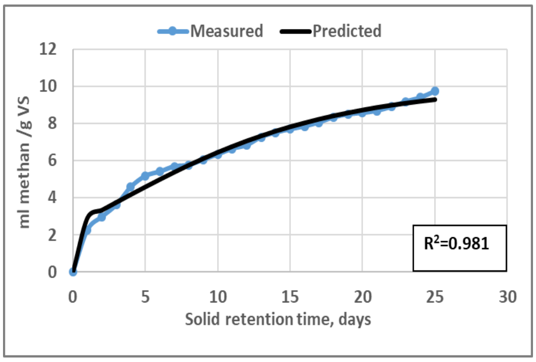
b. Methane yield from digester No. 2 (WS-4c) predictions and measurements



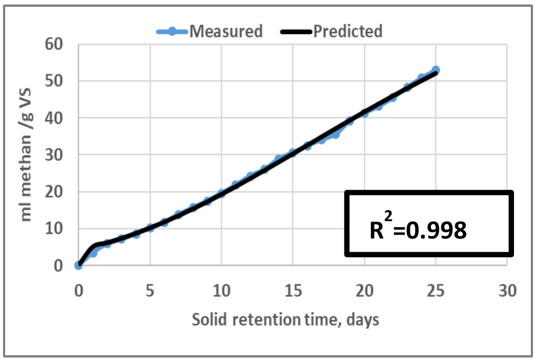
c. Methane yield from digester No. 3 (WS-1d) predictions and measurements



d. Methane yield from digester No. 4 (WS-4d) predictions and measurements



e. Methane yield from digester No. 5 (CS-1a) predictions and measurements



f. Methane yield from digester No. 6 (CS-4a) predictions and measurements

Fig. 7. The measured and projected values for digesters concerning both untreated and pretreated (WS and CS) samples.

4. CONCLUSION

The findings indicated that anaerobic co-digestion of pretreated wheat and maize stalks gives better biogas than untreated wheat and corn stalks. It is also noted that pretreatment using sodium hydroxide gave better results in the production of biogas and methane than untreated substrate. The increase was 317.90% and 277.99% in biogas production when using wheat and corn stalks with ostrich manure, respectively. Also, the anaerobic co-digestion of wheat and corn stalks with ostrich dung gave better results than other types of animal waste. Corn stalks also gave more production of biogas than wheat stalks. The analysis of the scanning electronic microscope also showed the change in the surface of the wheat and corn stems as a result of chemical pretreatment.

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