

## RESEARCH ARTICLE

# Assessment of the effect pre-treated using ultrasonic and hydrothermal technique for wheat and corn stalks on biogas generation and methane yield

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## ABSTRACT

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Current study aims to estimate the impact of physical pretreatment of wheat stalks and corn stalks on biogas production, where two types of pretreatment were used (ultrasonic pretreatment and hydrothermal pretreatment) and three types of animal manure were used as a source of bacteria (cow, sheep and ostrich manure). The results showed a high increment in the amount of biogas generated after pretreatment, as the increase when using ultrasonic pretreatment for wheat stalks inoculated with cow, sheep, and ostrich manure were 31.92%, 20.63%, 155.24%, respectively as compared with untreated samples of wheat stalks. As for the corn stalks, the increase in the biogas when ultrasonic pretreatment for corn stalks inoculated with cow, sheep, and ostrich manure was 57.12%, 43.38%, and 173.05%, respectively, as compared with untreated samples of corn stalks. In hydrothermal pretreatment, the increase in biogas generated by pretreatment for wheat stalks inoculated with cow and sheep manure was 30.57% and 6.10 %, respectively, compared with an untreated sample of wheat stalks. In the hydrothermal pretreatment for corn stalks, the increase was 29.69% and 3.67 %, respectively as compared with untreated sample of corn stalks with the same manure above. An increase was also observed in the amount of methane generated after each of the pre-treated, as the increase in the ultrasonic pretreatment for wheat stalks inoculated cow, sheep, and ostrich manure were 25.25%, 21.96% and 160.78 %, respectively. Whereas, when ultrasonic pretreatment for corn stalks the increase was 88.06%, 54.13%, and 210.91 %, respectively. In hydrothermal pretreatment, the increase in the amount of methane for wheat stalks inoculated with cow and sheep manure was 13.59% and 13.67 %, while, in the corn stalks inoculated with cow and sheep manure was 42.90% and 27.23 %, respectively. Finally, the highest biogas and methane production was obtained when ostrich dung was used as inoculum with wheat and corn stalks pre-treated using ultrasound compared to cow and sheep manure. A substantial accord was seen between the values that were measured and the values that were expected by the modified Gompertz model, as indicated by correlation coefficients that were  $\geq 0.96$ .

**Keywords:** Animal waste; biogas generation; Corn stalks; Wheat stalks

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## 1. Introduction

Biogas, a byproduct of the anaerobic digestion of the organic constituent of biomass, such as animal waste, sewage sludge, or industrial effluents, is a sustainable energy resource that can serve as a substitute for conventional energy sources. Methane (CH<sub>4</sub>) is the most essential component of biogas due to its high energy content per mole. Therefore, the high concentration of methane in biogas is crucial. The primary constituents of biogas are carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>), while the remaining components consist of hydrogen (1%) and nitrogen (10%), which may originate from air saturation in the influent. At thermophilic temperatures, biogas may have a higher concentration of vapor water (5-10%) and derived from medium evaporation. Additional components of the mixture include hydrogen sulfide (1-3%), formed by the reduction of sulfate present in certain waste streams, and ammonia (NH<sub>3</sub>), as reported by [1]. Many steps involving biological and chemical process that occur in absence of oxygen is anaerobic digestion. Organic matter such as human excreta, cow manure, and food waste through this process, was broken down via complex interactions microorganisms, resulting in the production of biogas, as showed by [2]. Lignocellulosic biomass is a plentiful organic material suitable for the sustainable generation of bioenergy and biofuels, including biogas with approximately 50-75% CH<sub>4</sub> and 25-50% CO<sub>2</sub>, as reported by [3]. Wheat straw is a widely available crop residue that has a variety of uses, including consumption and animal bedding, incorporation of the soil, and production of the energy through biogas or bioethanol etc, as cited by [4]. Wheat straw is composed of the dry wheat stalks that remain after the grain has been removed, as showed by [5]. The complicated structure of agricultural biomass, such as WS, presents another challenge to its effective start of the anaerobic digestion process. Breaking down this structure is crucial for improving the process. To degrade the structure of the lignocellulosic for wheat straw, it is necessary to use an appropriate pretreatment method, as mentioned by [6]. Physical pretreatment indicates processes that do not utilize chemicals or microorganisms through the pre-treatment methods. Typically, physical pre-treatment can enhance methane production from lignocellulosic biomass, like agricultural residues. Previously modified physical pretreatment methods involved the pretreatment with fluid hot water (hydro-thermolysis), the comminution (such as grinding and milling), steam-explosion (autohydrolysis), and extrusion, and irradiation (microwave and ultrasound), as proved by [3]. A kind of the physical pre-treatment used to pretreat organic waste is called thermal pretreatment. The temperature is applied to this pretreatment technique within the range of 50 to 250 °C. Numerous studies have shown that the thermal treatment of lignocellulosic residues increased the methane generated. During 1 to 15 minutes, the thermal pre-treatment of WS was performed at temperatures between 150 and 220 °C. In WS pre-treated at 220 °C for 1 minute, a 20% increase in CH<sub>4</sub> generation was observed, as mentioned by [6]. Ultrasonic pretreatment is a technology that degrades and destroys the feedstock. The chemical and physical impacts of ultrasound waves were made to change the morphology of the lignocellulosic substrate in this technique. Ultrasound pretreatment creates tiny bubbles of cavitation that bursting the hemicellulose and cellulose fraction, thereby increasing the accessibility of degrading enzymes to the cellulose, facilitating its efficient dislocation into simpler homogenous sugars, as reported by [7]. Sorghum and wheat straw are two types of substrates for anaerobic digestion (AD) in agricultural biogas facilities. Nevertheless, their methane production is dependent on their complicated structure, which is mostly made of cellulose, hemicelluloses, and lignin. Unlike cellulose and hemicelluloses, lignin does not degrade during the AD process, and its structure restricts the accessibility of cellulose and hemicelluloses to hydrolytic enzymes, preventing their destruction, as mentioned by [8]. The current study aimed to evaluate the impact of physical pre-treatment (ultrasonic and hydrothermal) for the wheat stalks and corn stalks inoculated with cow, sheep, and ostrich manure on the biogas generation and methane yield, properties of raw and pretreated substrates as total solids and volatile solids, and nitrogen & carbon content, and C/N ratio for raw and pretreated substrates.

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## 2. Materials and methods

### 2.1. Preparation of substrates and inoculum

In this study, wheat stalks (WS) and corn stalks (CS) were used as substrate, Wheat stalks was collected in November 2023 from a local farm in the city of Hilla (Babil, Iraq). After collecting agricultural residues, they were cleaned manually to remove unwanted sand and particles. Then it was dried in the air and then milled using an electric household grinder into grinder into 1 mm particles size, according to [8]. The piece size required in this study ranged from 0.3-0.6 mm as the same size, adopted by [9]. The characteristics of the chemicals for WS and CS are explained in **Table 1**.

Cow manure, sheep manure, and ostrich manure were three types of inoculums that were used as sources of microorganisms for this study. All animal manures were passed through a 3-mm sieve to elimination coarse fibers before the experiment. Subsequently, the samples were dried using air and then a mechanical mill to transform them into a fine powder, according to the practical method adopted by [10]. Three different kinds of manure were utilized as inoculum in the present research, were stored in an incubator at 37°C for 1 week to decompose with materials that are easily biodegradable present in the inoculum, in accordance with the procedure that was chosen by [11]. To prepare the inoculum slurry that was used in this study by mixing 15 grams of animal manure with 300 ml of the distilled water. To mix the slurry using a glass rod by moving the homogenate manually, according to the procedure adopted by [12,9], whereas, the weight of the inoculum was modified to obtain homogenized slurry. The chemical properties of wheat stalks, corn stalks, and inoculums is clarified in **Table 1**.

**Table 1.** The properties chemical for wheat stalks, corn stalks, and inoculums.

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

### 2.2. Methods for pretreatment of substrate

To enhance biogas generation from lignocellulosic biomass, it is essential to implement a pretreatment process that can effectively destroy the originality recalcitrant carbohydrate-lignin shields that weaken the reach of enzymes and microbes to hemicellulose and cellulose [3]. Two pretreatments, hydrothermal and ultrasonic were used. According to practical method adopted by [13], the ultrasonic (US) treatment was conducted using a bench ultrasonic cell with a low frequency of 20 kHz. The pretreatment time (15 min) were selected depending on the previous study reported by [18]. Thirty (30) grams of the milled substrate were added to a volume of 300 milliliters of the mixture, which was then placed in a cylindrical Pyrex beaker with a US probe positioned in the middle and immersing up to two centimeters. The tests were carried out at ratio of solid to distilled water or inoculum slurry (1/10) were selected based on the prior study, reported by [14]. Hydrothermal pretreatment was carried out using an autoclave (Model: DAIHAN LABTECH, Korea), the selected of pre-treatment time 15 min was depending on [15], while the chosen of temperature was 121°C (1 atm). Hydrothermal pretreatment involved placing 30 grams of finely crushed substrate into a 500 ml glass bottle containing 300 ml of distilled water, resulting in a solid-liquid ratio of 1/10 (weight of biomass (w) to

volume of distilled water (v)) was selected, as recommended by<sup>[16]</sup>. **Figure 1** show the air dried and milled samples of the wheat stalks and corn stalks.



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

### 2.3. Biochemical methane potential (BMP) setup

A BMP experiment is an assay utilized to estimate the biodegradability and methane generation potential of organic materials in anaerobic environments<sup>[17]</sup>. In a batch system, three digesters were carried out. The digesters included wheat stalks and corn stalks in case of untreated (WS-1) & (CS-1) and in two cases of pre-treatments: WS-2&CS-2 (ultrasonic pretreated), and WS-3&CS-3 (hydrothermal pretreated). **Figure 2** shows the experimental setup system, is the same as the arrangement of the lab-scale system it is highlighted in the studies' experimental work by<sup>[12,18]</sup>, with a few changes to the measurements. The digester consists mainly of the preparation of 500 ml heat-resistant Pyrex borosilicate glass bottles for anaerobic digestion. In each digestion, the contents were preserved in a ratio of 1:10. This percentage is equivalent to 30 grams of substrate: 300 ml of slurry inoculum. The bottles of singular digester were sealed utilizing plastic stoppers with two holes: the first hole with a diameter of 5 mm was linked with a rubber tube to pass nitrogen gas to the digester. The other hole of the digester was linked to a rubber tube connected to a valve to prevent the release of the produced gas, according to applicable previous research by<sup>[2]</sup>. Before the beginning of every batch, the pH of all the reactors was measured after blending the inoculum and substrate and adjusted to a neutral level if required. The reactors were flushed with nitrogen gas to elimination oxygen and sealed securely<sup>[11]</sup>. Reactors were manually shaken once a day while the digestion was placed in a temperature-controlled water bath to maintain a constant temperature, according to practical method by<sup>[19]</sup>. Keep the digestion at the desired a thermophilic temperature of 50°C, according to pervious research by<sup>[20]</sup>. The high microbial activity in the thermophilic temperature range occurred as a result of the thermophilic condition, as reported by<sup>[21]</sup>.



**Figure 2.** The experimental setup for the biogas generation system consists of the following basic components: (1) bath of water, (2) digester, (3) thermostat, (4) nitrogen purging rubber tube, and (5) manometer.

## 2.4. Analytical parameters

Before and after pretreatment total solids, volatile solids, and carbon /nitrogen ratio of WS and CS were analyzed. TS refers to the amount of residue left in the crucible after the sample has been evaporated and subsequently dried in a laboratory oven at 105°C for one hour, in accordance with the procedure implemented by<sup>[22]</sup>. The residue that was obtained from the total solids determination has been cooled in the room's atmosphere, weigh them using an electronic scale (Germany), then burned at 550-600°C for 4 h. to expel VS in the sample. Kjeldahl method is employed to quantify nitrogen. The digestion, distillation, and titration processes were the three primary phases that were utilized in the Kjeldahl analysis (Germany) operation, according to adopt by<sup>[9,23,12]</sup>. As shown in **Figure 2**, the manometer method was used to measure the biogas production in this study, as reported by<sup>[24]</sup>.

## 2.5. Kinetic Study

In order to accurately verify the methane production and complex metabolic process of anaerobic digestion, the modified Gompertz model is implemented to compare the cumulative methane production obtained from the BMP test results. The modified Gompertz model is primarily used to verify the cumulative methane production, which is determined by the experimental results. The modified Gompertz model is the most commonly used for modeling the growth curve of a target, as described by<sup>[25]</sup>. A nonlinear least-square regression analysis was applied utilizing SPSS [IBM SPSS statistics 26 (2019)]. Equation (1) represents this model, as mentioned by<sup>[26]</sup>.

$$G(t) = G_0 \cdot \exp \left\{ - \exp \left[ \frac{R_{max} \cdot e}{G_0} (\lambda - t) + 1 \right] \right\} \quad (1)$$

Where:

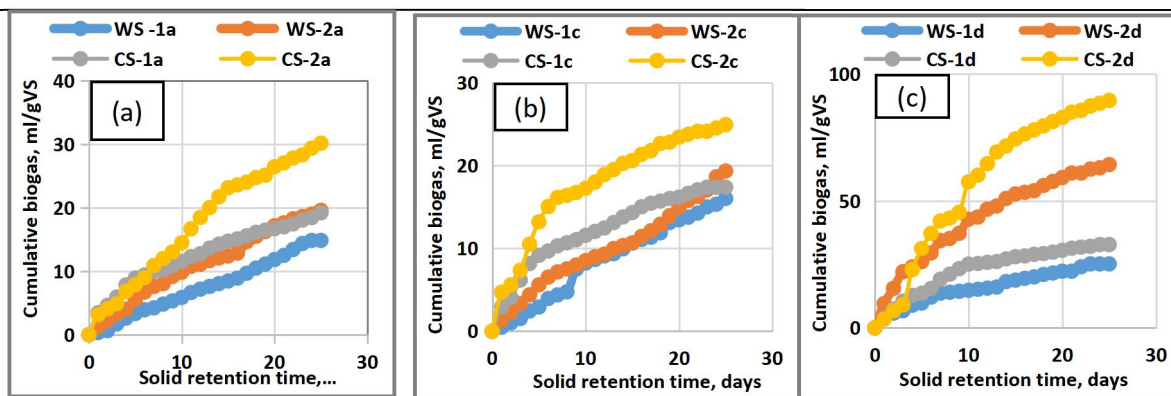
$R_{max}$ : The maximum rate of methane production, ml/g VS. day;  $G(t)$ : The cumulative biogas yield at a given digestion time (mL/g VS);  $G_0$  = The potential for biogas generation from the substrate (mL/g VS);  $\lambda$ = lag phase (day), day; and  $\exp$ : is  $\exp(1) = 2.7183$ ;  $t$ = time of digestion

## 3. Results and discussion

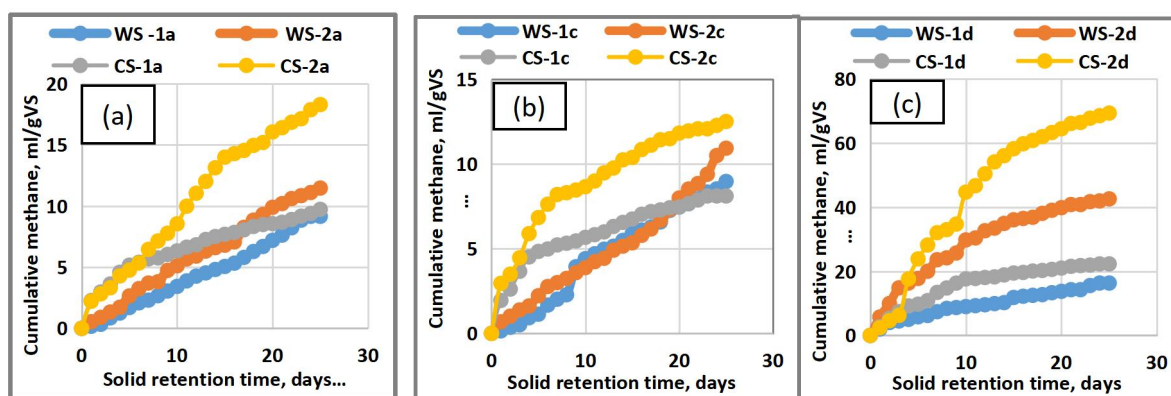
### 3.1. Effect of physical pre-treatment on the Biogas generation

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In this study, three types of inoculums that were used with the pretreated and untreated wheat stalks (WS) and corn stalks (CS). **Figures (3) and (4)**, together with **Table (2)** show the cumulative generation of methane and biogas for all types of inoculums with pretreated and untreated for wheat stalks (WS) and corn stalks (CS). Two types of pretreatments (ultrasonic pretreatment (WS-2) & (CS-2) and hydrothermal pretreatment (WS-3) & (CS-3)) were used to improve the generation of biogas. The amount of biogas produced and methane yield of control WS-1a, WS-1c, and WS-1d tests (when untreated wheat stalks co-digested with cow manure, sheep manure, and ostrich dung, respectively) were 14.85 & 9.15 ml/g VS; 16.04 & 8.97 ml/g VS and 25.178 & 16.32 ml/g VS. It was noticed that the sequences of the maximum amount of biogas produced when using ostrich dung, sheep manure, and cow manure, as inoculums mixed with untreated wheat stalks as follows: WS-1d>WS-1c>WS-1a. Whereas the order of the optimal of methane yield for the same tests as: WS-1d >WS-1a>WS-1c. It is clear that the ultrasonic pretreated wheat stalks which co-digested with cow manure, sheep manure, and ostrich dung (WS-2a, WS-2c, and WS-2d respectively), increased both biogas generation and methane yield as compared with control tests (WS-1a, WS-1c, and WS-1d) by yielding 19.59 & 11.46 ml/g VS; 19.35 & 10.94 ml/g VS; and 64.266 & 42.56 ml/g VS, respectively. When comparing these tests WS-2a, WS-2c, and WS-2d, the yield of methane and the production of biogas, it can be seen that the biogas generation and methane yield, when inoculated ultrasonic pretreated wheat stalks with ostrich dung (WS-2d test) increased amount to 64.266 & 42.56 ml/g VS, respectively, corresponding to an increase biogas generation and methane yield increment of 155.24% and 160.78%, respectively, as compared with the control WS-1d, as shown in **Table 2**. 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, sheep manure, and ostrich dung, respectively) were 19.19 & 9.72 ml/g VS; 17.401 & 8.116 ml/g VS and 32.778 & 22.289 ml/g VS. When ultrasonic pretreatment corn stalks co-digested with cow manure, sheep manure, and ostrich dung (CS-2a, CS-2c, and CS-2d, respectively), the values of biogas and methane generation were equal to 30.166&18.28 ml/g VS; 24.95&12.51 ml/g VS; and 89.503&69.299 ml/g VS, respectively. It was noted that the higher generation for the biogas and methane obtained when ultrasonic pretreatment corn stalks co-digested with ostrich dung (CS-2d). Because of a little produces bio-methane through anaerobic digestion, ultrasound has been employed as a pre-treatment to enhance the bio-methane production by solubilizing organic residues, breaking down cell structure, and disrupting the cellulose crystalline structure, to improve the bio-methane produce, as cited by<sup>[27]</sup>. These findings were consistent with the other studies.<sup>[13]</sup> exhibited that because of the ultrasonic pretreatment enhancement of organic matter solubilization, biogas was produced more in the sonicated mixture than in the untreated one. The biogas generation of the sonicated mixture (Test C) was 24%, 60%, and 93% higher than that of the untreated mixture (Test A), the organic fraction of municipal solid waste (OFMSW) (Test D), and sewage sludge (SS) (Test E) after 32 days. <sup>[18]</sup> found that the ultrasound pretreated for rice husk (RH-2 test) was hemicellulose and lignin reduction about 15 and 20%, respectively. Hence, the total generation of biogas and methane for RH-2 amounted to 44.19 ml/g VS and 30.73 ml/g VS, respectively. This corresponds to an increase of 50.72% and 66.11% in biogas and methane content, respectively, compared to the control RH-1 which had values of 29.32 ml/g VS and 18.5 ml/g VS for biogas and methane production, respectively.



**Figure 3.** Impact of ultrasonic pre-treatment for wheat stalks and corn stalks on the generation of biogas that co-digested with (a) cow manure, (b) sheep manure, and (c) ostrich dung.



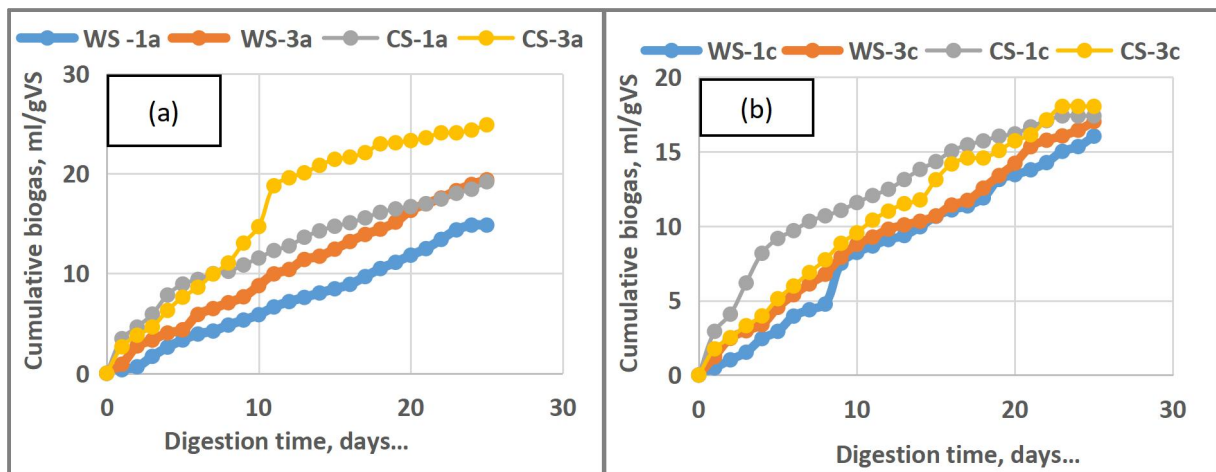
**Figure 4.** Impact of ultrasonic pre-treatment for wheat stalks and corn stalks on the amount of methane produced that co-digested with (a) cow manure, (b) sheep manure, and (d) ostrich dung.

**Table 2.** Impact of ultrasonic pretreatment (WS-2& CS-2) on methane yield and biogas production.

Pre-treatment	Max. Biogas generation (ml/g VS)	Max. Methane yield (ml/g VS)	Biogas increment %	Methane increment %
WS-1a	14.85	9.15	-	-
WS-2a	19.59	11.46	31.92	25.25
CS-1a	19.199	9.72	-	-
CS-2a	30.166	18.28	57.12	88.06
WS-1c	16.04	8.97	-	-
WS-2c	19.35	10.94	20.63	21.96
CS-1c	17.401	8.116	-	-
CS-2c	24.950	12.51	43.38	54.13
WS-1d	25.178	16.32	-	-
WS-2d	64.266	42.56	155.24	160.78
CS-1d	32.778	22.289	-	-
CS-2d	89.503	69.299	173.05	210.91

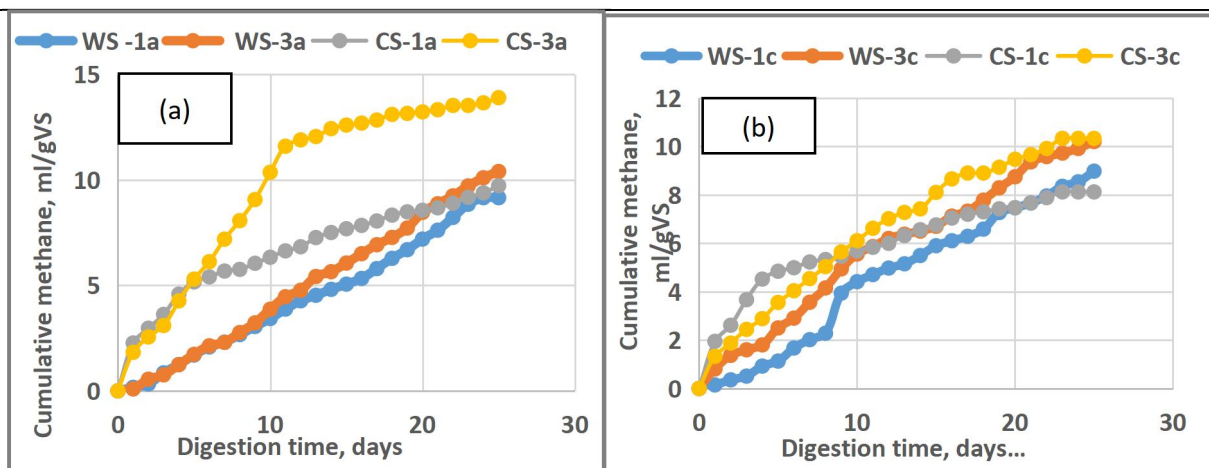
*Note:* a: Cow manure, c: Sheep, d: Ostrich, WS: Wheat Stalks, and CS: Corn Stalks.

For hydrothermal pretreatment (WS-3&CS-3), the maximum rates of biogas generation and methane content in the pretreatment of WS-3a, and WS-3c (when hydrothermal pretreated wheat stalks which co-digested with cow manure and sheep manure, respectively) were clearly 19.39&17.02 ml/g VS and 10.394 & 10.196 ml/g VS, respectively, in comparison with the control WS-1a and WS-1c which had values of 14.85& 16.04 ml/g VS and 9.15& 8.97 ml/g VS for biogas production and methane yield, respectively, as shown in **Figure (5)** and **(6)** together with **Table (3)**. It was noticed from **Table (3)** that the percentage of biogas increment between WS-3a&WS-1a; and WS-3c&WS-1c were 30.57% and 6.1%, respectively. The highest percentage of biogas increment was achieved when hydrothermal treated wheat stalks inoculum with cow manure, followed by inoculum with sheep manure. Whereas, the percentage of methane increment for the same tests above was 13.59% and 13.67%, respectively. Also, the highest two values were obtained when inoculum with cow manure and then with sheep manure. The amount of biogas produced and methane yield of control CS-1a, and CS-1c tests (when untreated corn stalks co-digested with cow manure and sheep manure respectively) were 19.19 & 9.72 ml/g VS and 17.401 & 8.116 ml/g VS. When hydrothermal pretreatment corn stalks co-digested with cow manure and sheep manure (CS-3a, and CS-3c, respectively), the values of biogas and methane generation were equal to 24.9&13.89 ml/g VS and 18.04 &10.326 ml/g VS, respectively. Finally, it may be said that the hydrothermal pretreatment contributed to increment of the production of biogas and methane for wheat stalks and corn stalks that inoculum with cow than with sheep manure used in this study. It can be seen that the biogas generation, when inoculated hydrothermal pretreated wheat stalks and corn stalks with cow manure (WS-3a &CS-3a test) increased amount to 19.39& 24.9 ml/g VS, respectively, corresponding to an increase biogas production of 30.57 and 29.69%, respectively, as compared with the control WS-1a &CS-1a tests, as shown in **Table (3)**. Whereas, in WS-3c &CS-3c, the amount of biogas (17.02 and 18.04 ml/g VS) was lower than in WS-3a &CS-3a (19.39& 24.9 ml/g VS). The findings in this section are consistent with the conclusions drawn in earlier research. [28] proved that the hydrothermal pre-treatment of wheat straw substrate resulted in a significant enhancement of 9.2% in biogas production and a 20.0% increase in methane generation relative to the untreated wheat straw (WS) substrate.



**Figure 5.** Impact of hydrothermal pretreatment for wheat stalks and corn stalks on the generation of biogas that co-digested with (a) cow manure, and (b) sheep manure.





**Figure 6.** Impact of hydrothermal pretreatment for wheat stalks and corn stalks on the amount of methane produced that co-digested with (a) cow manure and (b) sheep manure.

**Table 3.** Impact of hydrothermal pretreatment (WS-3& CS-3) on methane yield and biogas production.

Pre-treatment	Max. Biogas generation (ml/g VS)	Max. Methane yield (ml/g VS)	Biogas increment %	Methane increment %
WS-1a	14.85	9.15	-	-
WS-3a	19.39	10.394	30.57	13.59
CS-1a	19.199	9.72	-	-
CS-3a	24.90	13.89	29.69	42.90
WS-1c	16.04	8.97	-	-
WS-3c	17.02	10.196	6.10	13.67
CS-1c	17.401	8.116	-	-
CS-3c	18.04	10.326	3.67	27.23

### 3.2. Influence of pretreatment on properties of wheat stalks and corn stalks

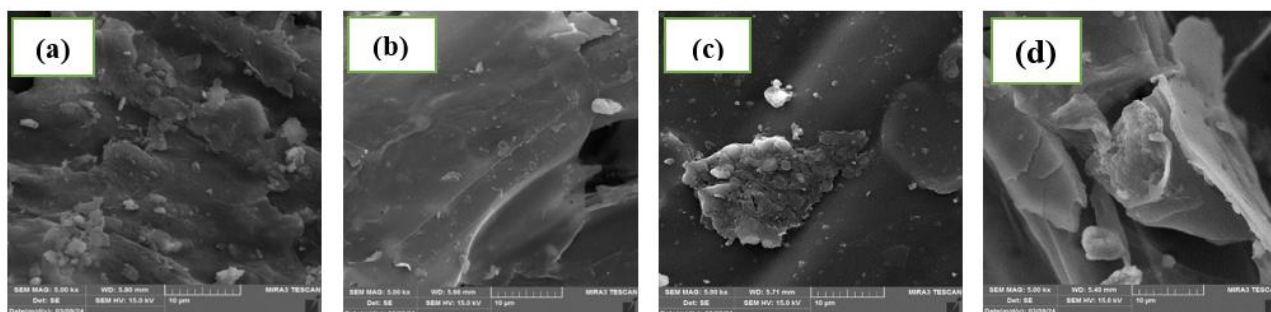
**Table 4** shows the TS, VS, ratio VS/TS, and C/N ratio of untreated and pretreated (ultrasonic and hydrothermal) wheat stalks and corn stalks. It was noticed that the value of VS/TS was higher for three tests for WS-1, WS-2, and WS-3, which were equal to 0.92, 0.99, and 0.94, respectively. And, for CS-1, CS-2, and CS-3 as equal to 0.96, 0.97, and 0.99, respectively. The results of this study were consistent with the previous research conducted by<sup>[10]</sup>, which showed that the high VS/TS ratios in the feedstock's (tomato plant residue (TPR), food waste (FW), and sheep manure (CM)) had similar and high VS/TS ratios. Consequently, the TPR, FW, and CM's high VS/TS ratios indicated that they possessed biodegradable potential for the AD process.

**Table 4.** Impact of pretreatment on characteristics of wheat stalks and corn stalks.

Variables	TS(%)	Reduction (%)	VS(%)	VS/TS	C (%)	N (%)	C/N
WS-1	93.02	-	85.74	0.92	49.72	0.91	54.63
WS-2	90	3.35	89.33	0.99	51.81	0.90	57.56
WS-3	92.4	0.67	87.04	0.94	50.48	0.93	54.27
CS-1	92.46	-	89.08	0.96	51.66	1.3	39.73
CS-2	97.6	5.27	95.08	0.97	51.14	1.2	42.61
CS-3	95.2	2.88	94.53	0.99	54.82	1.8	30.45

### 3.3. The impact of ultrasonic pretreatment on wheat stalk and corn stalks structure

**Figure 7** (a, b, c, and d) shows scanning electron microscopy (SEM) for untreated and pretreated wheat stalks and corn stalks substrates (ultrasonic pretreatment). For the 10  $\mu\text{m}$  magnification scale, SEM images. It is noticed from **Figure 7** (a and c) that the surface of the untreated wheat stalks and corn stalks, respectively, was connected without spaces between them and appeared as rough and thick. Whereas, in **Figure 7** (b and d) the surface of wheat stalks and corn stalks after being treated with the ultrasonic technique, respectively. The surface of wheat stalks was shown as smooth and slim in **Figure 7** (b), while it appeared as separate by spaces between them in **Figure 7**(d).<sup>[29]</sup> reported that physical structure through FE-SEM for untreated and pretreated rice straws. The FS-SEM picture of untreated rice straw shows a smooth and compacted surface, illustrating a rigid cell wall likely because of its intricate structure. Following the ultrasonic treatments, the surface structure has altered to have remarkably roughness with spaces and ruptures. An ultrasonic application can generate shock waves with sufficient amplitude to induce particle size reduction and degradation of greater molecules in the medium. In addition, the cavitation effect could potentially account for the alteration in the physical structure of the pre-treated rice straw.



**Figure 7.** SEM images of (a) untreated & (b) pretreated for wheat stalks and (c) untreated & (d) pretreated for corn stalks.

### 3.4. Impact of ultrasonic pretreatment on wheat stalk and corn stalks composition

According to previous research by<sup>[30]</sup> indicated that the absorption peaks in all regions decreased to some extent after the pretreatment of wheat straw. which implies that the lignocellulosic structure of wheat straw was got distorted. Consequently, these pretreatments altered the chemical structure of WS as compared to untreated wheat straw (raw material), due to the broken protective layer (hemicellulose-lignin) of cellulose. **Figure 8** (a &b) show Fourier transform infrared spectroscopy (FTIR) of untreated and ultrasonic pretreated for the wheat stalks and corn stalks. The absorption profile for untreated wheat stalks and corn stalks is greater than for treated wheat stalks and corn stalks with ultrasonic technique.

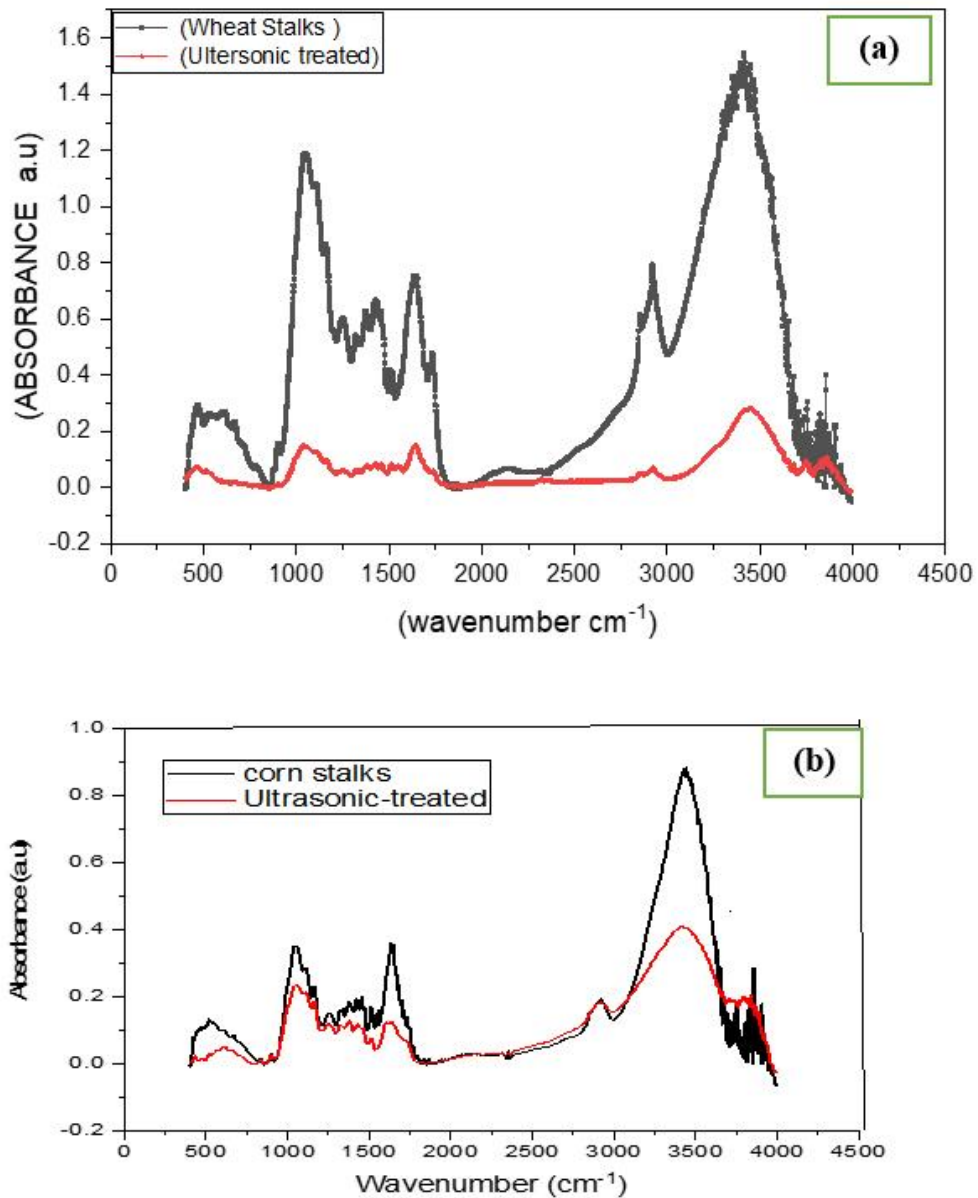
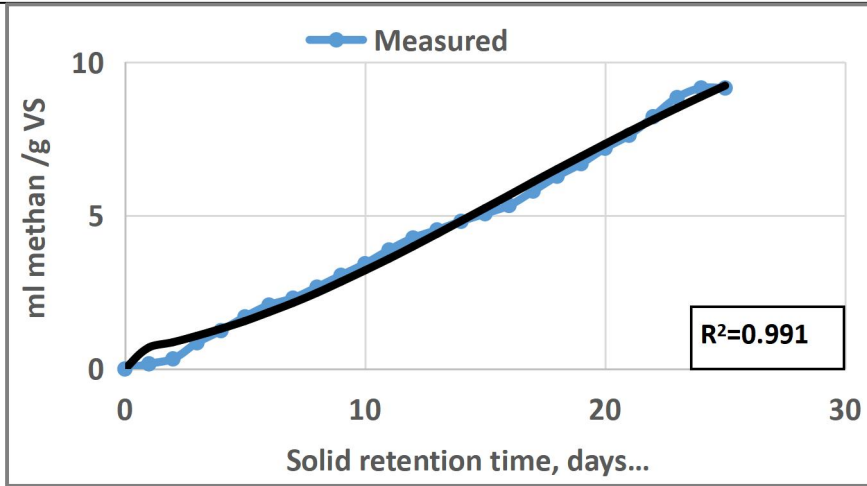


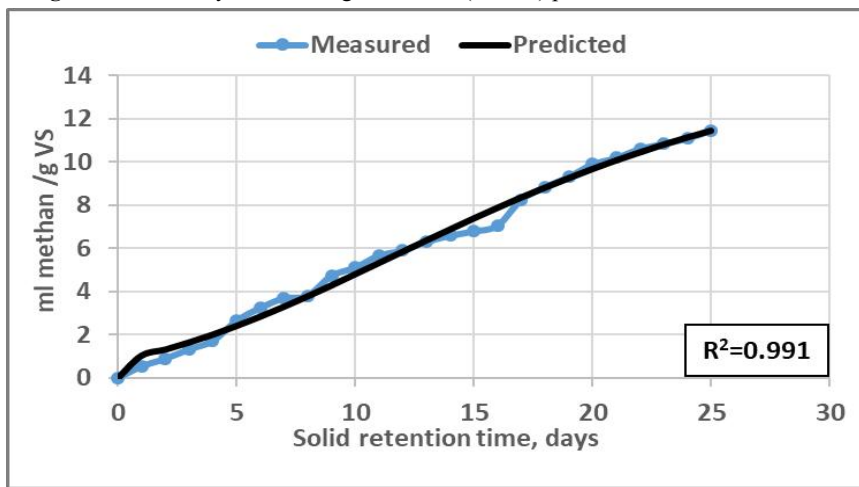
Figure 8. FTIR of untreated and ultrasonic pretreated for: (a) wheat stalks and (b) corn stalks.

### 3.5. Kinetic model

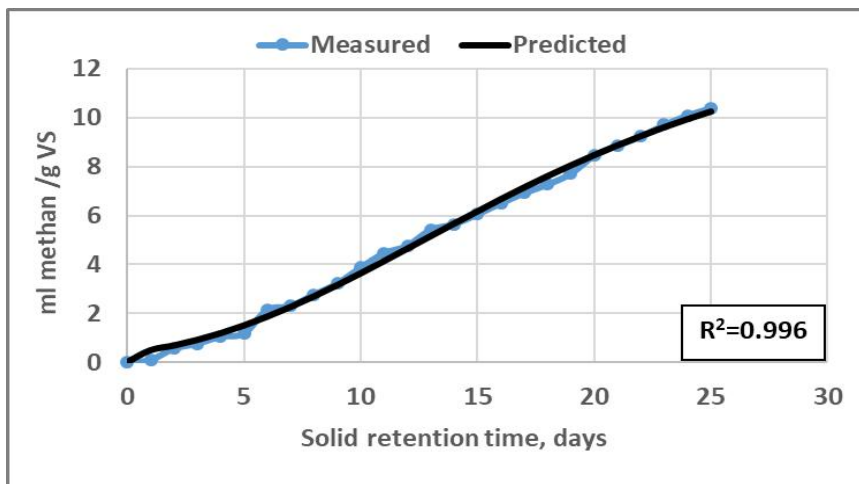
Figures (9-24) illustrate the extent of agreement between the predicted and measured values for digesters (1-16) which includes (WS-1a, WS-2a, WS-3a, WS-1c, WS-2c, WS-3c, WS-1d, WS-2d, CS-1a, CS-2a, CS-3a, CS-1c, CS-2c, CS-3c, CS-1d, and CS-2d), respectively. Moreover, Table 5 illustrates the Modified Gompertz Model's outcomes and kinetic constants. The predicted and measured values of biogas recovery were in good agreement, which was in accordance with the previously published research. The actual experimentation results were consistent with those results of the kinetic prediction using a modified Gompertz model as demonstrate by<sup>[25]</sup>. Also study by <sup>[24]</sup> showed that the kinetic investigate of the co-digestion process of GR for biogas recovery explained a strong agreement between the measured and predicted values obtained by the Modified Gompertz Model. The correlation coefficients valued at > 0.98 indicate that the conditions for co-digestion of inoculated GR are favorable.<sup>[31]</sup> proved that the values predicated of the biogas production from using modified Gompertz model were well fitted with experimental values with R2 > 0.96, suggesting favorable conditions of the digestion process.



**Figure 9.** Methane yield from digester No. 1 (WS-1a) predictions and measurements.



**Figure 10.** Methane yield from digester No. 2 (WS-2a) predictions and measurements.



**Figure 11.** Methane yield from digester No. 3 (WS-3a) predictions and measurements.

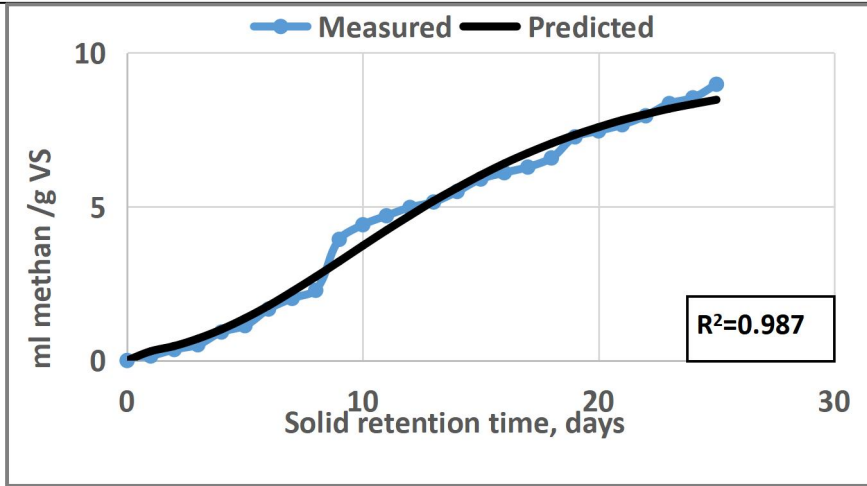


Figure 12. Methane yield from digester No. 4 (WS-1c) predictions and measurements.

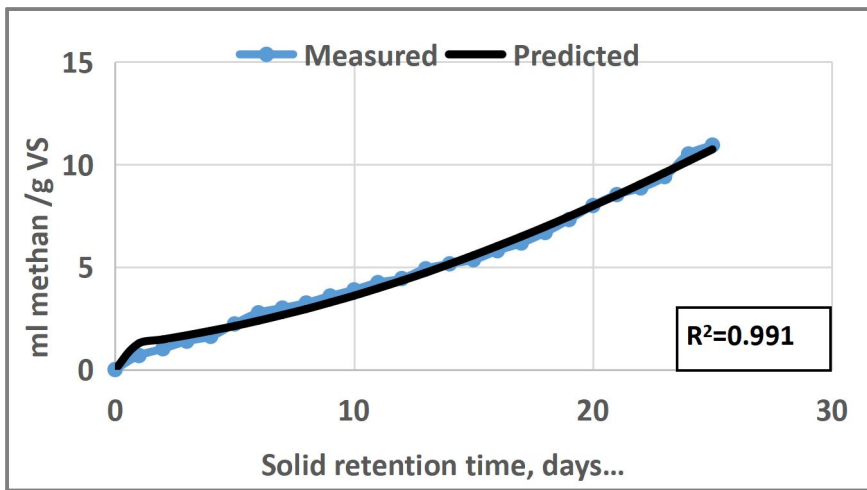


Figure 13. Methane yield from digester No. 5 (WS-2c) predictions and measurements.

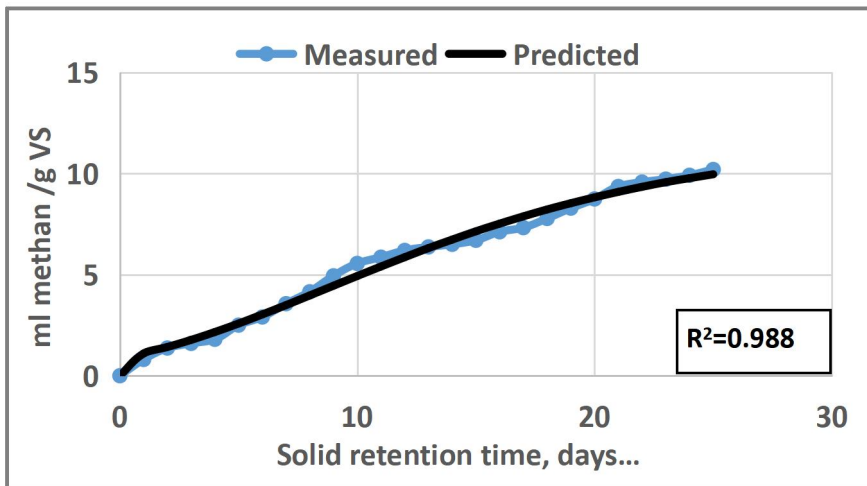


Figure 14. Methane yield from digester No. 6 (WS-3c) predictions and measurements.

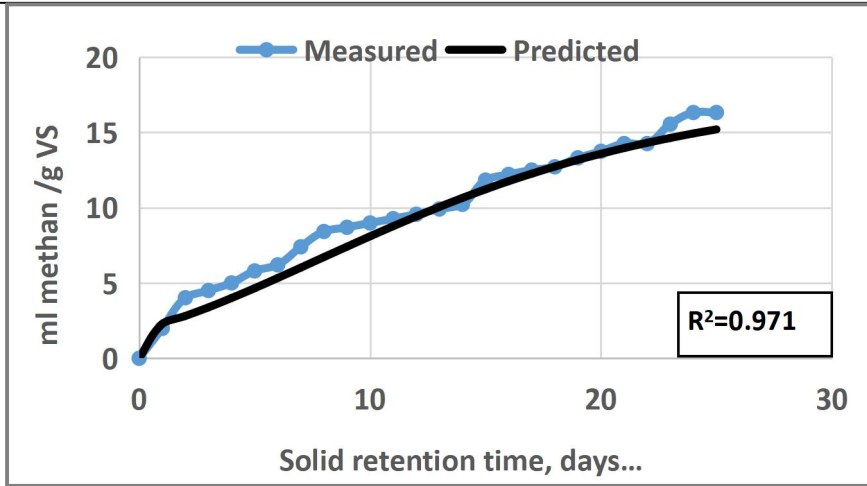


Figure 15. Methane yield from digester No. 7 (WS-1d) predictions and measurements.

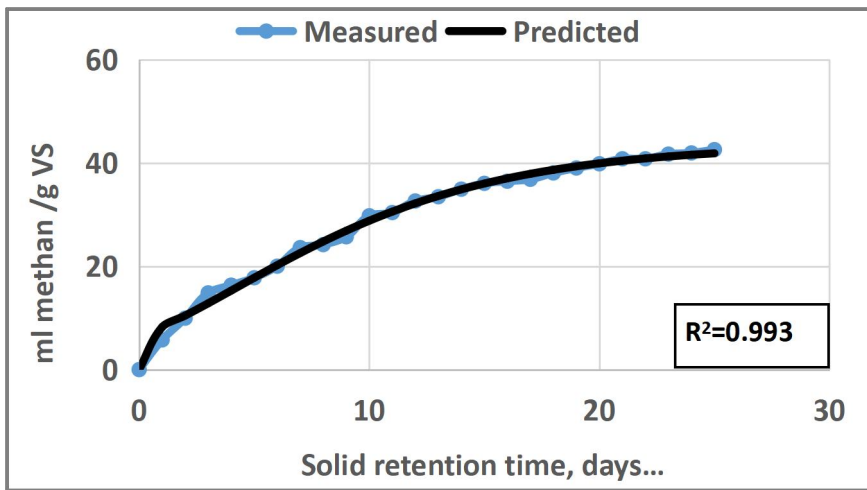


Figure 16. Methane yield from digester No. 8 (WS-2d) predictions and measurements.

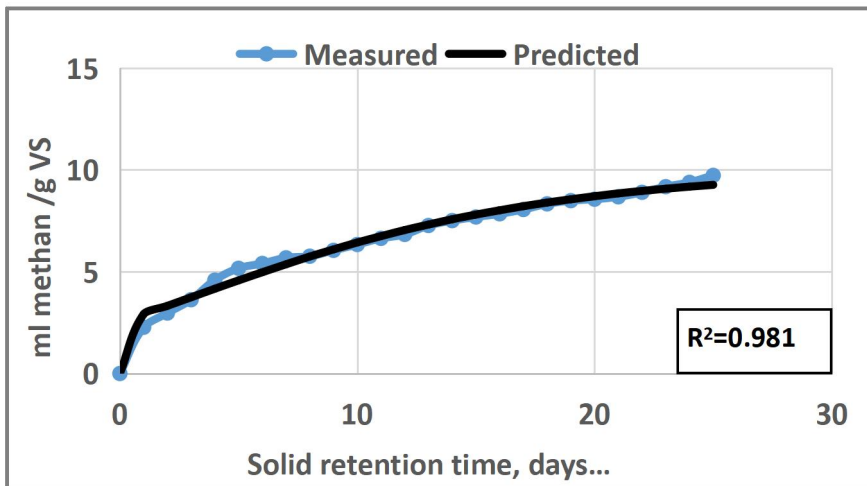


Figure 17. Methane yield from digester No. 9 (CS-1a) predictions and measurements.

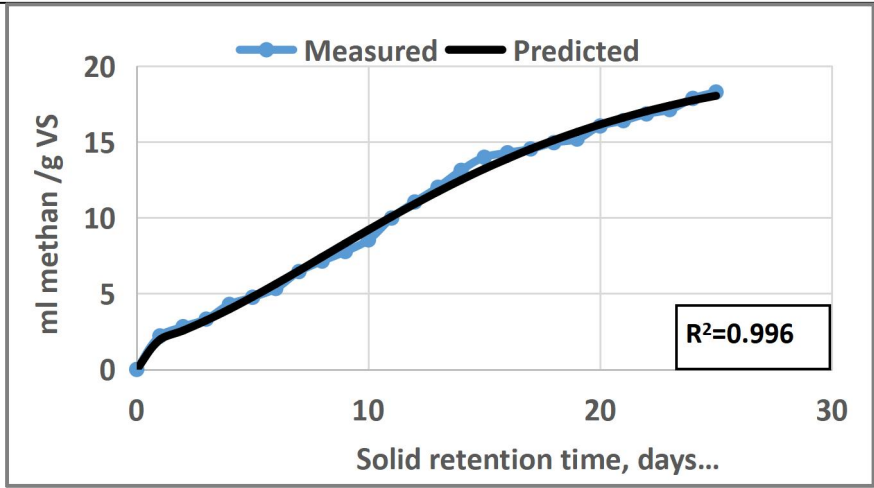


Figure 18. Methane yield from digester No. 10 (CS-2a) predictions and measurements.

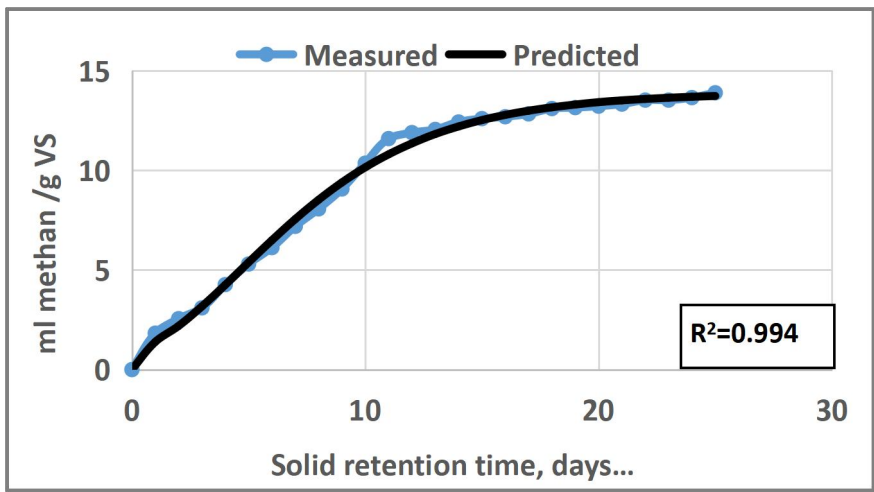


Figure 19. Methane yield from digester No. 11 (CS-3a) predictions and measurements.

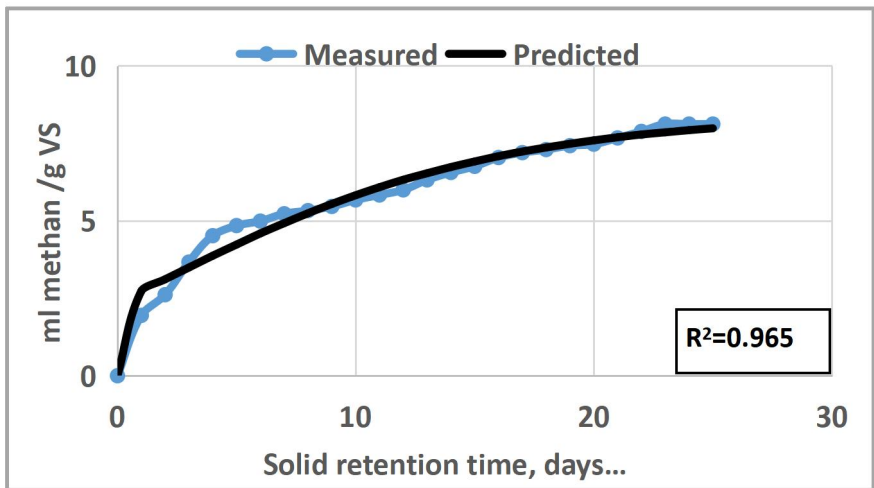


Figure 20. Methane yield from digester No. 12 (CS-1c) predictions and measurements.

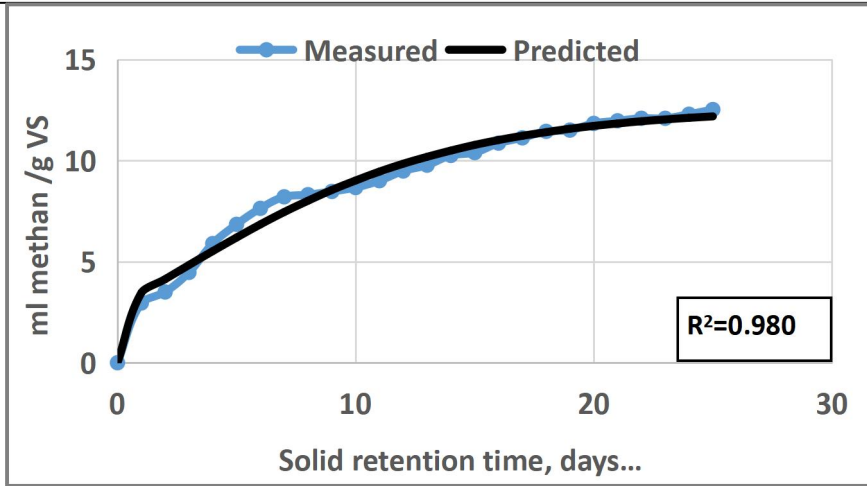


Figure 21. Methane yield from digester No. 13 (CS-2c) predictions and measurements.

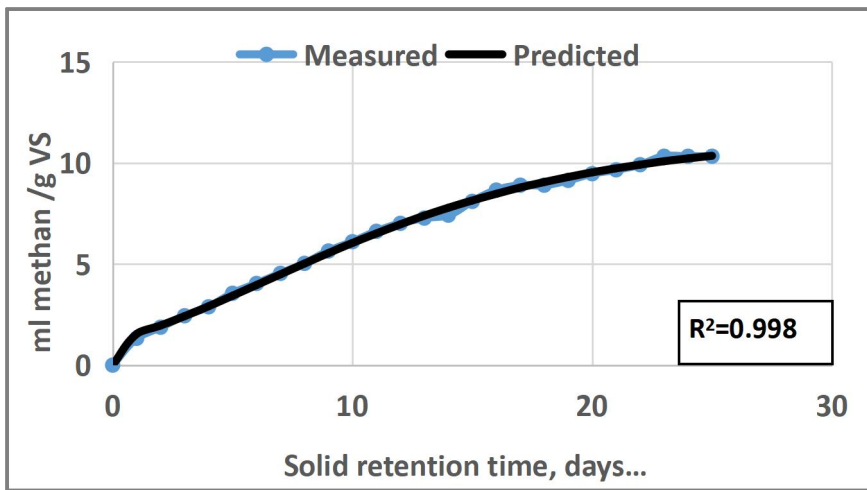


Figure 22. Methane yield from digester No. 14 (CS-3c) predictions and measurements.

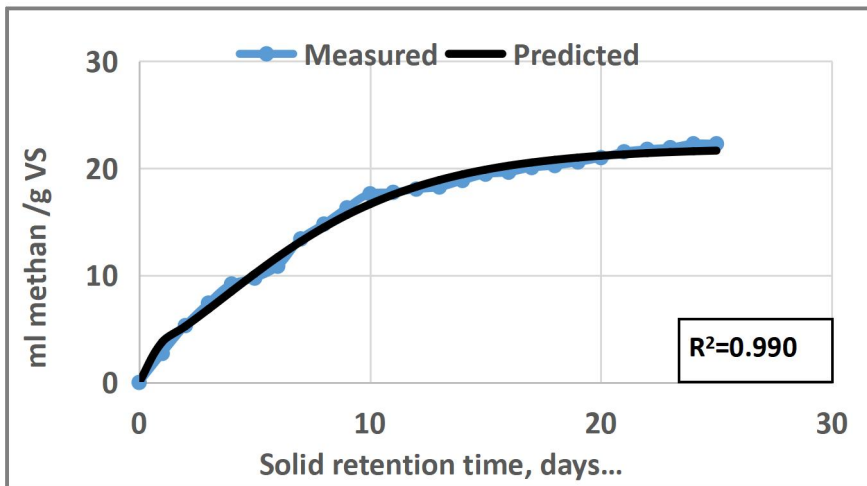


Figure 23. Methane yield from digester No. 15 (CS-1d) predictions and measurements.



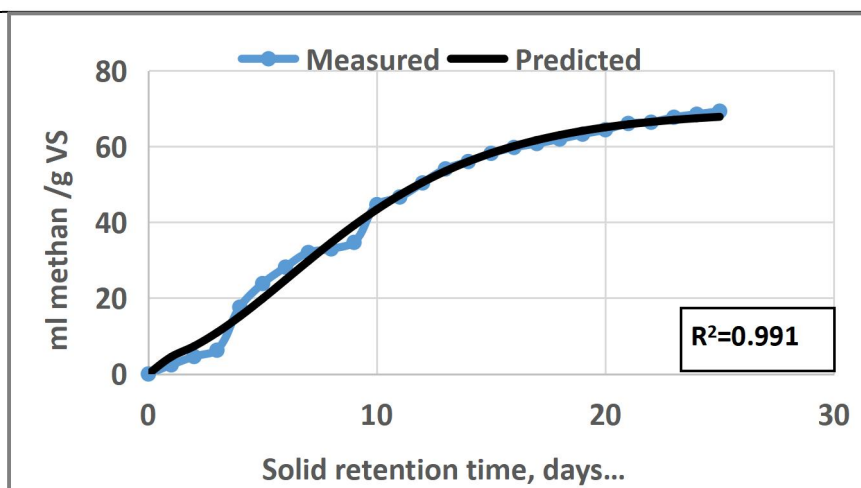


Figure 24. Methane yield from digester No. 16 (CS-2d) predictions and measurements.

Table 5. The modified Gompertz model yielded the following results.

Digester No.	G(t) exp. (ml methane/g VS)	Gompertz model parameters				R2
		$\lambda$ (day)	R max. (ml methane/g VS)	G0 (ml methane/g VS)	G(t) model (ml methane/g VS)	
1	9.15	2.648	0.424	9.15	9.23	0.991
2	11.46	0.837	0.523	11.46	11.43	0.991
3	10.39	2.957	0.513	10.39	10.24	0.996
4	8.97	2.640	0.506	8.97	8.47	0.987
5	10.94	7.709	0.614	10.94	10.73	0.991
6	10.20	0.255	0.481	10.20	9.96	0.988
7	16.32	1.622	0.628	16.32	15.20	0.971
8	42.56	2.161	2.480	42.56	41.85	0.993
9	9.72	5.910	0.419	9.72	9.26	0.981
10	18.29	0.212	0.902	18.29	18.04	0.996
11	13.89	0.251	1.132	13.89	13.73	0.994
12	8.12	6.136	0.382	8.12	7.98	0.965
13	12.52	3.929	0.696	12.52	12.18	0.980
14	10.33	1.400	0.535	10.33	10.35	0.998
15	22.29	1.112	1.663	22.29	21.65	0.990
16	69.30	1.035	4.997	69.30	67.81	0.991

## 4. Conclusions

The results obtained from the anaerobic co-digestion of wheat and corn stalks pretreated and inoculated with animal manure were better than those of untreated wheat and corn stalks. In addition, the ultrasound pretreatment of wheat and corn stalks gave better results than the thermal pretreatment in the generation of biogas and methane. The co-digestion of wheat and corn stalks with ostrich manure produced higher amounts of biogas and methane than cow and sheep manure. Higher biogas and methane production was observed for corn stalks than for wheat stalks. The scanning electron microscope analysis results showed clear distortion in the composition of both agricultural wastes (wheat and corn stalks).

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## Author Contributions

Israa Nsaif Jasim (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. Amal. Khalil (supervisor) compiled the manuscript preparation.

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## Conflict of interest

The authors declare no conflict of interest.

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