Methane Production Potential of Rumen Pretreated Lignocellulosic Wastes

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Abstract

Bioenergy production from lignocellulosic biomass is challenging due to its structure and a pretreatment is required before methane production. In this study, biological pretreatment by using rumen microorganisms was applied for different types of lignocellulosic wastes: wheat straw, cotton stalk, reeds and sunflower stalk. The reactors were pretreated for 2, 5, 10, 15 and 20 days. After the pretreatment stages and gas measurements were done, reactors were separated into two phases as lower solid phase and upper liquid phase. The reactors were installed for the methanation stage, gas measurements were made at regular intervals and graphs were drawn using the cumulative results. Modified Gompertz equation was used to estimate potential biogas production. According to the results, the reactor containing 5 days pretreated wheat straw became prominent among the other reactors in terms of biogas and methane production with 163 ml and 102 ml, respectively. It was followed by 20 days pretreated reeds with 104 ml biogas and 80 ml methane, 2 days pretreated sunflower stalk with 88 ml biogas and 52 ml methane, and 2 days pretreated cotton stalk with 87 ml biogas and 50 ml methane.

1. Introduction

The rapid increase in population and industrialization brought about the need for alternative energy resources. Biomass energy is one of the most important resources to be used in order to provide energy in a sustainable way without causing environmental pollution. Biomass is a non-fossil organic matter of biological origin. Food industry residues and wastes, forest residues, agricultural wastes, animal wastes, municipal wastes and all plants are within the definition of biomass. The improper management of biomass origin wastes results in environmental pollution. Especially the wastes caused by the processing of agricultural products occupy space in the facility sites due to storage problems and sometimes cause environmental problems by being given to surface waters (Baran et al. 1995). According to the U.S. Energy Information Administration (2020), biomass can be used to obtain energy directly by burning as well as converted into liquid biofuels or biogas; in turn it contributes economically while reducing the environmental damage. Biomass is a renewable energy source and can reduce the demand for fossil fuels in energy. The energy produced from biomass is clean, so that it can improve the environment, economy and energy security (Gokcol et al. 2009). Lignocellulosic waste is mostly generated in agricultural and food industries, and is an abundant type of biomass (Oh et al. 2018). Lignocellulose is present on woody or herbaceous plant cell walls and consists of two complex carbohydrates, cellulose and hemicellulose, that are enclosed by a lignin matrix (Zhang et al. 2016). Lignocellulosic biomass can be converted into biofuels by biological conversion, since cellulose and hemicellulose present in lignocellulose are fermentable sugars (Chuetor et al. 2019). However, the breakdown and hydrolysis of the lignocellulosic biomass becomes a rate limiting step during anaerobic digestion. The complex matrix structure of lignocellulose gives rise to need for pretreatment, where it is fractionated into lignin, hemicellulose and solid substrate (Islam et al. 2020). Fractionation of biomass ensures maximization of substrate accessibility and increases sugar and biogas yields (Van Fan et al. 2019; Leu and Zhu et al. 2013; Zhao et al. 2017).

There are 4 main types of lignocellulosic pretreatment: physical, chemical, physico-chemical and biological pretreatment. Physical pretreatment methods include particle size reducing processes such as chipping-milling (Ramos 2003), microwave (Jackowiak et al. 2011), and freezing (Chang et al. 2011). Physical pretreatment methods increase cellulose digestion for hydrolysis. However, these technologies are not economically feasible for the use of a large-scale bio-refinery in terms of capital and energy costs (Kumari and Singh 2018). In chemical pretreatment methods; acids (Sen et al. 2016), oxidizing agents (Song et al. 2016), NaOH (Antonopoulou et al. 2016) and organic solvents (Zhao et al. 2009) are the most commonly used substances. Chemical pretreatment methods promote methane production, but cannot be applied to large-scale fermenters due high energy consumption, neutralization of wastewater, production of fermentation inhibitors, high reagent costs and low pretreatment rates (Hendriks and Zeeman 2008). Biological pretreatment methods include using enzymes (Romano et al. 2009), fungi (Song et al. 2013), and microbial consortium (Zhang et al. 2011) for dissociation of lignocellulosic biomass. These methods have important advantages compared to other pretreatment methods since they are environmentally friendly and safe. Biodegradation of lignocellulose is already taking place in a known natural cycle. The removal of lignin from lignocellulose occurs naturally by the microorganisms in the rumen of ruminant animals such as sheep and cows (Güllert et al. 2016).

Rumen bacteria found in the digestive system of ruminant animals are also used for the biological pretreatment of lignocellulosic biomass. Ruminant animals use cellulosic materials in plants as an energy source by converting them into volatile fatty acids (VFA) by fermentation as a result of the symbiotic relationship of the microbial population in their digestive systems (Forsberg et al. 1997). High cellulolytic activities of rumen bacteria constitute a significant advantage in the degradation of lignocellulosic wastes (Quintero et al. 2012).

Due to its environmental friendly and inexpensive properties, several studies have focused on biological pretreatment methods such as using fungal consortium on hydrolysis of biomasses such as corn stover (Song et al. 2013) and straw (Taha et al. 2015). Satisfactory increase in hydrolysis rates were obtained in these studies.

In this study, the effect of biological pretreatment on biomethane production from lignocellulosic wastes was examined. 4 different wastes, which are cotton stalk, wheat straw, reeds, and sunflower stalk, were pre-treated with rumen microorganisms for 2, 5, 10, 15, and 20 days to determine the optimum retention period of pretreatment. Pretreated samples were fed to an anaerobic bioreactor to produce biomethane and obtained results were modeled with the Modified Gompertz equation to compare the effect of pretreatment time.

2. Materials And Methods

2.1 Sample Collection and Characterization

4 different agricultural wastes; cotton stalks, sunflower stalks, wheat straw, and reeds were used for biological methane potential analysis. Cotton and sunflower stalk wastes were provided from Sanliurfa

and Kayseri, which are cities located in the southeast and central of Anatolia, respectively. Wheat straw and reed wastes were obtained from Istanbul.

The samples were carried to the laboratory within plastic bags and were dried at a constant temperature of 60ºC overnight. Dried wastes were ground with a grinder to 1-2 cm and stored in plastic bags at room temperature until the experiment.

Elemental carbon, nitrogen, and sulfur analysis of dried samples were carried out following TS ISO 10694, TS ISO 13878, and ASTM E 775-15, respectively. The characterization of agricultural wastes is given in Table 1. The carbon content of wastes was quite high and nitrogen content was low, which promises a high biogas production potential. Cotton stalks had the highest carbon content among all wastes.

Table 1 Elemental characterization of wheat straw, reeds, sunflower stalk and cotton stalk wastes

Waste / Parameter	Wheat Straw	Reeds	Sunflower Stalk	Cotton Stalk
C (%)	43.2	43.7	43.9	46
N (%)	0.75	0.76	0.45	0.84
S (%)	0.31	0.28	0.14	0.27
C/N	57.6	57.5	97.6	54.8

2.2 Rumen and Methanogenic Seed

Rumen fluid was obtained from a healthy, non-medicated sheep that was kept at a veterinary faculty of a university. Rumen fluid was obtained 2 hours after morning feeding by the orogastric collection method and fresh sample brought to the laboratory immediately. Rumen culture was used directly for reactor setup without any enrichment process.

Granular anaerobic sludge inoculum was supplied from a gum factory treating its wastewater via anaerobic digestion under mesophilic conditions.

2.3 Pretreatment of Wastes

The effect of pretreatment on biomethane production of agricultural wastes was examined by the set-up of a two-stage reactor. The first stage was pretreatment and second biomethane production reactors.

In the first stage, 1 g of ground agricultural waste, 1.5 ml of rumen fluid, 45 ml nutrient solution was added to a glass reactor which having 100 mL total volume. 1 liter of nutrient medium required for microbial activity was prepared as follows: 8g/L $NaHCO_3$; 1 g/L KH_2PO_4; 3g/L K_2HPO_4; 0.03 g/L $CaCl_2.2H_2O$; 0.08 g/L $MgCl_2.6H_2O$; 0.18 g/L NH_4Cl; 0.173 g/L L-cysteine. 1 liter of metal solution was prepared as follows: 0.1 g/L $ZnSO_4.7H_2O$; 0.03 g/L $MnCl_2.4H_2O$; 0.3g/L H_3BO_3; 0.2 g/L $CoCl_2.H_2O$; 0.01

g/L $CuCl_2.2H_2O$; 0.02 g/L $NiCl_2.6H_2O$; 0.03 g/L $NaMoO_4.2H_2O$ and 1.5 g/L $FeCl_2.4H_2O$ (Hu and Yu 2006). 0.2 g/L 2-bromoethanesulfonic acid (BESA) was also added to prevent methanogenic activity.

Different reactors were conducted to observe the effect of pretreatment time on biomethane production from lignocellulosic wastes. Therefore, different reactors were prepared for 2, 5, 10, 15, and 20 days incubation times for 4 different agricultural wastes. Each reactor was studied in duplicate to observe the consistency of the experiment data. 40 pretreatment reactors were flushed with N_2 gas to remove dissolved oxygen and provide the anaerobic condition. Reactors were incubated under mesophilic conditions in a shaker at 145 rpm. Biogas production followed with the help of syringes. After the pretreatment period complements, and then the reactors were opened.

2.4 Reactor Setup for Pretreated samples

The reactors whose pretreatment times have expired were opened. Reactors were centrifuged to separate the solid and liquid phases. The biomethane potential of solid and liquid phases was investigated separately.

Each pretreated solid and liquid sample was fed to biomethane reactors with nutrient medium and 0.5 g of granular anaerobic seed sludge to support methanogenic activity. So, 80 biomethane reactors composed of 40 liquid phases and 40 solid phases were obtained in total. Blank reactors were also prepared for each non-pretreated agricultural waste by adding raw waste, media, and inoculum. All reactors were flushed with N_2 gas for 5 minutes to provide the anaerobic condition and sealed with rubber stoppers. After the anaerobic conditions were provided, the reactors were continuously stirred at 120 rpm under mesophilic conditions.

Biogas production was followed and the biogas composition of all reactors was analyzed by gas chromatography (GC) (GC-2014, SHIMADZU Gas Chromatograph, SHIMADZU Corporation, Japan) to determine methane and carbon dioxide composition.

2.5 Modeling

Modified Gompertz equation was used to determine the kinetic constants of biogas production curves. Kinetic parameters were calculated through the nonlinear regression method by using the MS Excel tool developed by Demir et al. (2018). The kinetic coefficients obtained from the model played an important role in analyzing the effect of biological pretreatment on biogas production efficiency from agricultural wastes. The modified Gompertz equation given in Equation 1 can be used in a wide range of study from microbial activity to biogas production and given as follows:

$$B = P * exp\left\{-exp\left(\frac{R_m * e}{P}(\lambda - t) + 1\right)\right\} \qquad \text{Equation 1}$$

Where B is the cumulative biogas yield in ‘t’ days (L/kg waste); P is the maximum biogas yield potential (L/kg waste); R_m is the maximum specific biogas yield (L/kg waste/day); λ is lag phase period (days) and t is the time for biogas production (days).

3. Results And Discussion

3.1 Pretreatment Stage

The wastes were pre-treated with rumen microorganisms for 2, 5, 10, 15, and 20 days and at the end of this period, the wastes were fed to anaerobic batch reactors for biomethane production. Since a part of biomass can be consumed by mixed culture for biogas production during pre-treatment, the biogas formation was also monitored in this step. The data obtained from the pretreatment was examined based on the type of lignocellulosic waste.

Since BESA was added to the pretreatment bioreactors in the reactor set-up, the biogas contains only CO_2. Although the gas generated during the pretreatment was valueless, monitoring biogas production was important in terms of controlling the biodegradation of biomass. The graphs showing amount of biogas produced in pretreatment stages were given in Fig.1.

According to the biogas amounts generated during the pretreatment given in Fig.1 were examined, the amount of biogas production during the pretreatment increases as the pretreatment time increases.

The best pretreatment result for wheat straw was observed in the 20 days pretreatment with 65.75 ml biogas formation. This result was followed by 58 and 32.1 ml biogas formations in 15 and 10 days pretreatments, respectively. A gradual increase in biogas production was observed for wheat straw waste as the pretreatment time increased. The lowest biogas production was observed from reactors pretreated for 2 days with 3.3 ml biogas. The effect of persistent action of cow rumen pretreatment on degradation of wheat straw has been studied by Xing et al. (2020). At the end of 33 days pretreatment, approximately 240 ml of biogas was obtained with the volatile fatty acids (VFAs) yield of 0.453 g COD g^{-1} VS. The best pretreatment and degradation result for cotton stalk was obtained from 20 days pretreatment period with 24.5 ml biogas. Biogas formation in 15 and 10 days pretreatments of cotton stalk were 10.5 and 4.75 ml, respectively. The pretreatment for 2 days had no effect on the degradation of cotton stalk and biogas formation was not observed. The effect of a thermophilic microbial consortium (MC1) pretreatment on degradation of cotton stalk has been studied by Yuan et al. (2016). The substrate with 4.0% load pretreated for 8 days resulted with the largest amounts of soluble chemical oxygen demand (COD). The best pretreatment and, consequently, the degradation result for reed was observed in the pretreatment period of 20 days with 43.25 ml. 19 ml and 13.55 ml biogas was obtained in 15 and 10 days pretreatments. Biogas output was not observed in the reactors pretreated for 2 and 5 days, in other words, 2 and 5 days pretreatments had no effect on the degradation of reed. The largest amount of biogas formation in pretreatments of sunflower stalk was obtained from the 10 days pretreated reactor with 29.8 ml. Thus, 10 days pretreatment for sunflower stalk waste was observed as the best degradation period. It is followed by 15 and 5 days pretreatments with 28 and 26.5 ml biogas formations, respectively. 2 days pretreatment was observed as the pretreatment time that has the least effect on the structure of the sunflower stalk waste with 7.25 ml of biogas. Biological pretreatment of sunflower stalk with a type of fungus *T. reesei* has been studied and compared with alkali pretreatment for bioethanol production by Manmai et al. (2019). However, the biological pretreatment was below the alkali treatment in terms of sugar yields.

3.2 Biological Methanation Stage

The upper and lower phases of the reactors whose pretreatment period has been completed were separated and methanation reactors were installed for each of them. Gas measurements were made at regular intervals with the help of syringes, the values were recorded and plotted. The gas measurement data was compared with non-linear regression using the modified Gompertz equation to estimate the kinetic parameters. The kinetic parameters are shown in Table 2.

Table 2 Kinetic parameter results of raw and pretreated reactors

Waste Type	Pretreatment Time	P	R_m	λ	R^2
Wheat Straw	Raw	141.30	4.04	5.19	0.995
	2 days	190.27	10.84	2.24	0.989
	5 days	184.91	4.84	0	0.983
	10 days	144.25	4.65	0	0.945
	15 days	120.15	3.54	0	0.994
	20 days	116.25	4.21	1.21	0.994
Cotton Stalk	Raw	59.02	1.54	4.27	0.980
	2 days	68.37	2.60	0.52	0.985
	5 days	73.89	2.42	0	0.964
	10 days	87.37	1.35	0	0.939
	15 days	74.62	1.69	0.97	0.981
	20 days	51.87	2.92	2.51	0.994
Reeds	Raw	93.94	1.68	9.29	0.985
	2 days	102.78	2.16	4.67	0.993
	5 days	100.09	3.40	5.13	0.991
	10 days	93.87	2.49	2.84	0.995
	15 days	94.55	2.19	1.84	0.987
	20 days	94.30	2.21	3.94	0.993
Sunflower Stalk	Raw	80.77	1.82	3.98	0.981
	2 days	91.18	3.57	0.05	0.949
	5 days	87.80	3.24	0	0.957
	10 days	65.79	2.98	0	0.860
	15 days	49.97	1.61	0.73	0.987
	20 days	49.72	1.79	2.83	0.995

The correlation coefficient (R^2) of the modified Gompertz equation was generally higher than 0.95, suggesting that the modified Gompertz equation fits the biomethane production profile and the final biomethane yield (Table 2). But it was insufficient to model biogas production curves completed in two-stage, such as pretreated sunflower and cotton stalks for 10 days.

The pretreatment of lignocellulosic wastes allowed faster hydrolysis and a shorter acclimation time for microorganisms (Mao et al., 2017; Bianco et al., 2020), as approved by the reduction of λ value. Even though, the optimum pretreatment time changed with the type of the waste. Based on lag phase reduction of wastes, the optimum pretreatment time was 2 days, 5 days, 5 days, and 15 days for sunflower stalks, wheat straws, cotton stalks, and reeds, respectively.

The bottom phase of the wheat straw pretreated for 2 days reached 150 ml of biogas in 60 days of methanation stage. The biogas formation in the upper phase started to rise on the 10th and 40th days. It is understood that the lignocellulosic material in the upper phase breaks down in 2 stages. A total of 160 ml biogas was obtained from the combination of the upper and lower phases of straw waste after 60 days.

146 ml of biogas was obtained after 80 days when the bottom phase of 5 days pretreated wheat straw was examined. An increase in production is observed as of the 36th day. In the upper phase, 17 ml of biogas was produced in 43 days. This value is 3 times more efficient than the upper phase of the straw, which was pretreated for 2 days. This shows that more substances dissolved in the upper phase in 5 days pretreatment. The total biogas yield from straw is 162 ml in 80 days.

The methanation stage of wheat straw lower phase after 10 days of pretreatment resulted in 90 ml of biogas in 72 days. Production started after the 4th day. A fluctuation occurred after the 33rd day. The production is completed in 45 days in upper phase, resulting in 49 ml of biogas. As in the lower phase, fluctuation occurred in the upper phase on the 33rd day. The yield from straw in total is 139 ml.

Continuous biogas production was observed during methanation of 15 days pretreated straw bottom phase and 69 ml of biogas is obtained after 40 days. In the upper phase, 22 ml of biogas was produced in 68 days. A decrease was observed according to the 10-day pretreatment straw upper phase. In total, the yield from the straw is 91 ml of biogas. A low yield was obtained for 10 days compared to pretreatment.

Considering the lower phase of 20 days pretreated wheat straw values, approximately 73 ml of biogas production was obtained in 74 days. The straw bottom phase production did not reach equilibrium for the first time in the biogas measurement process. Production continued with the fluctuation on the 60th day. Nevertheless, 2 and 5 days pretreatments are more advantageous for straw than 20 days pretreatment. According to the straw waste upper phase values, approximately 44 ml of biogas was produced in 60 days. There was an increase in the yield compared to the upper phase of the straw pretreated for 15 days. The total yield from the straw is 116 ml of biogas. 20 days pretreatment time for straw is more advantageous than 15 days pretreatment.

Comparison of biogas generated in methanation stages of raw and pretreated wheat straw reactors and model results was given in Fig.2. Pretreatments for 2, 5 and 10 days had an increasing effect on biogas production. This shows the biological pretreatment induced the degradation of wheat straw waste with lignocellulosic structure (Ferraro et al. 2018). However, lower biogas production was observed in the reactors pretreated for 15 and 20 days when they are compared with biogas generated in the methanation of raw wheat straw waste.

The biogas production continued up to 80 days by the lower phase of cotton stalk during the methanation process after 2 days of pretreatment. 75 ml of biogas was produced in 60 days and 86 ml of biogas was produced at the end of 80 days. After reaching the 40th day, an increase was observed after stabilization. Finally, the biogas production stopped completely when methanation time was reached up to 80 days. The biogas production in the upper phase of cotton stalk started on the 7th day and ended on the 14th day. However, the amount produced was approximately 1 ml and it was neglected.

The amount of biogas produced by methanation of the bottom phase of cotton stalk pretreated for 5 days reached 53 ml of biogas in 50 days and then production stopped. There was an increase in biogas production after the first two days. In the upper phase of cotton waste, there was a fluctuation following a delayed production on 7th, 14th and 35th days. In 49 days, 12 ml of biogas was measured and production ended. After combining the lower and upper phase of the cotton stalk values after 5 days of pretreatment, 65 ml of biogas yield was obtained from the cotton stalk in 49 days.

The biogas performance of the cotton stalk lower phase after 10 days of pretreatment resulted in 69.5 ml of biogas in 60 days. A significant fluctuation was observed on day 33. Production started after the 4th day in the upper phase. Biogas production was not taken into account for the cotton upper phase, since the amount obtained in more than 40 days was limited to about 6 ml of biogas. A total of 75 ml of biogas was obtained in 60 days from 10 days pretreated cotton stalk phases.

56 ml of biogas was obtained from the methanation of 15 days pretreated cotton stalk lower phase in 50 days, and the production was completed. 15 ml of biogas was formed in 50 days in the upper phase. Fluctuations occurred on the 21st and 41st days in the lower and upper phases. This indicates that the lignocellulosic biomass was gradually breaking down. Upper phase cotton stalk yield increased compared to the upper phase which was pretreated for 10 days. The total yield obtained from cotton stalk with 15 days of pretreatment is 71 ml of biogas in 50 days. A yield close to the total yield of cotton stalk that was pretreated for 10 days was obtained.

When the lower phase values of 20 days pretreated cotton stalk are examined, approximately 29 ml of biogas was produced in 6 days and the production stopped. In the upper phase, a biogas yield of 5 ml was obtained in 24 days and the production was completed. The total yield from cotton stalk is approximately 34 ml of biogas in 40 days. 20 days were the most inefficient pretreatment time for pretreatment cotton stalks. Comparison of biogas generated in methanation stages of raw and pretreated cotton stalk wastes and model results was given in Fig.3. Biogas production was observed to be increased significantly when cotton stalk waste was pretreated until 15 days.

After 2 days of pretreatment and methanation, 27.75 and 46.9 ml biogas was produced in 30 and 60 days, respectively, in the bottom phase of reed waste. Production increased between the 40th and 50th days. Lignocellulosic biomass was fragmented in 2 stages and a peak was observed in the biogas production after 80th day. It is assumed that the reason for this may be internal breathing. In the upper phase, the fragmentation started on the 7th day and ended on the 14th day. A low yield of 3.8 ml was obtained. This result shows that almost all of the lignocellulosic biomass that had dissociated in the pretreatment stage remained in the lower phase.

Fluctuations in the biogas production were observed on the 7th, 14th, 35th and 85th days of the 5 days pretreated reed bottom phase. 86 ml of biogas was obtained on the 90th day. These results are similar with the 2 days pretreated bottom phase. In the upper phase, there are fluctuations on the same days. However, due to the lack of lignocellulosic biomass in the upper phase, the amount reached in 42 days is only 7.5 ml of biogas. The total yield from combination of biogas produced in 5 days pretreatment of lower and upper phase reed waste is 93 ml in 91 days.

67.55 ml biogas was obtained from the methanation of the bottom phase of reed in 84 days after 10 days pretreatment. Fluctuations were observed on 33rd, 55th and 77th days. The upper phase completed production in 45 days and 25 ml of biogas was produced. A total of 93 ml of biogas was produced in 84 days from both phases of reed waste that was pretreated for 10 days.

Approximately 82 ml biogas was produced in 78 days, during methanation of 15 days pretreated reeds bottom phase. During the biogas measurement process, the continuous production was observed as in the previous reeds phases. However, when the pretreatment time increases, an increase was observed in biogas production performance for reeds. Approximately 23 ml of biogas was measured in 40 days in the upper phase. Total yield obtained from reed waste that was pretreated for 15 days is approximately 105 ml of biogas in 78 days. According to the total yield of the reed that was pretreated for 10 days, the yield was increased in 15 days pretreatment.

Considering the lower phase of 20 days pretreated reeds data, approximately 84 ml of biogas was produced during 60 day incubation period and the production was completed. The reeds lower phase has reached the equilibrium at the end of the measurement for the first time. Considering the upper phase values, approximately 20 ml of biogas was produced in 40 days. Production started to increase on the 14th and 30th days. The total yield obtained from the reeds waste is 104 ml of biogas in 60 days. 15 and 20 days of pretreatments are the best methods for reeds, in terms of biogas volume. Comparison of biogas generated in methanation stages of reactors which contain pretreated reeds wastes and model results was given in Fig.4.

Biogas production reached 40 ml on the 10th day, 61 ml on the 30th day and 72 ml on the 60th day when the lower phase of sunflower stalk was examined after 2 days of pretreatment. 16.25 ml of biogas was obtained and production no longer continued as of the 45th day in the methanation reactor of upper phase sunflower stalk waste. Looking at the combined graph, it is seen that a total of 88 ml of biogas was obtained from the sunflower stalk in 60 days.

The amount of biogas produced by methanation of the bottom phase of sunflower stalk pretreated for 5 days was obtained as 45.6 ml in 49 days with continuous production. In the upper phase, 26 ml of biogas production was observed in 49 days. Fluctuations were observed on the 7th and 42nd days, as a result of the increase in production. The total yield from the sunflower stalk with 5 days pretreatment was 72 ml in 49 days.

33 ml of biogas were produced from methanation of 10 days pretreated lower phase of sunflower waste in 46 days and production was completed. There was a fluctuation on the 33rd day. In the upper phase, 28 ml of biogas was produced in 46 days. Again, the fluctuation occurred on the 33rd day. The lower phase and upper phase values for 10 days pretreated sunflower stalk are quite close to each other.

As the pretreatment time increases, the amount of biogas production in lower phase decreases, while the increase in upper phase production is observed. This shows that as the pretreatment time increases, the amount of lignocellulosic biomass dissolved in the liquid supernatant increases. The total amount of biogas produced from the lower and upper phases for the sunflower stalk pretreated for 10 days is 62 ml in 41 days.

28.3 ml of biogas was produced from the methanation of 15 days pretreated sunflower stalk lower phase in 50 days, and the production was completed. The upper phase produced 21 ml of biogas in 50 days. The total yield from 15 days pretreated sunflower stalk is 49 ml of biogas in 50 days. A decreased amount of biogas was obtained compared to the biogas production of the sunflower stalk that was pretreated for 10 days.

Considering the lower phase of 20 days pretreated sunflower stalk values, it is seen that approximately 44 ml of biogas was produced in 40 days. An increase in yield was observed compared to the lower phase of sunflower stalk pretreated for 15 days. Approximately 5 ml of biogas was produced in 24 days in the upper phase. There was a decrease in yield compared to the upper phase of sunflower stalk waste that was pretreated for 15 days. When looking at the total sunflower stalk yield, it is approximately 49 ml of biogas in 60 days. This shows that 20 days pretreatment does not cause an increase on yield compared to 15 days pretreatment. Comparison of biogas generated in methanation stages of reactors which contain pretreated reeds wastes and model results was given in Fig.5.

It was observed that a great majority of the biogas was obtained from the bottom phase, which is the solid phase, for all waste types. In addition to that, more biogas production was observed in the earlier stages of pretreatment for all of the wastes, except reeds. The reason for the higher biogas yield in the earlier stages of pretreatment is the presence of higher soluble organics to be used for methanation stage at the beginning of pretreatment process (Yuan et al. 2016).

3.3 Biogas Content

After the steady-state condition, the methane and carbon dioxide content of biogas were examined. Methane content was above 45% in almost all reactors, similar to typical biogas content (Asadollahzadeh et al. 2020; Markou et al. 2017) and was an indication that lignocellulosic wastes can be used in biomethanation with high efficiency (Mathew et al. 2015). Also, there was no clear relationship between the amount of biogas formation and pretreatment time.

The most appropriate pretreatment times for each type of waste have been observed with the best biogas and methane yields they have reached. According to Fig.6, the highest amount of biogas obtained in the methanation of pretreated wheat straw was observed for 5 and 2 days pretreatments with 162.8 and 161.8 ml, respectively. In terms of methane content, 5 days pretreatment reached the highest results with 101.7 ml methane. In addition, these values represent the highest efficiency among all wastes and pretreatments. The highest amount of biogas yield and methane produced in the methanation of pretreated cotton stalk wastes was observed for 2 days pretreatment with 87.2 ml biogas and 50 ml methane. As the pretreatment time of reeds waste increased, the efficiency continued to increase. The highest biogas yield with 104.8 ml was observed for 15 days, and highest methane content with 76.15 ml was observed for 20 days pretreated reeds waste. The highest amount of biogas and methane produced in the methanation of pretreated sunflower stalk was observed for 2 days pretreatment with 87.8 biogas and 52 ml methane.

4. Conclusion

Lignocellulosic biomass is an energy alternative to fossil fuels and has a high potential. However, the difficulties in anaerobic digestion process due to the structural complexity of these substances lead to the requirement of pretreatment. In this study, biological pretreatment was preferred because of its cost-effective and sustainable nature. Pretreatment with sheep rumen liquid was applied to the lignocellulosic wastes for 2, 5, 10, 15 and 20 days. The structural degradation of each type of wastes by pretreatment and best biogas and methane yields has been observed and compared with the model results. Majority of the biogas was obtained from the solid phases of all types of wastes. The wheat straw waste pretreated for 5 days was yielded the most biogas, and followed by 20 days pretreated reeds, 2 days pretreated sunflower stalk and 2 days pretreated cotton stalk wastes.

Pretreatment with rumen microorganisms are recommended as a method that should be studied for efficient methane production from lignocellulosic biomass. This method has the advantage of being cost-effective and renewable. Therefore, pretreatment studies with rumen should be increased. Detailed research is recommended on the effect of rumen and how it can be made more effective in the production of different types of bioenergy.

Declarations

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Figures

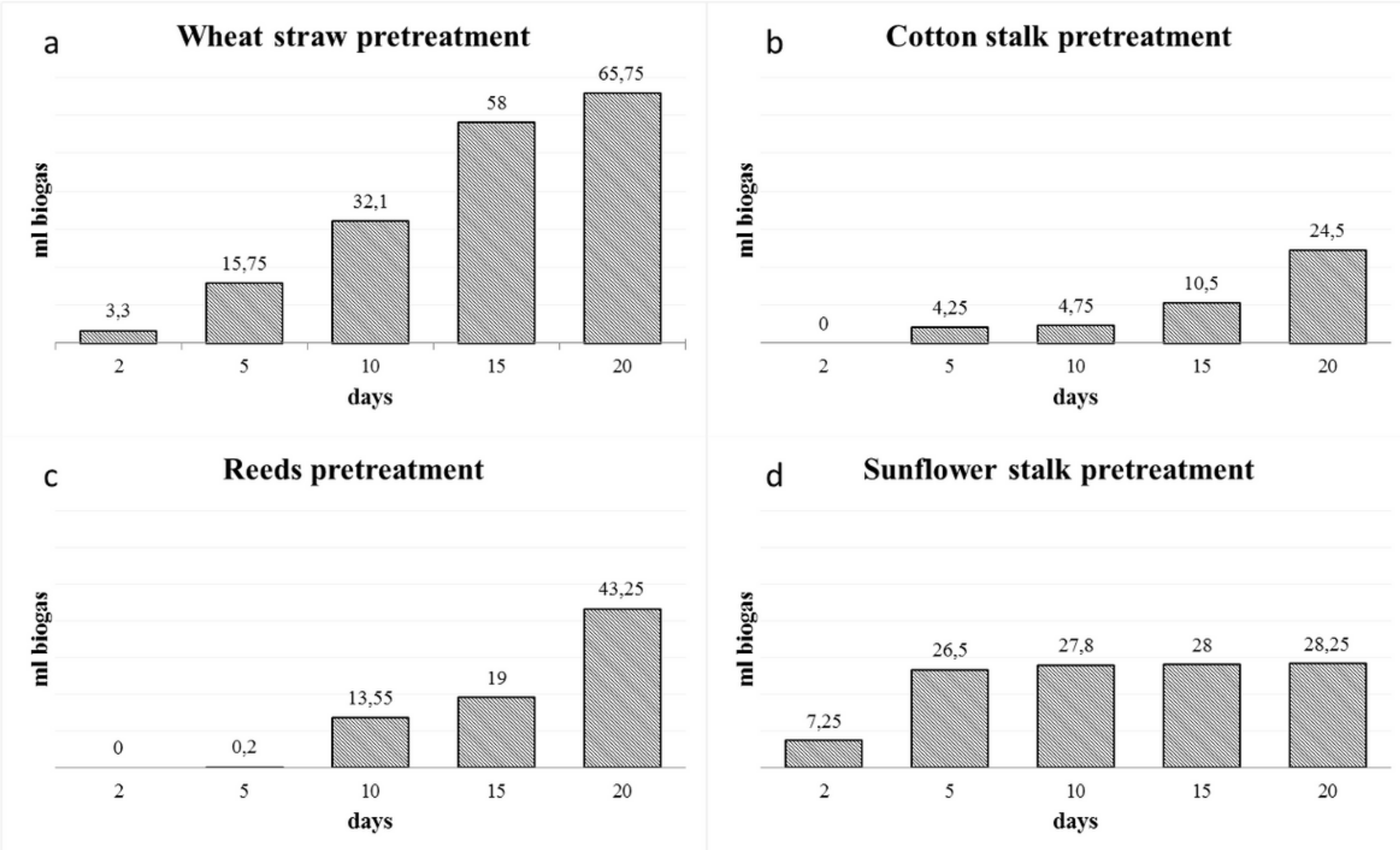

Figure 1

Biogas generated in pretreatment stages of (a) wheat straw, (b) cotton stalk, (c) reeds and (d) sunflower stalk wastes, respectively

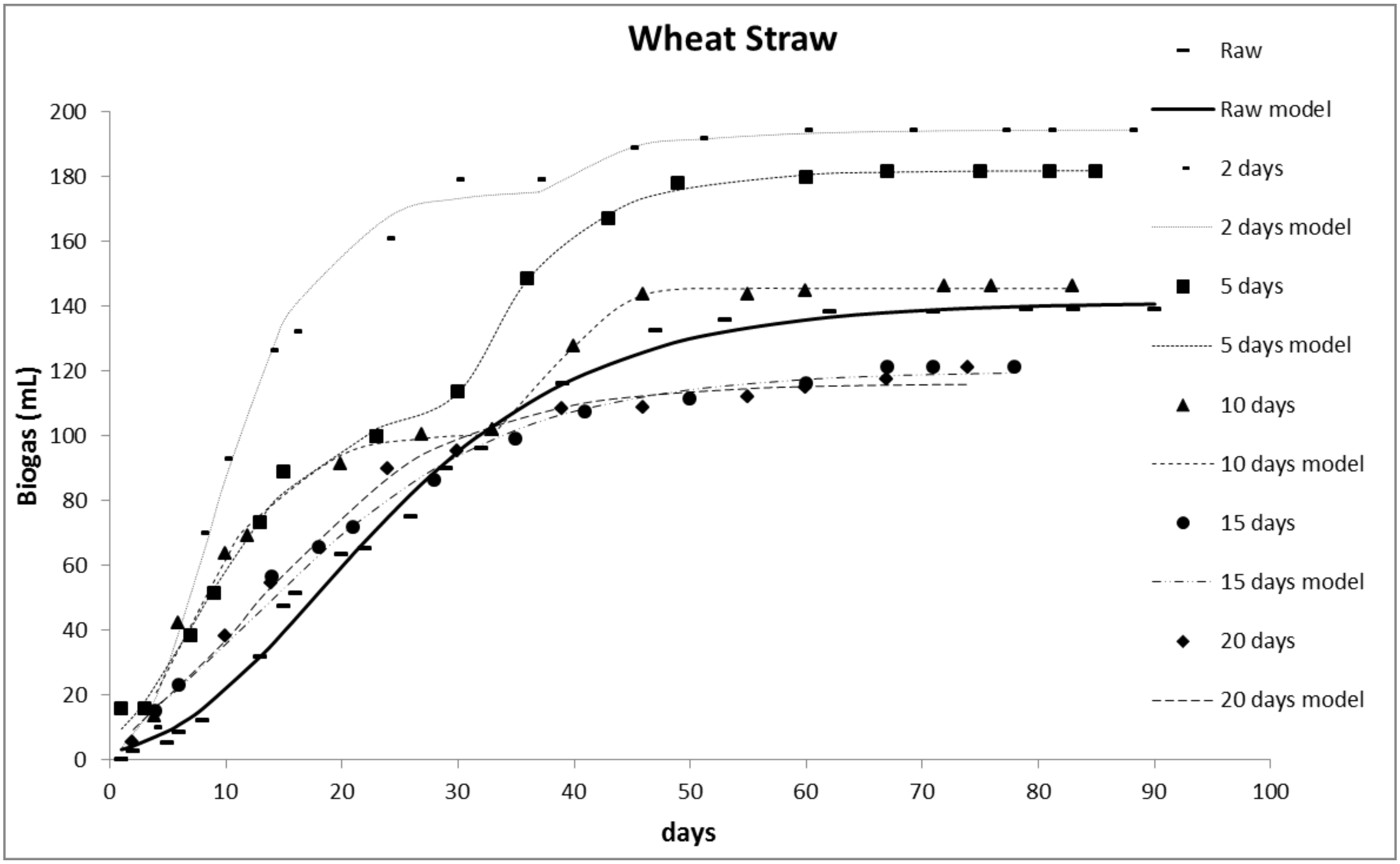

Figure 2

Biogas amount produced during methanation of wheat straw and comparison with model results

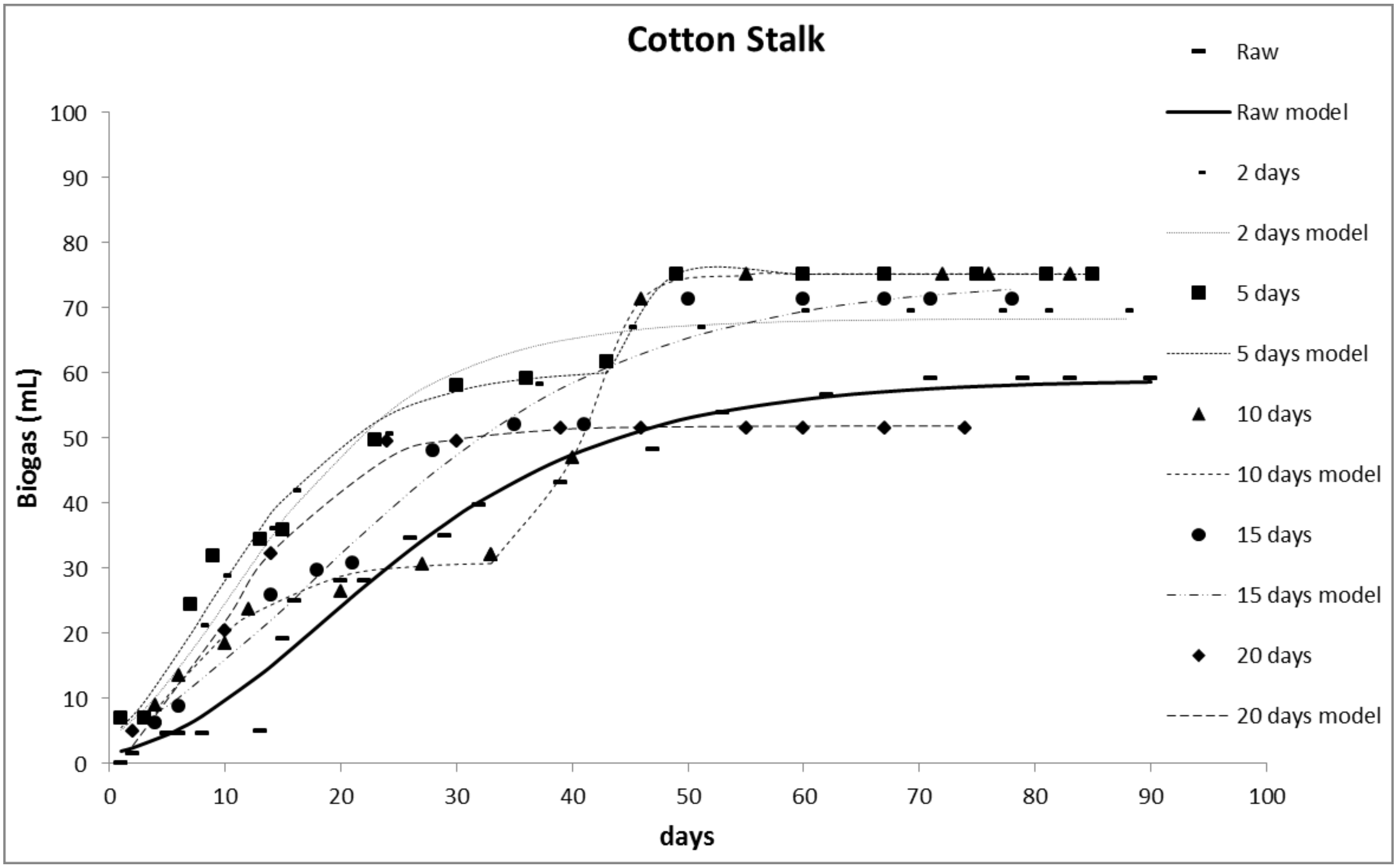

Figure 3

Biogas amount produced during methanation of cotton stalk and comparison with model results

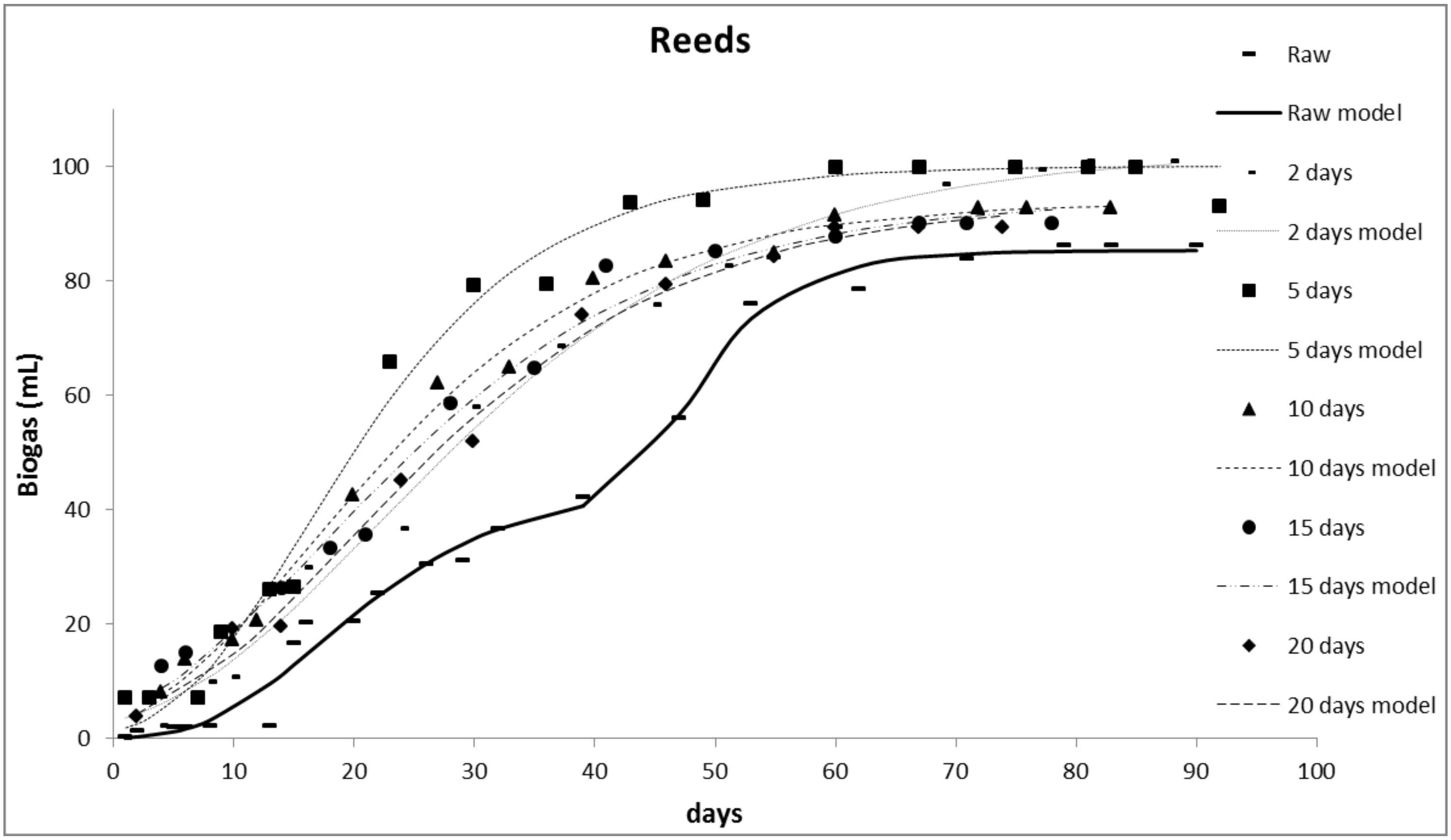

Figure 4

Biogas amount produced during methanation of reeds and comparison with model results

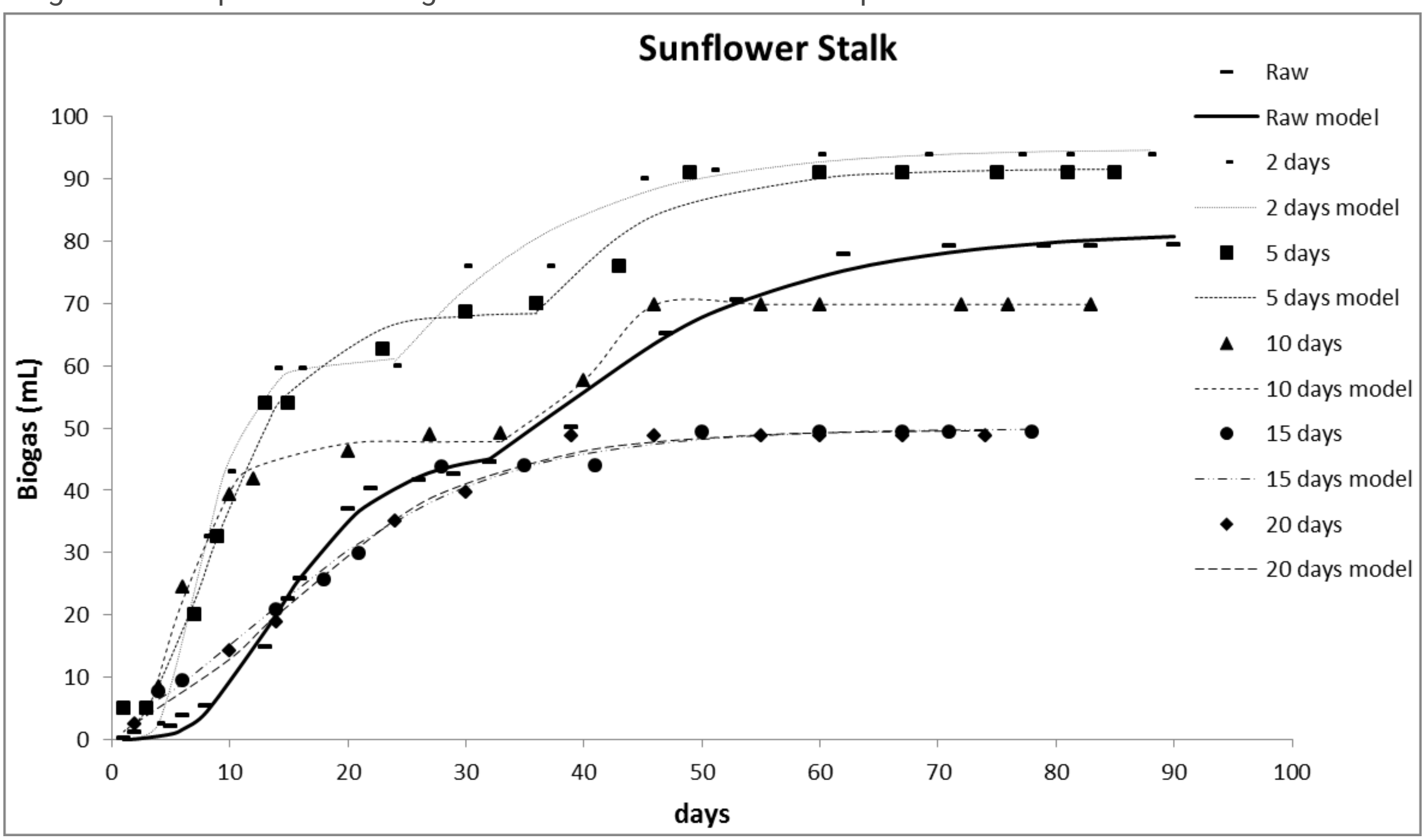

Figure 5

Biogas amount produced during methanation of sunflower stalk and comparison with model results

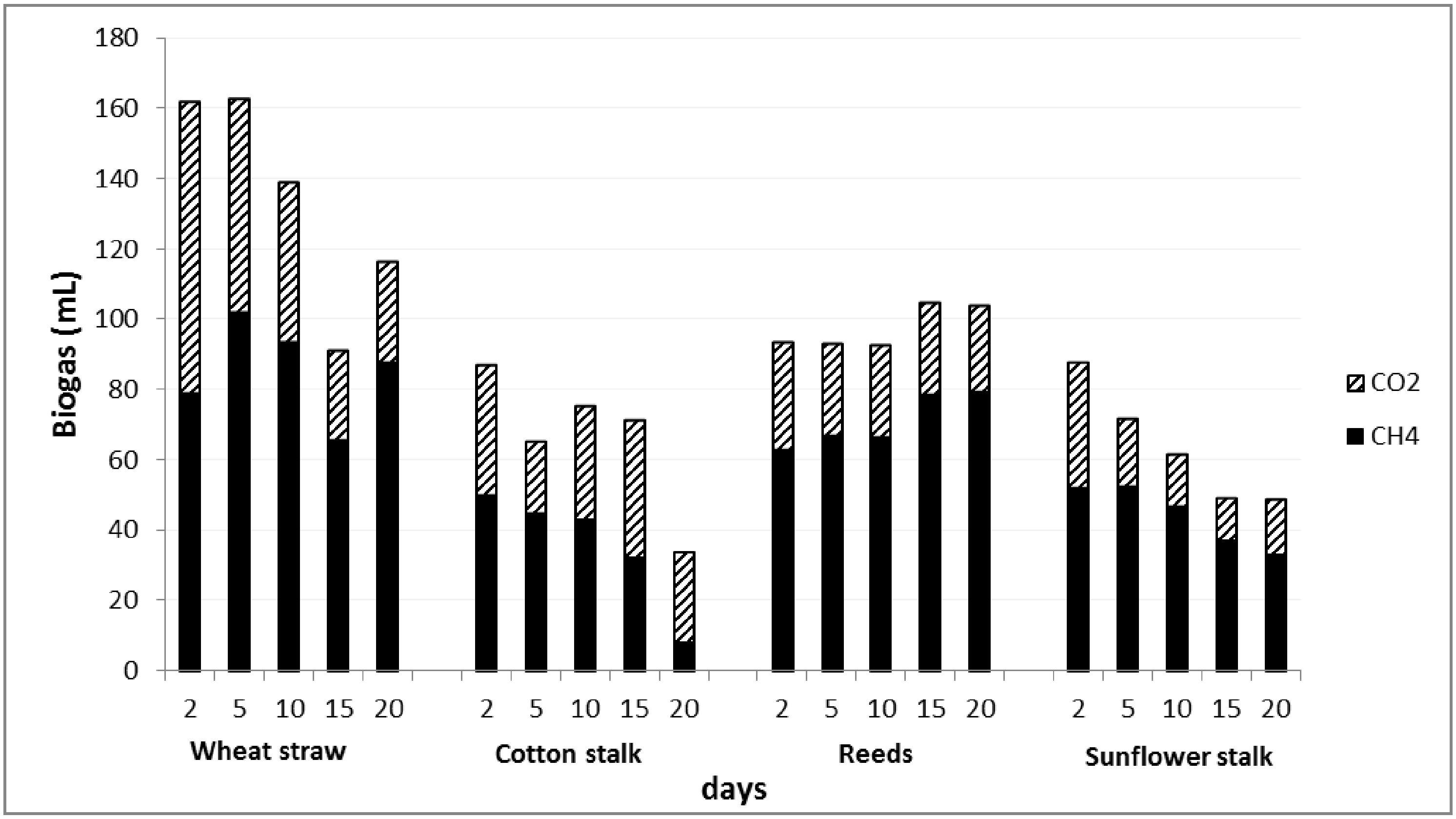

Figure 6

Biogas amounts and contents of pretreated wastes

Supplementary Files

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