

Anaerobic Digestion of Wheat Straw Pretreated with Soaking in Water and Alkali Medium

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ABSTRACT

The influence of the alkaline pretreatment of wheat straw with use 0.05M solution of NaOH by 22 hours at 25 °C temperature on the effectiveness of anaerobic digestion under mesophilic condition was the subject of this study. The water soaked straw was used as a control. The results showed that alkaline pretreatment improved the solubility of the straw biomass, increasing dissolved COD and concentration of VFA in the feedstock of 10% and 21%, respectively, compared to the water soaked samples. Consequently, the cumulative biogas yield was enhanced by 21.5%, reaching 412.1 mL g_{VS}⁻¹ for the pretreated biomass. The digestates were similar in terms of TS and VS, but it significantly differed in terms of phenols concentration, the content of which was considerably higher both in the feedstock as also as in digestate containing alkaline pretreated straw. Initial concentration of phenols in the feedstock of 27 mg L⁻¹ did not block the anaerobic digestion, although its inhibiting effect is forecasted. It was concluded that soaking the straw at low loading of NaOH (4% of raw mass (w/w)) under ambient conditions can efficiently improve its usability for biogas production.

Keywords: alkaline pretreatment, wheat straw, anaerobic digestion, biogas production, phenols.

INTRODUCTION

Using renewable sources for bioenergy production is increasingly frequent nowadays. It is caused by a growing demand for energy and the need to decrease the greenhouse gases emission generated by burning fossil fuels, which are the predominant energy source around the world, supplying 85% of the primary energy in 2018 [SRWE, 2019]. Using the biomass as the energy source has a great advantage over the fossil fuels, among which the renewability is the most important, because it allows balancing the CO₂ emission generated during fuels combustion by absorption of CO₂ into biomass *via* photosynthesis. Additionally, the widespread availability of biomass on the globe, allows obtaining partial energy independence for the countries that are poor in natural fossil resources. The important advantage of biomass is a low acquisition cost, but low energy density in the case of the majority of biomass types is a significant limiting factor.

The high moisture content is often the reason for the low value of this parameter.

Anaerobic digestion seems to be a promising way for recovering the renewable energy from biomass, especially the wet one. The natural origin (there is no need to inoculate the special microorganisms), low energy input in the case of the mesophilic conditions, available and known technology with high adaptability to different substrate properties, and wide substrate spectrum are the strong arguments for choosing this type of biomass conversion into energy. However, high amount of lignocellulose, which is resistant to the enzymatic hydrolysis poses a problem. A high content of lignin has particularly negative consequences: it decreases biogas yield and considerably extends the decomposition time. However, this component is a common building component of plants tissue, which means that many materials potentially useful for biogas production, such as grass biomass, straw residues, garden waste, are rarely used in biogas plants. This is for example

the case of wheat straw, which is one of the most abundant materials in the world (the amount of which in global scale is estimated at 556.3 million Mg per annum) and has a great potential for biofuels production [Saha et al., 2005]. Although it contains around 60% (dry weight) sugars, its availability to enzymatic hydrolysis is relatively low, which can be explained by a high content of crystalline cellulose, high degree of cellulose polymerization, and low accessibility of polysaccharides for cellulose enzymes because of lignin protection [Zhang and Lynd, 2004]. Thus, in the anaerobic digestion of lignocellulosic materials, the rate of hydrolysis is usually the limiting step.

In order to improve the bioavailability of the lignocellulosic materials prior to anaerobic digestion, effective pretreatment methods are required. The purpose of these methods is to destroy the compact structure of lignocellulose and enhance the cellulose accessibility for efficient enzymatic hydrolysis [Lu et al., 2013]. Many biological, chemical and physical processes and combination of them have been studied, including alkali hydrolysis, acid hydrolysis, the organosolv process, steam explosion, ammonia fiber explosion (AFEX), hot water treatment, and microorganism treatment [Galbe et al., 2007; Corredor et al., 2008].

Alkaline pretreatment (sodium, potassium, calcium and ammonium hydroxides adding) is commonly used in the lignocellulosic biomass with high lignin content, e.g. sugarcane bagasse and wheat straw [Taherdanak and Zilouei, 2014]. Nowadays, it is the leading pretreatment method of the biomass intended for anaerobic digestion. It causes the swelling of the fibers and saponification of the uronic bonds linking lignin to hemicelluloses. Alkaline pretreatment may also induce partial delignification and hemicellulose solubilization increasing the access of enzymes to a larger part of cellulose structure. The effectiveness of the process depends on the physical structure and chemical composition of the biomass and process conditions. It was stated that alkaline treatment is more effective with the subsequent anaerobic digestion than the acidic one; moreover, these methods are commonly conducted at lower temperatures and require less specialist equipment, which means lower capital costs [Sun and Cheng, 2002]. A high pH feedstock created during alkaline pretreatments can counteract acidification, induced by the fermentation end-products [Massanet-Nicolau et al., 2013]. On the other hand, the alkaline methods elongate the time of pretreatment and

result in lower sugar degradation [Kumar et al., 2009]. Among all alkaline chemicals used for the pretreatment of biomass before methane fermentation, NaOH has been preferential and most frequently studied [Zhu et al., 2010; Chandra et al., 2012; Taherdanak and Zilouei, 2014]. However, some researchers draw attention to the disadvantages related to the alkaline lignocellulosic biomass pretreatment. Barakat et al. [2012] stated that high doses of NaOH may cause the production of large amounts of furfurals, vanillin and lignin polymers acting as inhibitors to methanogens. Zhang and Pienkos [2009] claimed that using NaOH at high temperatures and pressures leads to the production of inhibitors, including phenolic compounds, hydroxymethylfurfural (HMF) and furfural. Both high levels of NaOH and the generated inhibitory byproducts may have a negative influence on the anaerobic digestion. Moreover, the NaOH usage and removal can be environmentally hazardous and may cause soil salinization and water pollution.

These inconsistent statements about the alkaline pretreatment as the method of preparing the hardly biodegradable biomass for biofuels production are the rationales for conducting the further studies. Additionally, there are very few studies on soaking the wheat straw with low concentrated NaOH, and no research comparing the weak alkaline pretreatment of this biomass with the effects of its soaking in water medium has been found.

The aim of this study was to assess the influence of low temperature alkaline pretreatment of wheat straw using low loading NaOH on the efficiency of the biogas/methane production and properties of the digestates, as well as evaluate the problem of phenols production during the wheat straw hydrolysis.

MATERIALS AND METHODS

Examined material

The biomass of wheat straw used in the experiment was collected in August 2017 from a private-owned farm localized in Lubelskie Region (Poland). The straw was stored in laboratory, in open bags. Before the experiment, the samples of biomass were tested in order to determine total solids (TS) and moisture content, volatile solids (VS) content, and pH. The parameters of wheat straw are presented in Table 1.

Table 1. Parameters of wheat straw and digested sewage sludge used in the experiment

Specification	Unit	Wheat straw	Digested sewage sludge (inoculum)
pH	-	7.07±0.2	7.7±0.2
Total solids (TS)	g kg ⁻¹	882.4±2.1	30.1±0.3
Volatile solids (VS)	g kg ⁻¹	824.4±10.8	18.5±0.5
COD _{total}	gO ₂ L ⁻¹	-	3.9±0.01

*measured in the water solution (1:10, mass of straw/ 10 volume of distilled water)

Wheat straw pretreatment

The samples of mechanically fragmented (<10 mm) air-dried wheat straw with the weight of 5 g were placed in 8 glass flasks (250 mL). Four flasks were filled with 100 mL 0.05 M solution of NaOH (pH 12.7), and the others were filled with 100 mL of distilled water (pH 7.2). The latter were used as the control samples (pretreated in distilled water). All the samples were placed in a shaking incubator (Stuart SI 500) and left there at the speed of 100 rpm, at a temperature of 25 °C for 22 h. After that time, two samples of each type were taken for the physicochemical analysis, and two others were submitted to the process of anaerobic digestion. The analyzed samples were centrifuged for 20 minutes at 4000 rpm (MPW-350 Med Instruments). Next, the supernatants were poured through a filter paper (84 g m⁻²) to the glass vials. Chemical oxygen demand (COD), phenolic compounds concentration and pH were analyzed in the obtained filtrates. Examinations were carried out in two replications.

Anaerobic digestion assays

The NaOH-pretreated and control samples of wheat straw were digested in the laboratory batch digesters in batch assays. The anaerobically digested sewage sludge taken from municipal wastewater treatment plant “Hajdów” (Lublin) from the pipeline transporting the sludge from the anaerobic digester was used as an inoculum. The parameters of the digested sewage sludge are presented in Table 1. The experiment was

conducted in the BioReactor Simulator (Bioprocess Control, Sweden) consisting of six continuously stirred glass bioreactors, each with the total volume of 2 L (1.8 L working volume). Each bioreactor was supplemented with 1200 mL of anaerobically digested sewage sludge (inoculum), then the wheat straw samples (entire content of the flasks, namely hydrolysate and solid phase), both pretreated with NaOH and with distilled water, were placed into the bioreactors (Table 2.). Mass ratio of the bioreactors components was 1:240 (wheat straw:digested sewage sludge). The bioreactors were stirred in a semi-continuous mode - 10 minutes of mixing (80 rpm) and 20 minutes break. The experiment was conducted under mesophilic conditions (37±1 °C) for 40 days. The biogas production was monitored automatically. The system was controlled by web-based software running on an efficient cloud computing solution accessible from any computer or mobile device with an Internet connection.

Analytical methods

The TS content was determined by drying the samples for 24 h at 105 °C in a drying chamber SUP-4 (Wamed, Poland). The VS content was determined after burning the dried samples to ashes for 24 h at 550 °C in a muffle furnace FCF 2.5 S (Czylok, Poland). The value of COD, concentration of phenolic compounds and volatile fatty acids (VFA) were determined by use of HACH cuvette tests (COD 100-2000 mg L⁻¹, phenols 0.05-5 mg L⁻¹, VFA 50-2500 mg L⁻¹). The values of pH were

Table 2. Characteristics of bioreactors feedstock (mixture of wheat straw and digested sewage sludge)

Parameter	Unit	Feedstock	
		with straw pretreated in distilled water (WH)	with straw pretreated in alkaline solution (AH)
COD _{dissolved}	mgO ₂ L ⁻¹	3810±40	4200±20
Phenolic compounds	mg L ⁻¹	22±0.3	27±1.0
Volatile fatty acids	mg L ⁻¹	660±12.0	800±8.1
Total solids (TS)	g kg ⁻¹	31.2±0.60	31.3±0.30
Volatile solids (VS)	g kg ⁻¹	20.2±0.01	20.2±0.02

measured by EasyPlus™ METTLER TOLEDO. The amount of biogas produced from each bioreactor was measured automatically on a daily basis during the anaerobic digestion period using the measurement equipment of BioReactor Simulator. The methane content was analyzed chromatographically at the end of the experiment using a Trace GC Ultra (Thermo Scientific) gas chromatograph with a RTQ-BOND column (30 x 0.25 mm ID, d_f 10 μ m), equipped with TCD detector.

Methods of the results interpretation

The specific cumulative biogas yield (CBY) and biochemical methane potential (BMP) were defined as follows (Eq. 1., Eq. 2.):

$$CBY = V_B/m_{VS} [\text{mL g}_{VS}^{-1}] \quad (1)$$

$$BMP = (V_B \cdot c_{CH_4})/m_{VS} [\text{mL}_{CH_4} \text{ g}_{VS}^{-1}] \quad (2)$$

where: V_B – volume of biogas produced during the test (mL); m_{VS} – mass of volatile solids added to the bioreactor with substrate (g_{VS}); c_{CH_4} – concentration of methane in biogas (-).

The removal efficiencies of total solids, volatile solids, COD and phenolic compounds were calculated according to Eq. 3.:

$$\eta = [(m_1 - m_2)/m_1] \cdot 100\% [\%] \quad (3)$$

where: m_1 – mass of parameter before the anaerobic digestion (mg); m_2 – mass of parameter after the anaerobic digestion (mg).

Statistical analysis

All analyses were conducted at least in duplicate (TS and VS content analyses in four repetitions). The differences in the values of parameters were determined by the mean and standard deviation. Microsoft Excel 2003 for Windows was used for statistical analysis.

RESULTS AND DISCUSSION

The biogas production and composition

The efficiency of hydrolysis of straw biomass at 25 °C with a low NaOH concentration of 0.2% (0.05M) for 22 hours was confirmed by the increase of COD and pH values measured in the hydrolysates (Table 3.). In the case of the wheat straw pretreated by soaking in distilled water, a slight increase of pH and high COD value after hydrolysis were observed. Conversely, the significant decrease of pH during the process, and over 48% higher COD value in the solution were noted in the case of the samples pretreated with NaOH. It indicates the release of organic substances, including the acids, due to hydrolysis of organic material containing in the straw. These changes affected the biogas production obtained during the anaerobic digestion of both types of straw samples.

The daily biogas production has been changed through the experiment, ranging from 0.99 to 149.5 mL d^{-1} in the case of the digestion of the control straw sample, and from 0 to 219.5 mL d^{-1} in the case of the NaOH-pretreated sample. The time-dependent changes of daily biogas production of both analyzed materials showed similar trends, rapidly increasing directly after the start of the experiment (the first peak after 1st day), and decreasing in the 2nd day, next reaching the highest values at 5th day. Afterwards, the production of biogas declined gradually up the end of the experiment (Figure 1a.). The highest maximum value of daily production was observed in the case of the digestion of the biomass pretreated under alkaline conditions. This value (53.25 $\text{mL d}^{-1} \text{ g}_{VS}^{-1}$) was of 47% higher than the maximum value measured in the control sample (36.27 $\text{mL d}^{-1} \text{ g}_{VS}^{-1}$). From 14th day onwards the biogas production in both the examined materials was almost identical and it stopped after the 40th day of the experiment. Shifting the graphs illustrating the time-dependent changes in the daily production of biogas only in the vertical direction relative to each other

Table 3. Chemical parameters of the solutions obtained after hydrolysis of wheat straw under different conditions

Parameter	Samples with wheat straw hydrolyzed in water		Samples with wheat straw hydrolyzed in NaOH solution	
	before the hydrolysis	after the hydrolysis	before the hydrolysis	after the hydrolysis
COD _{dissolved} ($\text{mgO}_2 \text{ L}^{-1}$)	n.a	4950±12	n.a.	7343±15
pH (-)	7.06±0.01	7.25±0.38	12.32±0.04	9.7±0.14

n.a.- not analysed

suggests that in terms of quality, the chemical composition of the bioreactor feedstocks did not differ significantly. Decomposition of the same substances occurred at the same time, while their concentration was significantly distinct. These observations indicate that alkaline hydrolysis has released more substances susceptible to microbial degradation to the solution than soaking the straw sample in distilled water, but the same types of chemicals were released to the solutions.

The cumulative biogas production followed similar values in both the examined substrates up to 4th day of the experiment. Next, the significant variation in the biogas production between the bioreactors containing the NaOH- and water-pretreated straw has been observed (Figure 1b.).

The alkaline pretreatment of wheat straw significantly increased the biogas yield compared to the water soaking. Digestion of both types of wheat straw biomass yielded onwards 1.7 L and 1.4 L of

biogas over the 40 days, respectively. Specific cumulative biogas yield was found as $412.1 \pm 19.4 \text{ mL g}_{\text{VS}}^{-1}$ for the alkaline pretreated wheat straw and $339.6 \pm 15.9 \text{ mL g}_{\text{VS}}^{-1}$ for the straw hydrolyzed in water. Thus, soaking the biomass in a NaOH solution caused 21% increase in the total biogas production. This increase may result from the physical and chemical changes in the lignocellulose contained in the straw. Remli et al. [2013] showed that alkaline pretreatment causes swelling of the straw and increasing in the internal surface area of substrate. Additionally, it is widely known that hemicelluloses are affected by alkaline compounds. After alkaline addition to the lignocellulosic substrate, the hydroxyl ions cause swelling of cellulose, breaking of the hydrogen bonds between cellulose and hemicelluloses as well as hydrolysis of the ester bonds connecting cell-wall polysaccharides. It results in the dissolution of hemicelluloses [Kim, 2018].

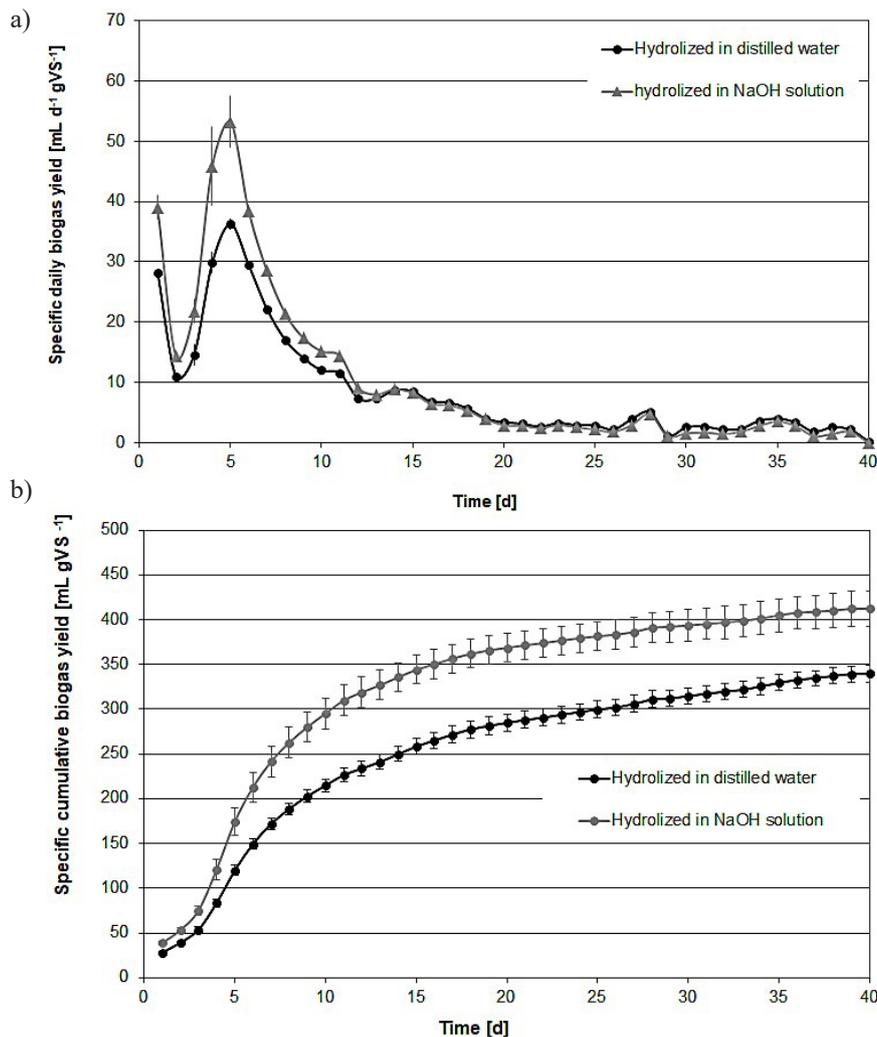


Figure 1. Specific daily biogas yield (a) and specific cumulative biogas yield (b) of the wheat straw hydrolyzed in distilled water and NaOH solution

The stimulating influence of weak alkaline pretreatment of wheat straw on the biogas production under mesophilic conditions was also observed by Mancini et al. [2018]. They hydrolyzed the biomass under harsh conditions (30 °C for 24 h, and NaOH loading 10% of raw mass) compared to this study (25 °C for 22 h, and NaOH loading 4% of raw mass) but they obtained lower enhancement of the biogas production (by 15%). Definitely more evident increase in the biogas production, reaching 87.5%, due to the alkaline pretreatment with the use of NaOH, was observed by Chandra et al. [2012] in a batch test conducted under mesophilic conditions. In their study, the specific cumulative biogas yield of the alkaline pretreated biomass was 353.2 mL g_{VS}⁻¹, which was 14% lower than the value obtained in this study for the alkaline pretreated sample. However, the biogas yield of untreated straw in their experiment was only 188.4 mL g_{VS}⁻¹, which was 45% lower compared to the value obtained in this study. This suggests that water soaking can be considered as a quite efficient and cost-effective method of straw pretreatment. The biogas yield of 339.6±15.9 mL g_{VS}⁻¹ obtained in this study in the case of the water-soaked sample was higher than all the values of this parameter given in the literature for the untreated wheat straw, even under thermophilic anaerobic digestion [Chandra et al., 2012; Taherdanak and Zilouei, 2014; Mancini et al., 2018].

An important improvement of the biogas generation due to the alkaline pretreatment of wheat straw, which was used as co-substrate for cattle manure, was observed by Krishania and Vijay [2012]. They stated that the biogas production from the feedstock with the untreated wheat straw was 488 mL g_{VS}⁻¹ while from the feedstock containing the wheat straw soaked with a solution of 2% NaOH for 72 h, under ambient temperature it was ca. 10% higher. Significantly higher efficiency of methane production (even by 94%) using the same substrates mixed in different proportions was observed by Krishania et al. [2013]. The NaOH loading used in this study was 20% of raw biomass.

According to the study of Zhu et al. [2010] conducted on corn stover, the effect of the pretreatment depended on the NaOH loading. No effect of the pretreatment was observed when the NaOH loading was 1% (w/w) for solid-state. Only increasing the loading to 5% caused a significant increase in biogas yield (by 37%) in comparison with the untreated sample. There

are insufficient data to confirm such dependence in the case of wheat straw. No data on the wheat straw pretreatment at the NaOH loading below 4% of raw mass have been found.

All the afore-mentioned studies showed that alkaline pretreatment significantly enhanced the degradability of wheat straw during the anaerobic digestion process making the alkaline pretreated wheat straw more easily accessible for the microorganisms involved in the anaerobic digestion process. However, comparing the changes in the COD values in both types of hydrolysates used in this experiment and in the biogas production obtained during the anaerobic digestion of these materials, an evident disproportion between the increases in these parameters is noted. As it was previously mentioned, the gas production increased by 21%, while the COD concentration in alkaline hydrolysate was 48% higher than in the water soaked one. Expecting that the biogas from one each milligram of COD added to the bioreactor will be the similar, the production of biogas should be higher than obtained. This disproportion indicates that not all substances released during the pretreatment of the biomass were converted into biogas. This suggests that the inhibition of methanogenesis occurred.

Lignocellulose is the main constituent of the wheat straw. Although the chemical composition of the straw biomass depends on several parameters, e.g. climate conditions, soil properties, plant species, generally, the contents of cellulose, hemicellulose and lignin are similar and range from 33 to 40, from 20 to 28, and from 15 to 20 (% w/w), respectively [Talebnia et al., 2010]. During the decomposition of lignocellulosic biomasses under alkaline conditions, phenolic compounds are generated [Penaud et al., 1999]. Zhu et al. [2010] showed that lignin degradation during the alkaline pretreatment of corn stover was in the range 9.1% to 46.2% (w/w) depending on NaOH loading that was changed from 1.0% to 7.5% w/w for solid-state. Many studies confirm that phenols may hinder the bacterial growth in the subsequent biological processes of biofuel production from the pretreated biomass [Kim, 2018; Xie et al., 2018]. The phenols concentration in the feedstock examined in this experiment was not very high (Table 2.) taking into account that the inhibitory effect of these compounds on methanogenesis was observed when their concentration exceeded 500 mg L⁻¹ [Fedorak and Hruday, 1984]. However, it is well-known that toxicity depends on the chemical structure of the phenolic compounds.

Generally, the greater the substitution of the phenolic ring, the greater the recalcitrance and toxicity of the substrate [Borja et al., 1997].

The cumulative biogas yields calculated on the basis of biogas volume released up to the 21 (CBY₂₁) and 40 (CBY₄₀) day of the experiment were significantly lower for the wheat straw soaked in water than for the alkaline pretreated substrate (Fig. 2.). The CBY₂₁ value for water hydrolyzed sample was 85% of total biogas yield obtained at the end of the experiment, and the CBY₂₁ value for the sample hydrolyzed in the NaOH solution constituted 90% of the CBY₄₀ value. It showed that the alkaline pretreatment accelerated the process of anaerobic digestion. Thus, it suggests that hydraulic retention time (HRT) for such a substrate can be shortened compared to the untreated straw.

Taking into account the average methane concentration in the biogas that were 48.23% and 50.73% in the case of soaked in distilled water and alkaline pretreated straw, respectively, the biochemical methane potential calculated on the basis of total biogas volume obtained after the 40th day of the experiment was 164 and 209 mL_{CH₄} g_{VS}⁻¹, respectively.

The time needed for achieving 80% of the maximum biogas production during anaerobic digestion is called technical digestion time (T₈₀). Calculating T₈₀ enables comparing the biodegradability of various kinds of substrates or substrates after different pretreatments. Lower T₈₀ suggests shorter digestion time and higher biomass conversion efficiency [Yang et al., 2014]. It was found that T₈₀ for the NaOH-pretreated wheat straw is 14 days, and it is 21% lower than the value obtained in the case of the straw hydrolyzed in water (17 days).

Digestate composition

The composition of the output material from the bioreactors was significantly different compared to the properties of the feedstock (Table 2., Table 4.). Decreases in TS, VS, dissolved COD and concentrations of phenolic compounds were observed. The analysis of the removal efficiencies calculated for the particular parameters showed that the straw pretreatment influenced the properties of feedstocks and digestates (Table 5.). Generally, the digestion of the alkaline pretreated wheat straw resulted in higher removal efficiencies of all the analyzed parameters in comparison to the water soaked straw. The highest difference in removal efficiencies was noted in the case of phenols concentration (ca. 77% higher value in the case of the pretreated sample), clearly lower in the cases of VS and dissolved COD (16% and 13% higher value, respectively), and the lowest one (ca. 6% higher value) in the case of TS.

This study confirmed that phenols are biodegradable under anaerobic conditions, although their removal efficiencies were below 20% (Table 5.). The low susceptibility to biodegradation can be explained by the complex composition of these compounds.

Unexpectedly, the value of pH measured in the wheat straw hydrolyzed in NaOH solution was lower than in the case of the water-soaked straw. It indicates that the concentration of organic acids that are main substrates for methanogenesis was higher in the former. This suggests the inhibition of the acid conversion into methane.

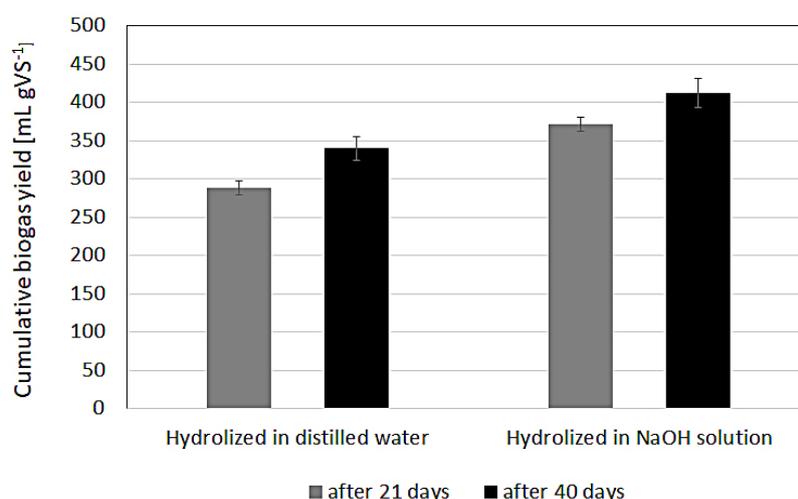


Figure 2. Cumulative biogas yield obtained after 21. and 40. day of anaerobic digestion of the wheat straw hydrolyzed in distilled water and NaOH solution

Table 4. Parameters of digestates obtained during the anaerobic digestion on the wheat straw hydrolyzed in water and NaOH solution

Parameter	Digestate	
	samples with wheat straw hydrolyzed in water	samples with wheat straw hydrolyzed in NaOH solution
COD _{dissolved} (mgO ₂ L ⁻¹)	2604±10	2694±70
Phenolic compounds (mg L ⁻¹)	19.1±0.4	22±1.15
Total solids (g kg ⁻¹)	27.13±0.13	26.89±0.50
Volatile solids (g kg ⁻¹)	17.44±0.21	16.99±0.16
pH (-)	7.76±0.01	7.64±0.09

Table 5. Removal efficiencies of total solids, volatile solids, COD and phenolic compounds during the anaerobic digestion on the wheat straw hydrolyzed in water and NaOH solution

Sample	Parameter			
	η_{TS} (%)	η_{VS} (%)	$\eta_{CODdiss}$ (%)	η_{phenol} (%)
Samples with wheat straw hydrolyzed in water	13.0	13.7	31.7	13.2
Samples with wheat straw hydrolyzed in NaOH solution	14.1	15.9	35.9	18.5

CONCLUSIONS

An analysis of the results of the laboratory batch experiment on the anaerobic digestion of wheat straw biomass allows stating that the alkaline pretreatment of the straw under low temperature and low concentrated NaOH solution significantly improved the biomass biodegradability and enhanced its biogas potential. Soaking the biomass in distilled water was not such as efficient in weakening the chemical structure of wheat straw as its soaking in 0.05M NaOH solution, which increased the solubility of organic compounds containing in straw, improving its availability to anaerobic microorganisms. Consequently, the biogas yield obtained during the digestion of the alkaline pretreated straw was 21% higher compared to the control samples. Simultaneously, both types of digestates did not differ significantly in terms of total solids and volatile solids, but the concentration of dissolved organic compounds was higher in the case of the digestate with the alkaline pretreated sample. It suggests that the potential of methane generation was not fully realized, probably because of the high content of phenols, known as toxicants for microorganisms. However, to confirm that assumption, the anaerobic digestion of the diluted hydrolysates should be conducted in the future.

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