

## Influence of Temperature and Reaction Time on the Efficiency of Alkaline Pretreatment of Hay Biomass

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### ABSTRACT

Low biodegradability caused by polymeric structure is the main barrier in the use of lignocellulosic materials in biofuels production by using biological methods. Pretreatment of the biomass is the way to improve the suitability of hardly biodegradable biomass for biogas or bioethanol production. Evaluation of the influence of thermal and thermochemical alkaline pretreatment on the efficiency of hydrolysis of hay (mixture of various grass species) was the aim of the study. The batch scale experiment was carried out with the use of NaOH and distilled water as solvents, and the changes in pretreatment time (2, 4 and 8 hours) and temperature (22 and 80°C) were also considered. The efficiency of biomass solubilisation was assessed based on the results obtained from the measurements of chemical oxygen demand (COD) and volatile fatty acids (VFA) concentration in the hydrolysates. The solubility of the biomass, expressed as a percentage of soluble COD in total COD, was calculated. The experiment showed that the highest solubilisation of hay biomass was observed at 80°C under alkaline conditions. In this case, the solubility of the COD was 3-times higher, and the VFA concentration in hydrolysates was 4-times higher in comparison to the distilled water-based test at 22°C. It was noted that time of the process significantly influenced the efficiency of biomass solubilisation only during the experiment carried out at 22°C. Extension of hydrolysis time from 2 to 8 hours increased the value of soluble COD of 70% and 55% for water and alkaline solvent, respectively. The process conducted at 80°C was not time-dependent over the considered period.

**Keywords:** lignocellulosic biomass, hay, alkaline pretreatment, thermal pretreatment

### INTRODUCTION

The recently observed intensive development in agriculture and industry has an enormous influence on the environmental conditions. It causes pollution of the environment and imperceptible disturbances in its functioning. Growing energy demand connected with this development results in a search for new energy sources. Burning of fossil fuels is one of the main factors causing the environment contamination, especially atmospheric pollution. Many technologies for generating energy using other sources gain much attention nowadays. Anaerobic digestion seems to be an alternative for the non-renewable sources of energy, as it results in the production of biogas, which can be used as an electrical or thermal energy source or as an ecological fuel. Lignocellulosic materials

are a promising feedstock for the biogas production. The lignocellulose-based biomass is a cheap and widely available renewable source of energy; moreover, it is characteristic because of high potential biogas yields [Li et al., 1985]. It consists of carbohydrates (cellulose and hemicellulose), lignin, and other components in smaller concentrations [Kim, 2013]. Its low biodegradability, caused by the polymeric structure, is the main barrier in the use of this biomass in biogas and biofuels production by means of biological methods. High content of lignin makes the lignocellulosic biomass hardly biodegradable for microorganisms; what is more, it results in lower methane production yield [Taherzadeh and Karimi, 2008]. The methods of pretreatment are used in order to transfer the structural components of lignocellulosic biomass from the water-insoluble phase to

soluble phase, so that microorganisms can utilize them in anaerobic digestion for the biogas or bioethanol production. Pretreatment, constituting the hydrolysis phase is believed to be the phase the rate of which is the limiting step of the whole anaerobic digestion process [Jackowiak et al., 2011; Liew et al., 2011]. The main goal of pretreatment of lignocellulosic materials is to reach the delignification and hemicellulose dissolution making the biomass more susceptible to enzymatic saccharification. The commonly used parameters indicating the efficiency of the pretreatments are soluble chemical oxygen demand (sCOD), soluble carbohydrate (sCH), soluble protein (sPC) concentrations and biogas production.

Numerous pretreatment methods involving: physical, chemical, physicochemical and biological have been investigated over the last few decades. One of the most effective and fast-acting methods of lignocellulose solubilisation is the chemical pretreatment using alkali [Kumar and Wyman, 2009]. The most frequently used alkali reagents are sodium hydroxide [Chen et al., 2013], potassium hydroxide [Sharma et al., 2013], lime [Kaar and Holtzapple, 2000] and ammonia [Prior and Day, 2008]. Out of these, sodium hydroxide has been studied the most. The alkaline pretreatment of lignocellulosic biomass using dilute NaOH increases the internal surface area, causes swelling, decreases the crystallinity and the degree of polymerization, separates the structural linkages between lignin and carbohydrates, as well as disrupts the lignin structure [Fan et al., 1987]. The process efficiency depends on reagent concentration, reaction temperature and residence time. Additionally, the reaction temperature and residence time are usually lower than in other pretreatment methods [Kim and Han, 2012].

The influence of temperature and time of the process duration on the efficiency of thermal and thermochemical alkaline pretreatment of hay (mixture of various grass species) using NaOH was the aim of the study.

## MATERIALS AND METHODS

### Examined material

First, the cut hay biomass used in the experiment was collected in May from a private-owned meadow localized in the Lubelskie Region (Poland). It was transported to the laboratory and

stored in open bags. The air-dried hay biomass was then mechanically fragmented (by using laboratory scissors) to 3–4 cm stem length. The total solids (TS) content and volatile solids (VS) content of the hay biomass were determined. The parameters of the hay biomass are presented in Table 1. The sodium hydroxide (POCH S.A.) was also used in the experiment.

### Hay biomass pretreatment

The batch scale experiment with use of NaOH and distilled water as the solvents was carried out. The samples of mechanically fragmented (ca. 3–4 cm) air-dried biomass (1.5 g) were placed in 24 glass flasks (250 ml). Half of them were filled with 125 ml of 0.01M NaOH solution (pH 12), and the others were filled with 125 ml of distilled water (pH 7.1). All the samples were mixed in order to immerse the whole substrate in the liquid. Next, six of the samples with NaOH solution and the same number of the samples with water were placed in the thermostated water bath at 80°C, while the rest were left at room temperature (22°C). After 2, 4 and 8 hours, the samples heated to 80°C were cooled down to room temperature. All samples were taken for the physicochemical analysis. The analyzed samples were centrifuged for 20 minutes by 4000 rpm (MPW-350 Med Instruments). Then, they were percolated through filter paper (84 g m<sup>-2</sup>) to the glass vials of 40 cm<sup>3</sup> of volume. Chemical oxygen demand (COD), concentration of volatile fatty acids (VFA) and pH in the obtained filtrates were analyzed. COD in the solid samples was also determined.

### Analytical methods

The TS content were determined after drying the samples for 24 h at 105°C in SUP-4 Wamed drying chamber. The VS content was determined after burning the dried samples to ashes for 24 h at 550°C in a FCF 2,5 S muffle furnace (Czylok). The measurements of pH were performed using Easy Plus™METTLER TOLEDO. Soluble

**Table 1.** Total solids and volatile solids of hay biomass

Parameter	Unit	Value
Total solids (TS)	g <sub>TS</sub> ·kg <sup>-1</sup>	940.80 ± 4.6
Volatile solids (VS)	g <sub>VS</sub> ·kg <sup>-1</sup>	909.66 ± 2.2

chemical oxygen demand and volatile fatty acids concentrations in hydrolysates were determined by using of HACH cuvette tests LCK514 and LCK365. COD in the solid samples was determined with the dichromate method.

### Methods of the results interpretation

The solubility of the biomass (S) was calculated according to Eq.1:

$$S = \frac{COD_{solution}}{COD_{sample}} \cdot 100\% [\%] \quad (1)$$

where:  $COD_{solution}$  – COD calculated on volume of solution [mg O<sub>2</sub>],  
 $COD_{sample}$  – COD calculated on mass of the hydrolyzed sample [mg O<sub>2</sub>].

### Statistical analysis

All analyses were conducted in duplicate. The means, standard deviations and confidence intervals for parameter values were calculated. The statistical analysis was performed using Microsoft Excel 2003 for Windows software.

## RESULTS AND DISCUSSION

### The pH of the hydrolysates

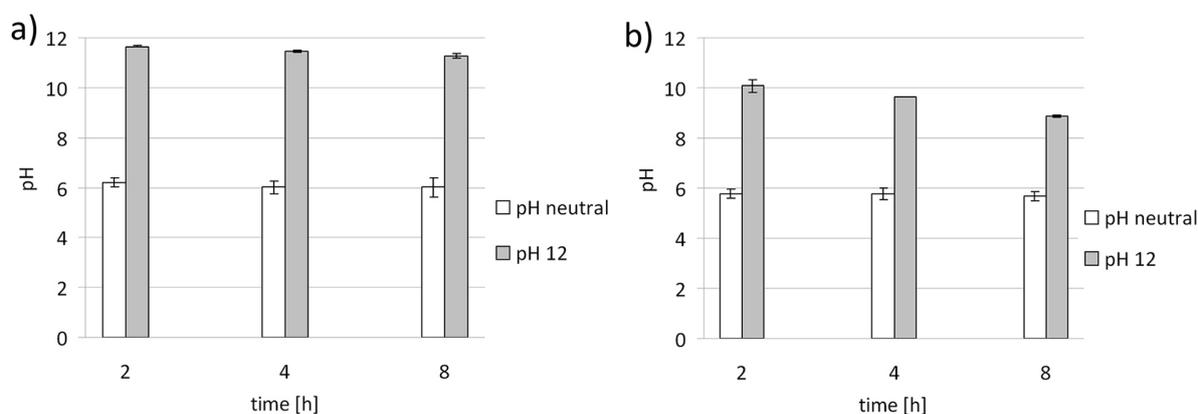
The pH of the solution in the samples soaked in different time intervals in distilled water treated at 22°C was in the range 6.01–6.21 (acidic pH range). After time of the process extension from 2 to 4 hours, the pH value decreased by 0.2; extension for 8 hours did not affect the pH value. A slight decrease of pH was observed in

the samples with distilled water hydrolyzed at 80°C – after 2 hours of soaking, the pH value was 5.78 and after 8 hours it dropped to 5.68. Generally, there were no significant differences in the pH of the solution in the samples treated at 22°C and 80°C in distilled water when the time of process was extended from 2 to 8 hours (Figure 1).

The use of NaOH caused strong alkalization of the solutions. The time of the process duration had a significant influence on the pH decrease in the samples hydrolyzed with alkaline solution at 80°C, from 10.08 after 2 hours of process, through 9.63 after 4 hours to 8.86 after the time of the process extension to 8 hours. It also affected pH in the samples treated with NaOH solution in 22°C, although to a lesser extent. After time of the process extension from 2 to 4 hours the pH value decreased from 11.65 to 11.44, and it was further reduced of 0.15 in the samples hydrolyzed for 8 hours.

### COD of the hydrolysates

The influence of the process duration on the COD release in hydrolysates depended on temperature. At 22°C, the soluble COD level increased with an extension of the process duration, both in the case of distilled water and NaOH solution. In the samples with distilled water, the value of 1085 mg<sub>CODsoluble</sub> l<sup>-1</sup> was achieved after 2 hours of process duration; when the time of the hydrolysis was extended from 2 to 8 h – 1850 mg<sub>CODsoluble</sub> l<sup>-1</sup> was measured. Extension of process time from 2 to 8 hours caused an increase of the soluble COD concentration of about 71%. While analyzing changes of soluble COD in samples hydrolysed in NaOH solution,



**Figure 1.** Values of pH in the solutions after the pretreatment at 22°C (a), at 80°C (b)

ca. 1.6-fold increase was observed when the time of the hydrolysis was extended from 2 to 8 h (Figure 2a).

The hydrolysis conducted at 80°C caused higher soluble COD levels in comparison to the relevant values obtained in the process conducted at 22°C (for all analyzed time intervals (Figure 2b)). The maximal increase was about 1030 and 1966 mg<sub>CODsoluble</sub> l<sup>-1</sup> for the samples treated in the water and alkali medium, respectively. The highest soluble COD concentration was observed in the samples treated with NaOH solution at 80°C for 8 hours and this value was almost 3.0-fold higher than the lowest COD concentration noted in the samples treated with water at 22°C for 2 hours. As far as the samples with distilled water are concerned, the process extension had a negative effect on soluble COD, the values of which dropped gradually from 2115 after 2 hours to 2070 mg<sub>CODsoluble</sub> l<sup>-1</sup> after 8 hours of the pretreatment.

In the samples treated at 80°C with alkali medium, in the time interval from 2 to 4 hours, the soluble COD concentration decreased of 226 mg<sub>CODsoluble</sub> l<sup>-1</sup>, but after time of the process extension from 4 to 8 hours, it increased of 462 mg<sub>CODsoluble</sub> l<sup>-1</sup>.

### VFA of the hydrolysates

Significant influence of the test duration on the VFA concentration in the hydrolysates was observed in the samples treated in the water and NaOH solution, both at 22°C and 80°C (Figure 3a, 3b). At 22°C, the differences between the VFA concentration in the relevant samples treated with particular solvents were lower than at 80°C. A statistically significant dependence at lower temperature was observed only after the

process duration for 8 hours. After the process extension from 2 to 8 hours, the VFA level increased from 284 to 366 mgVFA l<sup>-1</sup>. It gave the concentration rise by 29%.

More noticeable influence of the test duration was noted in the case of the samples treated with NaOH, where the process extension from 2 to 8 hours caused the VFA concentration increase by 80%. Similarly as in the samples with water, statistically significant dependence was observed only after the process extension from 2 to 8 hours.

In the samples subjected to heat treatment, substantially higher VFA concentrations were observed in comparison to the relevant values obtained in the samples at 22°C, both for samples with water and NaOH solution. In the case of the samples treated with water, an increase by even 48% was observed, whereas for the samples treated with alkali medium, ca. 2.6-fold increase was noted. After the process time extension from 2 to 8 hours, the VFA concentration rises only by about 20% (from 371 to 445 mgVFA l<sup>-1</sup>) and about 25.5% (from 899 to 1128 mgVFA l<sup>-1</sup>) were observed, in the samples hydrolyzed with water and NaOH solution, respectively. Statistically significant dependence was observed only after analyzing the values of VFA concentration in the samples hydrolyzed in alkali medium.

The highest VFA concentration was observed in the samples treated with the NaOH solution at 80°C for 8 hours. The value obtained under these conditions was 4.0-fold higher than the VFA concentration in the samples treated with water at 22°C for 4 hours (the lowest one). Higher share of VFA in released organic matter (express as VFA/COD ratio) was stated in the case of the samples hydrolyzed with the NaOH solution, regardless of temperature.

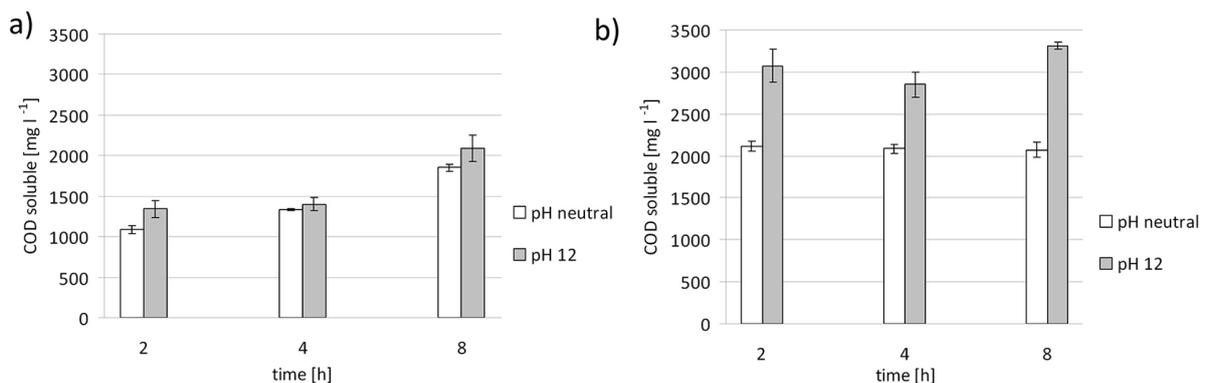
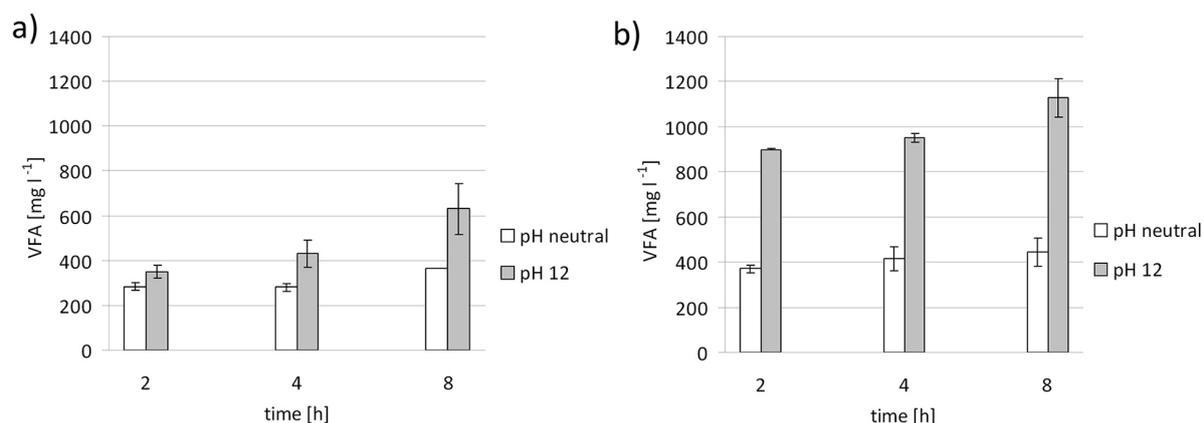


Figure 2. COD in the solutions after the pretreatment at 22°C (a), at 80°C (b)



**Figure 3.** Concentration of VFA in the solutions after the pretreatment at 22°C (a), at 80°C (b)

### Solubility of the biomass

The solubility of the organic matter, calculated on the basis of COD, for the hydrolysates obtained at room temperature grew with the experiment time extension. For the hydrolysates treated with water, the percentages values of the COD release were 6.15%, 7.57% and 10.48%, for 2, 4 and 8 hours, respectively. For the others, which were subjected to alkalization, they were slightly higher and amounted to: 7.62%, 7.93% and 11.84%, respectively.

A much higher solubility was observed in the tests that were subjected to thermal treatment in comparison to the solutions left at room temperature. In that case, any unequivocal relationship between the process duration and solubility was determined, both in the samples treated with water and the NaOH solution. In each analyzed period of time, adding NaOH solution resulted in higher values of the organic matter solubility in comparison to the samples treated with water.

Temperature significantly affected the solubility of the biomass (Table 2). The higher values were noted in the hydrolysates obtained at 80°C. The scale of the influence depended on the hydrolysis time. The lowest values were obtained in the sample hydrolyzed in the water solution at 22°C for 2 hours. In the samples with distilled water, the highest values were obtained at 80°C in the test carried out for 2 hours, and in the samples with the NaOH solution at 80°C in the test carried out for 8 hours.

The combinations introduced into the methods of pretreatment of lignocellulosic materials conducted at lower temperatures improve enzymatic saccharification by speeding up the pretreatment reaction, which was observed in many studies

[Keshwani et al., 2007; Hu and Wen, 2008]. It was proven that a relatively long residence time is needed in order to produce high sugar yields in a low temperature alkali pretreatment method [Agu et al., 2017]. On the other hand, many pretreatment methods of lignocellulosic biomass require a high temperature action, which can be achieved by conventional heating or microwave-based heating. Thermal pretreatment of various types of waste material has been studied in temperatures ranging from 60°C to 270°C [Appels et al., 2010]. Using higher temperature during alkali pretreatment gives such advantages as high conservation of cellulosic biomass, low costs, low production of fermentation inhibitors such as HMF and furfural [Kim et al., 2016; Rodrigues et al., 2016] as well as results in the more intensive organics release and biogas production enhancement [Appels et al., 2010; Liu et al., 2012].

**Table 2.** Solubility of the organic matter calculated on the basis of COD

Temperature [°C]	Solution	Time [h]	S [%]
22	Distilled water	2	6.15
		4	7.57
		8	10.48
	NaOH solution	2	7.62
		4	7.93
		8	11.84
80	Distilled water	2	11.99
		4	11.82
		8	11.73
	NaOH solution	2	17.43
		4	16.14
		8	18.76

In the experiment conducted by Pedersen et al. [2010] where the wheat straw was subjected to alkaline pretreatment (with NaOH, for 10 minutes at 140°C) and enzymatic hydrolysis, the pH decreased from 13 at the beginning of the experiment to 10.6 after pretreatment. As other catalysts were used in the experiment (acid and alkaline), the authors stated that initial pH influenced the monosaccharide yields and lignin removal. The same tendency was observed in our article, where in the samples pretreated with NaOH (both at 22°C and 80°C) a gradual pH decrease during the considered period of time was noted.

Volatile fatty acids are one of the main substrate for methane production. On the other hand, too high concentration of VFA in the waste subjected to anaerobic digestion can significantly reduce the efficiency of the process. Rising concentration of VFA during the pretreatment indicates the hemicellulose dissolution and delignification. In our article, both in the samples hydrolyzed in water and NaOH solution, and at both temperatures, a gradual increase in the VFA concentration with an extension of the process duration was observed.

The soluble chemical oxygen demand concentration is one of the parameters indicating the efficiency of the pretreatment. Arıcı et al. [2015] studied the influence of thermal (temperatures from 20 to 90°C, pretreatment times from 2 to 12 hours) and alkali conditions (0.5%, 1% and 2% (w/v) NaOH) on garden waste. The highest COD solubilisation was obtained at 90°C after 2-hour pretreatment time, which was the most effective and economical option of pretreatment. Regarding the results of our experiment, at 22°C, soluble COD level increased gradually during the experiment time, both in the case of distilled water and NaOH solution. This indicated the intensification of the lignocellulose hydrolysis process. At 80°C, in the samples hydrolyzed in distilled water, the soluble COD concentration dropped gradually during the experiment time. In the samples treated at 80°C with NaOH solution, test duration from 2 to 4 hours caused a decrease in the soluble COD value, but after time of the process extension from 4 to 8 hours, an increase in this parameter was noted.

In our article, similarly as in the experiment conducted by Cabrera et al. [2014], who studied the effect of various NaOH concentrations (0.5, 1.0, 3.0% w/w) and pretreatment times (12, 24, 48h) on the enzymatic hydrolysis of rice hulls and straw (lignocellulosic substrates), it was observed

that alkaline pretreatment improved enzymatic hydrolysis in all analyzed pretreatment conditions in comparison to the untreated samples. These authors observed that the effect of pretreatment time on the enzymatic hydrolysis of rice straw was significant only at 0.5% NaOH addition. It was noted that this concentration was too low to reach the needed hemicellulose dissolution and delignification in 12 h pretreatment time.

In the experiment conducted by Sun et al. [1995] the influence of alkaline pretreatments on the cell wall components of wheat straw was studied. The samples of lignocellulosic biomass were treated with potassium, hydrogen peroxide, lithium hydroxide, liquid ammonia and calcium (each at a 1.5% concentration (for 6 h at 20°C)) and various concentrations of sodium hydroxide. Taking into account NaOH, the following pretreatments were studied: (a) with 1.5% NaOH at 20°C in air for 0.5, 1, 2,3,4,6, 12, 24, 48, 72, 96 and 144 h, respectively; (b) with 1.5% NaOH for 6 h at 0,20,40,60 and 80°C, respectively; (c) with 0.5, 1.5, 3.0, 5.0 and 10.0% NaOH for 6 h at 20°C. The optimal pretreatment method for releasing lignin and hemicellulose were found to be this one with 1.5% sodium hydroxide addition for 144 h at 20°C.

Vásquez et al. [2015], who studied the influence of NaOH dose (2 and 4%), temperature (60 and 120°C), pretreatment time (10 and 30 minutes) and residue size on organic matter solubilization and biodegradability stated that more aggressive test conditions increased the lignocellulosic biomass structure degradation, whereby any significant influence of the pretreatment time was observed. On the other hand, lower reagent dose and smaller residue size maximized the release of the biodegradable organic matter.

However, in the studies conducted by Dąbkowska [2017], where the influence of 2% NaOH and 2% H<sub>2</sub>O<sub>2</sub> as a pretreatment agents on maize residues was analyzed (for 2,9 and 24 h), no significant influence of the process duration on the biomass composition and saccharification of cellulose and hemicellulose was noted.

## CONCLUSIONS

The experiment showed that the alkaline treatment with use of 0.01 M NaOH solution intensified the solubilisation of organic matter contained in hay biomass compared to its treatment

with distilled water, but a scale of the effect was depended on the temperature of the process. At 80°C under alkaline conditions, the solubility of the COD was 3-times higher, and the VFA concentration in hydrolysates was 4 times higher in comparison to the control sample (treated with distilled water at 22°C). The process duration significantly influenced the efficiency of the biomass solubilisation only in the case of the samples hydrolyzed at 22°C. Under these conditions, the extension of hydrolysis time from 2 to 8 hours increased the value of soluble COD, in the samples treated with water and alkaline solvent, by 70% and 55%, respectively. The process conducted at 80°C was not time-dependent over the considered period. The efficiency of the pretreatment carried out at 22°C did not depend on the solvent used in the experiment. Regardless of the test duration, the solubilization of organic matter contained in the hay biomass performed by means of the NaOH solution and distilled water was similar.

The research showed that the appropriate selection of the method of lignocellulosic biomass pretreatment allows increasing the degree of release of organic compounds from the hay biomass, and thus may contribute to increasing the efficiency of the methane fermentation process of this substrate.

It was stated that further studies regarding the chemical composition of raw and pretreated material are needed to confirm our observation about the temperature and reaction time influence on the hay pretreatment efficiency.

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