

Article

Efficiency of Chemical Pretreatment of Sugar Beet Pulp Biomass Intended to Energy Production via Biological Processes

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Abstract: Environmentally friendly anaerobic digestion (AD) of lignocellulose-based materials is becoming an increasingly popular alternative to non-renewable energy sources. It also corresponds with the principles of sustainable development. The structure of lignocellulosic materials, which is resistant to biodegradation, requires using pretreatment methods prior to subjecting them to anaerobic processes. The aim of the study was to evaluate the influence of temperature and type of hydrolyzing agent on the efficiency of chemical pretreatment of sugar beet pulp. Biomass samples soaked in distilled water and 0.05 M solutions of NaOH and H₂SO₄ were left for 20 h, both at room temperature (22 °C) and in a thermostated chamber (50 °C). The changes in pH, electrolytic conductivity (EC), concentration of volatile fatty acids (VFA), dissolved chemical oxygen demand (COD), and phenols were analyzed. The concentration of COD_{dissolved}, demonstrating the efficiency of pretreatment methods, obtained after alkaline hydrolysis conducted at 22 °C was almost at the same level as in the case of acid hydrolysis carried out at 50 °C: 3451 mg dm⁻³ and 3608.5 mg dm⁻³, respectively. Hydrolysis carried out in a NaOH solution at 22 °C appears to be the most economical option for sugar beet pulp pretreatment out of all analyzed ones, as there is no need to increase expenditure on heating samples.

Keywords: lignocellulosic biomass; sugar beet pulp; chemical pretreatment; temperature influence; renewable energy sources; energy production optimization



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1. Introduction

Combusting fossil fuels in order to obtain the energy required for developing industry and agriculture results in environmental contamination and large quantities of waste production [1]. As predicted, the energy consumption on the Earth will increase about three times in the next thirty years [2]. In order to maintain the principles of sustainable development, which meet the needs of the current generation without compromising the ability of future generations to meet their own needs [3], obtaining energy from less invasive sources should be considered. As using conventional sources causes their depletion, more and more attention is given nowadays to searching for methods of renewable energy production in order to provide its resources for posterity. Biomass is a substrate regarded as one of the alternative energy sources. It is a potentially sustainable and naturally available complex biological solid product sourced from living or dead organisms [4]. Biomass consists of agricultural and forestry waste, seaweed, and organic fractions of industrial, animal, and human waste [5]. Using biomass for energy production can solve environmental problems, especially by eliminating the emissions of greenhouse gases, when compared with burning fossil fuels [6]. There are two main groups of methods intended for converting this substrate into energy: thermochemical and biochemical. Thermochemical methods include combustion, pyrolysis, and gasification [5,7]. Such conversions are based on the use of heat and chemical catalysts. Ethanol fermentation, anaerobic digestion, and esterification belong to biochemical methods [8]. Among them, anaerobic digestion is the most popular

method of biomass and organic waste transformation into biofuels [4,9]. In the biochemical processes, organisms and biological catalysts play a major role. The techniques of the thermochemical conversion result in biochar, bio-oil, and gas production, while biogas, ethanol, methanol, and biodiesel are produced during biochemical processes [10,11]. The advantages of thermochemical processes are the high rate and yield of the conversion. In turn, biochemical methods, especially anaerobic digestion, leading to the production of biogas, which can then be upgraded to biomethane, are environmentally friendly and require lower costs [5]. Biomethane, as a high-calorific biomass-derived fuel, can successfully replace natural gas. In the current situation in the world fuel market, the wider deployment of biomethane production can be a way to ensure the continuity of the national economies and their partial independence from external sources of natural gas.

However, these methods also have limitations, which result from the low biodegradability of the substrates containing lignocellulose.

Although lignocellulosic materials (forestry and agricultural wastes, energy crops, and municipal solid wastes) are regarded as a substrate of high potential biogas yield [12], obtaining the high biogas production requires the substrate to be pre-treated to improve its biodegradability and increase the accessibility of hydrolytic enzymes to chains of cellulose and hemicellulose [13]. The process of biogas production by digestion of lignocellulosic material is complicated and needs optimal conditions to achieve high performance. It is limited mainly in the rate and the efficiency of the first phase of the process, enzymatic hydrolysis [14]. In order to shorten this phase and increase the efficiency of biomass conversion into biogas, substrate pretreatment methods are applied. Without the prior application of an appropriate pretreatment method, biomass decomposition in the phase of enzymatic hydrolysis usually occurs very slowly and may be incomplete. Disturbing that phase of anaerobic digestion leads to the formation of toxic products (e.g., formic, acetic, levulinic, and uronic acids, furaldehydes, formaldehyde, 4-hydroxybenzoic acid, vanillin, and phenol) which may negatively influence methanogenic microorganisms [15].

The goal of the pretreatment methods of lignocellulosic materials is to damage the dense structure of lignocellulose. As a result of the process, the substrate contact area with enzymes increases. Moreover, the degree of crystallization and polymerization of cellulose is reduced [15]. Pretreatment methods result in changing the structural components of lignocellulosic materials into a soluble phase which enables the anaerobic microorganisms to transform it into biogas or bioethanol. There are three main groups of lignocellulosic biomass pretreatment methods: physical, chemical, and biological [16,17]. Chemical methods, in comparison to others, are less expensive and more efficient in lignocellulose solubilization [18,19]. The type and concentration of the hydrolyzing agent, pretreatment time, and reaction temperature are the main factors influencing the process efficiency [18].

The most commonly used chemicals in alkaline pretreatment are sodium hydroxide, potassium hydroxide, and ammonium. Among them, the influence of NaOH on substrate solubilization has been studied the most. As a result of saponification and the linkages between lignin and carbohydrate cleavage, the structural components of lignocellulosic substrate become easily biodegradable [20]. Using alkali causes swelling of the lignocellulosic matrix, followed by the lignin structure disruption. Moreover, it leads to increased porosity and accessible surface area. At the same time, a cellulose crystallinity decrease is observed [21]. It was stated that alkaline pretreatment of biomass requires lower temperature and shorter reaction time than other pretreatment methods [22]. The main disadvantage of this method is the possibility of the formation of phenolic compounds, negatively influencing fermentative microorganisms [21]. Acid pretreatment of lignocellulosic biomass is carried out using sulfuric acid or, less frequently, hydrochloric acid. The advantage of this method is the reduction or elimination of the necessity of using hemicellulose-degrading enzymes in the anaerobic digestion process. This is because of the fact that this method results not only in solubilizing hemicellulose but also in transforming solubilized hemicellulose into fermentable sugars [23]. Regarding that, and additionally

considering that hemicellulosic sugars usually constitute about 30% of the total carbohydrate in most lignocellulosic materials, acid pretreatment increases the economy of the process of energy production in biorefineries [24]. The possibility of forming inhibitory compounds, e.g., furfural and 5-hydroxymethylfurfural (HMF), is the main drawback of this method [21].

Pretreatment of lignocellulosic biomass consists in breaking down the recalcitrant structure of the substrate. This process is considered to be necessary for lignocellulosic biomass to become attractive in AD. It is a very important stage in converting biomass into fermentable sugars, which can be further subjected to utilization by microorganisms in anaerobic processes resulting in the production of biofuels [25]. Pretreatment should be conducted with sufficient intensity to increase the availability of cellulose and hemicellulose in the subsequent enzymatic hydrolysis process [26]. Acid pretreatment favors the most hydrolysis of hemicellulose from the lignocellulosic substrate [27], while alkaline pretreatment provides mainly the lignin hydrolysis. What is more, it results in increasing the surface area of the lignocellulose-based material and changing its structural components into a soluble phase more readily available to microorganisms [28].

Sugar beet pulp is a residue remaining after sucrose extraction from sugar beets. Processing 1 ton of sugar beetroots gives about 70 kg of sugar beet pulp (on a dry weight basis [29]). It is characterized by high cellulose and hemicellulose content and low content of lignin. Sugar beet pulp consists of up to 75% *w/w* of carbohydrates (on a dry weight basis [30]). Its detailed composition is as follows (on a dry weight basis): 20–25% cellulose, 25–36% hemicellulose, 1–2% lignin, 20–25% pectin, and 10–15% protein [31,32]. High sugar and low lignin content make sugar beet pulp the desired substrate for biorefinery and/or bioethanol production [30]. The major problem during the biodegradation of this material is the high content of hemicellulose, which has to be removed to enable access to cellulose fibers.

Several methods of sugar beet pulp (SBP) management have been investigated. Puligundla and Mok [33] and Usmani et al. [34] described the valorization of this substrate through enzymatic hydrolysis, acid hydrolysis, and hydrothermal techniques into value-added products. What is more, they presented the biotechnological improvements concerning its valorization and showed the sugar beet pulp potential in the production of biodegradable plastics, pectic oligosaccharides, citric acid, lactic acid, alcohols, microbial enzymes, and biofuels. The possibility of sugar beet pulp use for biofuels, single cell protein, ethanol, methanol, etc., production was also shown by Özbaş and Özbaş [35]. Kamzon and Abderafi [36] conducted a simulation study on the influence of sulfuric acid pretreatment and hydrolysis of sugar beet pulp on bioethanol production, while Grabarczyk et al. [37] compared two methods of this biomass pretreatment applied before subjecting SBP to hydrogen fermentation. The influence of acid pretreatment of SBP on the extraction of carbohydrates under pressure and non-pressure conditions was studied by Gönen et al. [38]. They stated that pressure significantly increased the sulfuric acid effect and intensified sugar extraction. Kharina et al. [39] observed the positive influence of sulfurous acid pretreatment on the extraction of carbohydrates from SBP and on further enzymatic hydrolysis. The effects of several pretreatment methods of SBP (thermal-pressure processing, grinding, enzymatic hydrolysis, and a combination of these methods) on subsequent anaerobic digestion efficiency were studied by Ziemiński and Kowalska-Wentel [40]. They stated that the highest biogas yield (898.7 mL gVS⁻¹) was obtained from the SBP previously processed with grinding, thermal-pressure processing, and enzymatic hydrolysis. The efficiency of an anaerobic process based on sugar beet pulp subjected to thermochemical pretreatment was also studied by Şenol et al. [41]. The highest biogas yield of 458.4 mL gTS⁻¹ was obtained after KOH thermal pretreatment at 100 °C.

The influence of chemical pretreatment methods on the parameters of lignocellulose-based materials was the aim of many studies [18,22,23,30,42]. The results of the thermochemical pretreatment of this biomass were also analyzed [43–45]. In most of these papers, changes in lignocellulosic biomass composition (raw fiber fractions) and concen-

tration of particular monosaccharides released during the pretreatment processes were studied [15,22,45,46]. Very few papers in which other parameters, such as pH, concentration of volatile fatty acids, and dissolved chemical oxygen demand are examined, have been found in the literature [24,47,48], although these parameters can be used as indicators of biomass solubilization.

The aim of the study was to evaluate the influence of low-temperature chemical pretreatment on the efficiency of sugar beet pulp solubilization. The biomass was treated with diluted solutions of NaOH and H₂SO₄ at temperatures of 22 °C and 50 °C to assess the possibility of achieving the biomass solubilization effect without high energy input. The parameters that are important when considering the bioconversion of biomass in the anaerobic digestion process, such as chemical oxygen demand and concentration of volatile fatty acids in the obtained extracts, were examined to assess the efficiency.

2. Materials and Methods

2.1. Examined Materials

The research material was sugar beet pulp collected from the storage tanks of a bio-power plant in Siedliszczki near Piaski, Lublin Province, Poland (Figure 1). The biomass samples were transported to the laboratory and stored in a laboratory freezer at −17 °C. The frozen samples were then thawed under room conditions before the experiment. The sodium hydroxide (NaOH) pellets and sulfuric acid (H₂SO₄) 95% (Avantor, Gliwice, Poland, formerly POCH S.A.) were also used in the experiment.



Figure 1. Sugar beet pulp.

The total solids (TS) and volatile solids (VS) contents of sugar beet pulp were determined. The parameters of the analyzed material are presented in Table 1.

Table 1. Total solids and volatile solids of sugar beet pulp in air-dried sample.

Parameter	Unit	Value
Total solids (TS)	%	93.35 ± 3.51
Volatile solids (VS)	% d.w.	94.56 ± 0.69
COD	mgO ₂ g d.w. ^{−1}	1831 ± 169
C	% d.w.	40.09 ± 0.53
H	% d.w.	4.8 ± 0.01
N	% d.w.	1.57 ± 0.04
S	% d.w.	0.102 ± 0.003
Ash	% d.w.	5.22 ± 0.15
Fixed carbon	% d.w.	0.22 ± 0.03

d.w. dry weight.

2.2. Sugar Beet Pulp Pretreatment

The solutions of NaOH, H₂SO₄, and distilled water acted as the solvents in a batch scale experiment. The 12 samples of air-dried sugar beet pulp of 5 g mass were placed in glass flasks (250 mL of volume). Four of them were filled with 0.05M NaOH solution; another four were filled with 0.05M H₂SO₄, and the others with distilled water in the ratio of 1:20 (*w/v*). The content of the glass flasks was then mixed to ensure the moistening of the whole sugar beet pulp samples in the liquid. The samples with distilled water were used as a control.

Next, two samples with a NaOH solution, two samples with an H₂SO₄ solution, and two samples with distilled water were placed in a thermostated chamber for 20 h at 50 °C, and the other 6 flasks (two of each solvent) were left for 20 h at room temperature (22 °C). After that time, the heated samples were cooled down to room temperature. Then, all samples were subjected to centrifugation at 4000 rpm, which lasted 20 min (MPW-350 Med Instruments, Warsaw, Poland). The contents of the flasks were percolated through filter paper (84 g m⁻²) to the glass vials of 40 mL of volume. The analyses of pH and electrolytic conductivity, the concentration of volatile fatty acids (VFA), dissolved chemical oxygen demand (COD_{dissolved}), and phenols in the obtained filtrates were conducted.

2.3. Analytical Methods

The content of total solids (TS) was determined as a residue after drying the samples of biomass at 105 °C for 24 h in the SUP-4 Wamed drying chamber (Warsaw, Poland). The content of volatile solids (VS) was determined after the ignition of dried samples at 550 °C for 24 h in a Czylok FCF 2,5 S muffle furnace (Jastrzebie-Zdroj, Poland). The pH was measured by using an Easy Plus™METTLER TOLEDO (Greifensee, Switzerland) and electrolytic conductivity (EC) by using an Elmetron CPC-501 potentiometric detector (Zabrze, Poland). For heating the samples to 50 °C, the Stuart SI500 Bibby Scientific thermostated shaking incubator (Stone, UK) was used. The concentrations of dissolved chemical oxygen demand, volatile fatty acids, and phenols in the obtained filtrates were determined spectrophotometrically (VIS spectrophotometer DR 3900, Loveland, CO, USA) by using HACH cuvette tests (LCK514, LCK365, and LCK345, respectively) and HT 200S thermostat (Hach, Loveland, CO, USA). The determination of particular parameters was conducted in accordance with the guidelines provided by the producer.

2.4. Statistical Analysis

All the parameters measured in the obtained filtrates were analyzed in duplicates. The means, standard deviations, and confidence intervals for parameter values were calculated. The statistical analysis was conducted using Microsoft Excel 2013 for Windows software.

3. Results

3.1. The pH of the Hydrolysates

The pH measurement was carried out after 20 h of the hydrolysis, both in the samples left at room temperature and in the thermostated chamber (after cooling them down). The comparison of the mean pH values measured in the samples when the experiment was started and after their pretreatment under different conditions is presented in Figure 2a.

The pH values in the hydrolysates obtained after pretreatment conducted in distilled water at 22 °C and 50 °C did not differ significantly. In the case of hydrolysis carried out in H₂SO₄ solution at 22 °C and 50 °C, the pH values reached 0.95 and 0.85, respectively. The values of this parameter obtained after alkaline hydrolysis differed the most. Higher differences in the pH values after the treatment process, compared to the initial values of the solutions that were used in the experiment, were observed in the case of the alkaline treatment. The pH value in the samples hydrolyzed at 22 °C decreased by 0.8, and in the samples hydrolyzed at 50 °C by 1.7. At the same time, in the samples hydrolyzed with the acid solution, the pH dropped by only 0.3 and 0.4, respectively.

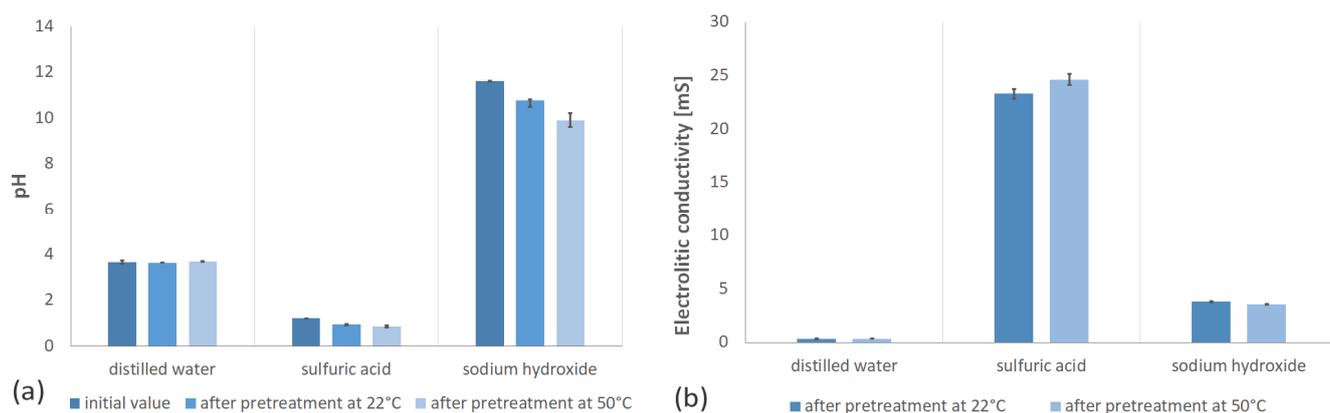


Figure 2. Values of (a) pH and (b) electrolytic conductivity in the samples hydrolyzed at various temperatures in distilled water, NaOH, and H₂SO₄ solutions.

3.2. Electrolytic Conductivity of the Hydrolysates

A graphical presentation of the differences in the EC values in the samples hydrolyzed at various temperatures and in different solvents is presented in Figure 2b. In the samples with distilled water, a slightly higher EC value (of about 5%) was observed in the samples hydrolyzed at 50 °C in comparison to those left at 22 °C. Similar results were obtained in the case of acid hydrolysis, where the EC value in the samples hydrolyzed at 50 °C was 24.65 mS, while at 22 °C, it amounted to 23.30 mS. However, in the case of alkaline hydrolysis, the value of electrolytic conductivity measured in the samples hydrolyzed at 50 °C was about 6% lower than in the samples hydrolyzed at 22 °C.

3.3. COD of the Hydrolysates

The values of dissolved COD levels in the samples after 20 h of hydrolysis are presented in Figure 3a.

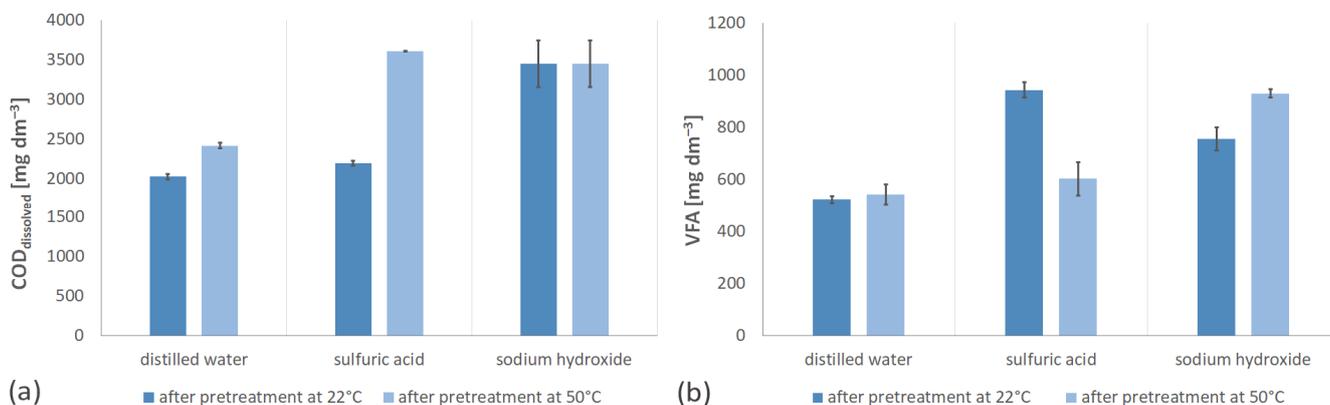


Figure 3. Concentrations of (a) dissolved COD and (b) volatile fatty acids in the samples hydrolyzed at various temperatures in distilled water, NaOH, and H₂SO₄ solutions.

The influence of temperature was the most apparent in the case of acid treatment. The COD value in the samples incubated at 22 °C was similar to that measured in distilled water, while when the temperature rose to 50 °C, the COD value increased by 65%. The mean value of COD measured under these conditions was the highest noted during the experiment. However, it was similar to the mean COD values obtained in both the samples hydrolyzed with the use of an alkaline solution.

Practically, any difference in the dissolved COD value between the samples left in various temperatures was observed in the case of alkaline hydrolysis.

The value of dissolved COD in these samples exceeded the COD_{dissolved} value measured in the samples hydrolyzed in distilled water at the same temperature by 1.7 times.

The influence of temperature occurred in the case of samples soaked with distilled water as a hydrolyzing agent. A higher concentration of dissolved COD was obtained in the samples left at the temperature of 50 °C. The concentration of this parameter was 19% higher compared to its value in the samples hydrolyzed at 22 °C.

3.4. VFA of the Hydrolysates

The graphical presentation of VFA concentrations measured in the samples hydrolyzed at various temperatures and in different solvents is shown in Figure 3b.

The values of VFA in the hydrolysates obtained after the pretreatment conducted in various temperatures in the samples with distilled water as the hydrolyzing agent changed slightly: at the temperature of 50 °C the concentration of VFA was only about 4% higher than at 22 °C. In the case of acid hydrolysis, a clearly higher concentration of volatile fatty acids (by 56%) was observed in the samples left at room temperature. In the samples subjected to alkaline hydrolysis, a higher value of VFA concentration (by 23%) was observed at the temperature of 50 °C.

The concentration of volatile fatty acids in acid and alkaline hydrolysates was much higher than in distilled water. At the temperature of 22 °C, when the hydrolyzing agent was sulfuric acid, the volatile fatty acids concentration was 80% higher compared to the value obtained after the process conducted in distilled water; in the case of alkaline hydrolysis, this value was 44% higher in comparison to the concentration obtained in the control samples. The VFA level in the samples hydrolyzed in sulfuric acid solution at 50 °C was 11% higher in comparison to the values measured in the control samples; in the case of the alkaline hydrolysis, this value was 71% higher. Considering the results of the hydrolysis conducted at 22 °C, acidic conditions favored releasing VFA from the sugar beet pulp. In the case of pretreatment carried out at 50 °C, the highest value of released VFA was obtained under alkaline conditions.

3.5. Concentration of Phenols in the Hydrolysates

The phenol concentration values, which were measured only in the samples subjected to hydrolysis conducted at 50 °C, were $7.3 \pm 0.5 \text{ mg dm}^{-3}$ and $12.4 \pm 0.2 \text{ mg dm}^{-3}$ under acidic and alkaline conditions, respectively. Thus, a significantly higher value of the phenol concentration (by 70%) was observed in the case of hydrolysis carried out in a NaOH solution in comparison to this conducted in an acidic environment.

4. Discussion

About 18 million tons of sugar were produced from sugar beets in Europe in 2019/2020 [49]. On average, the production of 1 ton of sugar from beets yields 1.6 tons of by-product—beet pulp—with a dry matter content of about 20% [29], translating into 28.8 million tons of beet pulp, requiring management, produced in Europe during this time.

Pulp biomass, although largely devoid of simple sugars, is still rich in organic compounds that can be useful in biofuel production. In the pulp studied, their content accounted for as much as 94.6% of its mass. However, as it is well known, a large part of this biomass, even more than 60% of its dry weight [22,32], consists of compounds such as cellulose and hemicelluloses, which are hardly biodegradable, limiting the possibility of efficient use of the chemical energy contained in biomass through its conversion by biological means (methane fermentation, alcoholic fermentation). Therefore, to increase the availability of organic compounds to microorganisms, they were pretreated to release simple organic substances into the solution.

The study was focused on chemical treatment with dilute (0.05 M) sulfuric acid and sodium base solutions because these types of pretreatments are known as efficient and more beneficial for industrial applications compared to the use of concentrated solutions [50]. The studies were conducted at chemicals to biomass ratios of 10% H_2SO_4 *w/w* and 4% NaOH *w/w* as well as low temperatures (22 °C and 50 °C), but with an extended reaction time of 20 h, compared to that typically used in research. According to Kassim et al. [50],

for acid hydrolysis with dilute acids (0.2–2.5% *w/w*) carried out at <120 °C, the hydrolysis time is in the range of 30–90 min. In contrast, concentrated acids are usually used for low-temperature processing (in the range of 30–60 °C). Alkaline treatment with dilute bases at lower temperatures mainly removes lignins [27], but at higher temperatures (>100 °C), it also causes changes in the structure of polysaccharides [51]. The conducted studies indicate that even at low temperatures, there was a release of volatile acids into the solution during alkaline hydrolysis.

When conducting the studies, COD was the primary indicator for assessing the effectiveness of organic matter solubilization. The advantage of this indicator is the ease of determination when compared to the raw fiber fraction of biomass. A comparison of the values of this parameter in the tested solutions shows that the release of organic matter during the action of acid and alkali solutions at 50 °C was similar, while at 22 °C, the alkali solution was 57% more effective than the acid. In practice, this shows that the use of alkali solutions would be preferable to acids, especially since the consumption of acid relative to the weight of the pulp is 2.5 times higher.

Despite the popularity of COD as an indicator of organic matter content in various media (e.g., wastewater, sewage sludge, digested biomass), its use to assess the effects of organic matter solubilization is rare. One of the few sources in which the authors cite changes in dissolved COD values to assess the impact of using pretreatment is the work of Ozkan et al. [47]. They compared the results of the effects of alkaline, thermal, microwave, thermal-alkaline, and microwave-alkaline treatments of sugar beet-pulp. The researchers found that the biomass treated with alkaline (pH 12 for 30 min) or combined thermal-alkaline (pH 12 for 30 min, then autoclaving at 121 °C, 1.5 atm for 30 min) yielded higher solubilization ($6739 \pm 587 \text{ mg dm}^{-3}$ and $20,884 \pm 818 \text{ mg dm}^{-3}$, respectively) than the others, and this translated into higher biohydrogen production. The highest concentrations of COD_{dissolved} received in the conducted study were two times lower compared to the results of alkaline pretreatment and six times lower compared to the results of thermal-alkaline pretreatment obtained by Ozkan et al. [47]. These significant differences in the levels of dissolved COD can be explained by the lower temperature and pressure used in the experiment conducted in the presented study.

According to the observations, an increase in temperature enhanced the efficiency of biomass solubilization for water and sulfuric acid solution, while it had no effect on the effects of sodium base. Zheng et al. [24] also observed an increase in sugar beet pulp hydrolysis due to the increase in temperature during the acid pretreatment. Based on the literature data [52], they stated that it is caused by a significant positive effect of temperature on the solubilization of hemicellulose. The significant increase in dissolved COD value after the biomass pretreatment in the higher temperature examined in the conducted study confirms this theory.

Acid treatment conducted at 22 °C had very little effect on COD leaching in biomass compared to soaking in water. Conducting this treatment at a lower temperature is therefore economically and environmentally unjustified.

Considering the further planned use of biomass for biogas, the effect of the applied treatment on the release of VFA, which constitute the basic substrate for acetotrophic methanogenesis, was also studied. Thus, the release of VFA can be considered to be an indicator of pretreatment efficiency.

In the case of pretreatment conducted in distilled water and sodium hydroxide solution, the growth of VFA concentration dependent on increasing temperature was observed. The temperature influence was more significant in the case of alkaline pretreatment, whereas in the case of distilled water soaking, it was slight. Ziemiński et al. [48] observed the stimulating effect of temperature on the solubilization of beet pulp in water, but they used a liquid hot water treatment, in which the temperatures ranged from 120 to 200 °C, and high pressure was maintained. What is more, they noted a pH drop while increasing the process temperature. The same tendency was reported in the presented study during the hydrolysis conducted under acidic and alkaline conditions. The pH drop was probably

the effect of the rising concentration of acids being the degradation products of lignin and hemicelluloses occurring during the pretreatment.

The greater differences in pH values between the samples tested in the same solutions suggest a greater effect of temperature on the solubilization of organic matter under alkaline than in acidic environments. However, the decrease in pH for alkaline treatments cannot be attributed only to the release of acids (although a temperature-stimulated increase in VFA concentration was observed during this treatment) but also to a decrease in the concentration of OH⁻ ions in solution due to the occurring reactions.

EC was measured to assess the changes in the solution that may indicate a loss of reactants. Electrolytic conductivity depends mainly on the type of ions, their concentration, and the temperature of the solution [53]. Generally, EC increases along with temperature; however, the type of ions present in the solution and the motion of ionic charge have a significant influence on this parameter. Individual ions are characteristic because of varying mobility, so the temperature effect on their behavior is also different. In the conducted study, higher EC levels at 50 °C were obtained in the samples hydrolyzed with distilled water and acid solution, but in the case of alkaline pretreatment, electrolytic conductivity dropped with temperature rise. Probably, higher temperature favored ion mobility increase in the samples soaked in distilled water and H₂SO₄ solution but negatively influenced the mobility of ions present in an alkaline environment. At 50 °C for acid hydrolysis, electrolytic conductivity reached 24.65 mS, while under alkaline conditions at the same temperature—only 3.59 mS. It was the highest difference in the EC values obtained during the pretreatment carried out in different environments at the same temperature. However, this difference results mainly from the different abilities to conduct electricity of particular ions presented in soaking solutions. Generally, H⁺ ions conduct electricity better than Na⁺ and OH⁻ ions; thus, the EC values in acid solutions are the highest.

Due to the toxicity of phenols to microorganisms, their concentration in solution was measured. Higher concentrations of phenols were measured in the samples hydrolyzed with NaOH solution, which can be attributed to the fact that phenols are the result of lignin decomposition, the intensification of which occurs after alkaline pretreatment of lignocellulosic biomass [54,55]. However, the concentrations of these compounds, although more than 1.5 times higher after alkaline treatment, did not pose a threat to methanogenic microorganisms. According to a study by Kayembe et al. [56], the concentrations of phenols at which toxic effects were observed on the methanogens conducting the anaerobic digestion of pig manure were 1249 mg dm⁻³. Similar toxic values (1400 mg dm⁻³) were observed by Wang et al. [57]. The concentrations obtained in the conducted study were more than 100 times lower, and it can be concluded that they do not pose a threat to the microorganisms conducting the fermentation process. The low level of phenolic release in the experiment also conducted under alkaline conditions is associated with the low lignin content of beet pulp, which usually does not exceed a few % [31]. Therefore, alkaline treatment can be safely used to prepare the pulp for the biogas production process.

Biomass pretreatment leading to an increase in the production of biogas, the main component of which is methane, is one of the ways for faster achieving of the annual production of biomethane in the European Union at the level of 35 billion cubic meters. According to the guidelines set out in the REPowerEU plan [58] published on 18.05.2022, this goal must be achieved by 2030. Implementation of this requirement was dictated primarily by the urgent need for diversification of gas supplies and reducing the EU's dependence on Russian fossil fuels. Moreover, scaling up the production and application of biomethane helps to mitigate the climate crisis.

The waste biomass should be a basic substrate for biomethane production. Such an approach allows not only for protecting the environment but also for achieving goals of sustainable production and realizing the models of circular bioeconomy. According to the economic and social analysis conducted by D'Adamo et al. [59] and González-Castaño et al. [60], biomethane is an energy resource that can meet sustainable production requirements.

5. Conclusions

The recalcitrant structure of lignocellulosic substrates, such as sugar beet pulp, limits the efficiency of their conversion to biofuels by biological methods. For this reason, looking for the efficient and most cost-effective methods of lignocellulosic biomass pretreatment is needed in order to intensify the production of biofuels, which is particularly important from the point of view of implementing the strategy related to increasing the share of biomethane in the European economy by 2030.

The laboratory studies which were aimed at assessing the influence of acid and alkaline hydrolysis of sugar beet pulp showed that:

- The solubilizing effect of these pretreatment methods depended on temperature, even at its low range, as shown by the similar solubilization efficiency of beet pulp biomass obtained as a result of alkaline pretreatment (4% NaOH *w/w*) at 22 °C and after pretreating it with acid (10% H₂SO₄) at 50 °C.
- To obtain a comparable solubilization effect, it would be more advisable to use the alkaline pretreatment at 22 °C due to its lower costs. The reduction of costs results from savings on heating and lower consumption of NaOH than H₂SO₄ taking into account the unit mass of the pulp (the consumption of NaOH is 2.5 times lower, while the price for 1 kg of both reactants is similar).
- Under alkaline pretreatment at a lower temperature, the concentration of volatile fatty acids was lower, both when compared with the alkaline pretreatment carried out at 50 °C and the acid treatment at 22 °C.

The increase in biogas production, which can be obtained due to the applied biomass pretreatment method, cannot be estimated only on the basis of the results of solubilization tests. Thus, further research is needed to determine the composition of the products released into the solutions and then to determine the biogas and methanogenic potential of the treated biomass in batch assays.

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