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Comparison of acid and alkaline pre-treatment of lignocellulosic materials for biogas production

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Abstract: This work deals with the study of a pre-treatment method promoting degradability of lignocellulosic biomass and hence biogas yield therefrom, as this material is challenging to decompose due to its structure. The investigated pre-treatment methods are hydrolysis of the material in NaOH (0.5% and 5%) and H₂SO₄ (0.5% and 5%) at temperatures of 90–100 °C for 2 h. This work aimed to compare the effects of these pre-treatment methods on the lignocellulosic composition of maize waste (maize stalks, leaves and cobs), rapeseed straw and wheat straw and the biogas yields from its subsequent anaerobic digestion. Pre-treatment by 0.5% NaOH increased the biogas production the most (by 159% for rapeseed straw, 240% for wheat straw and 59% for maize waste) also the degrees of solubilisation was higher, at the same time the proportions of total organic carbon (TOC) in the solid component appear to be sufficiently high, it could be assumed that there has been sufficient disturbance of the material structure. Alkaline methods have proven to be more suitable compared to acid pre-treatment methods, also the use of alkali with a lower concentration has shown to be more efficient, which is more advantageous for use in practice from an environmental and economic point of view.

Keywords: agriculture; biogas potential; decomposition; methane; renewable energy source

With growing demand and declining fossil fuel supplies, finding renewable energy sources is a necessary task. An important renewable energy source is biogas obtained by anaerobic digestion of substrates such as lignocellulosic biomass. Lignocellulosic biomass includes waste from agricultural, forestry or agro-industrial production which is accumulated every year in large quantities (Isikgor and Becer 2015). The main components of lignocellulosic biomass are cellulose, hemicellulose and lignin (Lee et al. 2014). These components form a complex and compact structure that is difficult to degrade by anaerobic microorganisms. The factors affecting the enzymatic hydrolysis

of cellulose include lignin content, hemicelluloses content, acetyl group content, cellulose crystallinity, degree of polymerisation, specific surface area, and pore volume and particle size (Zhu et al. 2008). To remove barriers causing degradation resistance and to increase the enzymatic degradability of cellulose, changing the chemical composition and physical structure of the raw material, must be applied to pre-treatment methods.

In the last few decades, various biological, physical, physicochemical and chemical methods for the pre-treatment of lignocellulosic biomass have been developed. Biological methods include pre-treatment

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by fungi (Mustafa et al. 2016), bacterial systems (Xu et al. 2018), microbial consortium (Zhong et al. 2011) or enzyme pre-treatment (Schroyen et al. 2014). Physical methods are mechanical pre-treatment (Menardo et al. 2012), radiation (Kaur and Puthela 2016) or ultrasonic pre-treatment (Wang et al. 2012). Physicochemical methods include steam explosion (Lizasoain et al. 2017) and liquid hot water (Rajput et al. 2018). The most common group of pre-treatment methods are chemical methods, which use chemicals for the pre-treatment of lignocellulosic materials, such as oxidising agents (Song et al. 2012), organic solvents (Ostovareh et al. 2015), alkalis (Zhu et al. 2008) and acids (Badsham et al. 2012). In published studies focused on chemical pre-treatments, high concentrations of the solutions at low temperatures are mostly used. These processes are economically costly for the demanding maintenance of the equipment in which the pre-treatment is carried out, but especially for the complexity of the process taking place with the formation of inhibitors as 5-hydroxy methyl-furfural and 2-furfuralaldehyde (Mood et al. 2013) or the ancillary costs associated with their removal.

The aim of this work was to study the pre-treatment methods, acid (H_2SO_4) and alkaline (NaOH) hydrolysis of selected lignocellulosic materials (maize waste, rapeseed and wheat straw), which could represent easier and more economically applicable methods to real biogas plants with respect to the environment. Therefore, acid and alkaline pre-treatment was studied using a low concentration of solutions. The chosen pre-treatment methods were carried out at 90–100 °C, as in many cases there is a lot of unused thermal energy at biogas plants. So, such a heat source (in the form of cooling water of the cogeneration unit, which reaches a temperature of up to about 90 °C) is realistically available at biogas plants.

The degree of solubilisation, total organic carbon (TOC) in the materials before and after hydrolysis and the sugars released into the liquid fraction were

investigated to assess the degradation of the lignocellulosic materials. For a more efficient evaluation of the suitability of the methods, the production of biogas was observed from the solid part after hydrolysis and also from the liquid fraction after hydrolysis, as sugars are expected to be released into the liquid fraction, which would also be a possible substrate for biogas production.

MATERIAL AND METHODS

Raw materials and inoculum. After harvest, maize waste (stalks, leaves and cobs), wheat straw and rapeseed straw were dried and cut to a size of 0.5 to 1.5 cm. The materials were dried before harvesting in the field, so it was no longer specially dried under laboratory conditions. The main physicochemical characteristics of the studied lignocellulosic materials are reported in Table 1. The inoculum was anaerobically stabilised sludge from the wastewater treatment plant. The total solids (TS) dry matter of the anaerobic sludge was 15.5 g/L, the volatile solids (VS) portion was 62%, chemical oxygen demand (COD) of filtered reject water was 320 mg/L.

Pre-treatment methods. Two different pre-treatment methods of lignocellulosic materials, acid and alkaline hydrolysis were used in this study for the pre-treatment of lignocellulosic materials. The acid pre-treatment was performed using H_2SO_4 (at a concentration of 5% or 0.5%), where 1 g of lignocellulosic materials (rapeseed straw, wheat straw, maize waste) was mixed with 100 mL of this solution and heated at pre-treatment temperature 90–100 °C for 2 h. NaOH (at a concentration of 5% or 0.5%) was used in the alkaline pre-treatment, where also the substrates were mixed with the solution in a ratio of 1:100 and heated at a pre-treatment temperature of 90–100 °C for 2 h. To compare the effectiveness of these methods, as a control were used materials pre-treated in water.

Table 1. Characteristics of the maize waste, wheat straw and rapeseed straw

	Maize waste	Wheat straw	Rapeseed straw
TS (%)	94.85 ± 0.85	94.05 ± 1.45	91.90 ± 1.70
VS (%)	92.05 ± 2.85	92.25 ± 3.55	85.03 ± 4.98
COD (g/g)	1.31 ± 0.17	1.20 ± 0.29	1.24 ± 0.21
Cellulose (%)	45.15 ± 0.25	28.75 ± 1.65	22.00 ± 3.00
TOC (mg/g)	428.09 ± 10.38	455.77 ± 1.65	496.35 ± 20.69

TS – total solids; VS – volatile solids; COD – chemical oxygen demand; TOC – total organic carbon

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During the pre-treatment, samples of the liquid fraction were taken at time intervals of 0.25, 0.5, 1, 1.5 and 2 h for the analysis of COD and total sugars. The effect of the selected pre-treatment method on the solubilisation and degradation of the materials was compared based on the average values of COD, total sugars and TOC analysis results before and after the material treatment, respectively total sugars content released from 1 g of dry matter into the liquid fraction and subsequently calculated percentages of total sugars from the measured COD (from 1 g of dry matter) in the liquid fraction. The degree of solubilisation of the materials was expressed by the known released COD (g/g TS) into the liquid fraction of the reaction mixture (in a given time) from 1 g of total dry matter in the initial material and the initial COD (g/g TS) of materials not subjected to the pre-treatment (Penaud et al. 1999).

$$\text{degree of solubilisation} = \frac{\text{COD in liquid fraction from 1 g of initial material (g/g TS)}}{\text{COD of untreated initial material (g/g TS)}} \times 100 \quad (1)$$

Biogas potential test. After pre-treatment of materials with 5% H₂SO₄, 0.5% H₂SO₄, 5% NaOH, and 0.5% NaOH at the temperature of 90–105 °C for 2 h, the liquid fraction of the reaction mixture was separated from solids through a sieve with an aperture size of 1 × 1 mm. Both solid parts and liquid fractions after pre-treatment were used as substrates in the biogas potential test. To neutralise the solid parts of substrates prior to the biogas potential test, the pre-treated materials were rinsed with distilled water until the pH was equal to 7, similar to Liu et al. (2019). The liquid fractions after pre-treatment with 5% NaOH were neutralised with 37% HCl in an amount of 0.94 mL (maximum value) per 100 mL of the liquid fraction before starting the test. The liquid fractions after pre-treatment with 5% H₂SO₄ were neutralised with 50% NaOH in an amount of 0.73 mL (maximum value) per 100 mL of liquid fraction. For liquid fractions at lower concentrations (0.5% NaOH and 0.5% H₂SO₄), 10 times smaller amounts of neutralising agents were used.

The test was performed according to the method of Angelidaki et al. (2009). Gas-tight flasks were loaded in triplicate with 25 mL of inoculum with the concentration of 12 g/L and substrate (solid or liquid parts) to obtain an inoculum to substrate ratio of 2:1 (based on the organic matter content (VS)).

Subsequently, water was added to the flasks to an obtained final concentration of sludge of 1 g/L VS. Thus, the total volume in the flasks was 300 mL. To determine the biogas production of the inoculum, one triplicate of blank samples containing only inoculum was prepared, which was then subtracted from the production of the lignocellulosic samples. To evaluate the results, the average values of biogas production from triplicates were used. The sludge mixture was bubbled through with nitrogen for 1 min and then the bottles were hermetically sealed with a rubber septum. Biogas production was then measured at given time intervals by a volumetric method when by inserting the needle into the rubber septum of the bottle, the headspace of the bottle was connected to the gas burette. The volume of gas in the burette was recorded after releasing the biogas pressure from the bottle to the burette that was filled with a saturated solution of NaCl with HCl at pH 4.5 to avoid dissolving of CO₂ from biogas. The length of the test period was 25 days.

Analytic methods. For the determination of COD, the modified method of determination by potassium dichromate "semimicromethod" according to Eaton (2017) was used. The standard gravimetric method (Eaton 2017) was used to determine total solids (drying at 105 °C) and volatile solids (annealing at 550 °C). The percentage of VS was calculated according to the equation:

$$\text{VS (\%)} = \frac{\text{VS (g/g)}}{\text{TS (g/g)}} \times 100 \quad (2)$$

Total sugar concentration was determined by the standard Dubois spectrophotometric method described in Saha and Brewer (1994). The proportion of TOC in the investigated materials was determined on a Vario Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). The composition of biogas was measured by a gas analyser GA 2000 Plus (Geotechnical Instruments, Coventry, UK). The percentage of cellulose was determined and calculated according to the different weights on the frit according to Rivers et al. (1983). Each parameter was measured three times and the average values were taken.

RESULTS AND DISCUSSION

Effect of pre-treatments on the decomposition and solubilisation of lignocellulosic materials. Time courses of the degrees of solubilisation achieved during pre-treatment of lignocellulosic materials are

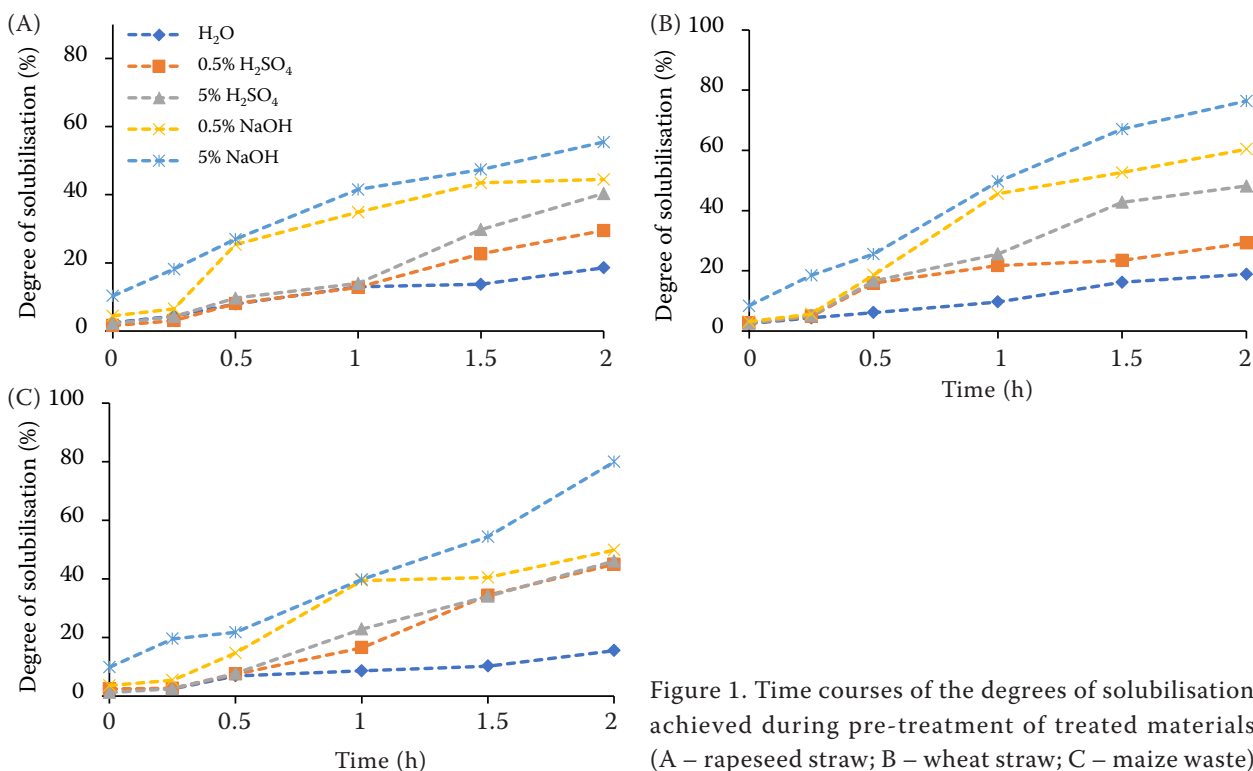


Figure 1. Time courses of the degrees of solubilisation achieved during pre-treatment of treated materials (A – rapeseed straw; B – wheat straw; C – maize waste)

shown in Figure 1. The lowest degrees of solubilisation (< 20%) were achieved for all treated materials during pre-treatment in water. For a pre-treatment time of 1 h, the degrees of solubilisation using acid pre-treatment in the case of rapeseed (0.5% H₂SO₄ and 5% H₂SO₄ at 90–100 °C) do not differ much compared to the pre-treatment of this material in water. For maize waste and wheat straw, the degrees of solubilisation were 8–15% higher compared to pre-treatment in water for 1 h. A significant effect on the solubilisation of lignocellulosic materials was achieved only after 1 h of the acid pre-treatment (especially when using 5% H₂SO₄), the solubilisation degree increased to 30–50% within 2 h.

Alkaline pre-treatment (0.5% and 5% NaOH) was much faster and the solubilisation of the studied materials (35–50%) increased most rapidly within 1 h of the pre-treatment process and after that, the solubilisation rate decreased. The highest degree of solubilisation, about 80%, was achieved using 5% NaOH for pre-treatment of wheat straw and maize waste.

During the experiment, the values of total sugars (in mg from 1 g of material) released from lignocellulosic materials into the liquid fraction were monitored at time intervals of 0.25, 0.5, 1, 1.5 and 2 h and thus the percentages of total sugars in the measured COD (from 1 g of material) in the liquid

fraction were calculated (Table 2). The sugar portion of the total COD content was quantified to evaluate the release of organic matter into the liquid fraction and to compare saccharification with solubilisation. Though according to Table 2, the degree of saccharification was higher for acid hydrolysis, Figure 1 shows that the overall degree of solubilisation was higher for alkaline hydrolysis.

Although the degree of material solubilisation was not high when using acid pre-treatment, a high ratio of dissolved total sugars to COD in the liquid fraction was observed after 30 min of treatment (> 80%, > 90%). This corresponds to the findings of various studies published (Solarte-Toro et al. 2019, Tan et al. 2019). During acid pre-treatment of lignocellulosic materials at higher temperatures, hemicellulose is effectively removed and monosaccharides (xylose, arabinose) are released in a short time, which increases the porosity of cell walls and facilitates subsequent enzymatic hydrolysis of cellulose.

Lower amounts of sugars in the liquid fraction of the mixture in alkaline pre-treatment indicate a lower degree of cellulose degradation. Since the highest levels of organic substances have been released into the liquid fraction during alkaline pre-treatment, degradation of lignin and partial degradation of hemicellulose and cellulose is assumed. The alkaline reagent cleaves the bonds in lignin and glycosidic

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Table 2. Time course of total sugars in chemical oxygen demand (%) released into the liquid fraction during the thermochemical pre-treatment of treated materials

Time (h)	H ₂ O	0.5% H ₂ SO ₄	5% H ₂ SO ₄	0.5% NaOH	5% NaOH
Rapeseed straw					
0.25	20.0	28.8	57.1	36.1	40.0
0.5	19.2	57.1	85.8	46.0	51.2
1.0	26.4	86.2	87.4	33.3	58.0
1.5	27.8	88.5	89.5	33.0	59.1
2.0	36.6	92.6	94.0	34.7	66.8
Wheat straw					
0.25	30.4	35.6	27.4	29.7	17.2
0.5	32.2	54.6	92.3	34.4	58.8
1.0	24.3	96.4	95.7	41.8	62.2
1.5	27.0	96.0	98.8	43.3	70.5
2.0	27.8	93.0	92.5	42.1	71.7
Maize waste					
0.25	8.6	18.4	52.6	31.6	31.6
0.5	13.1	79.4	90.7	40.8	32.8
1.0	23.6	87.4	94.3	43.9	42.5
1.5	28.2	91.9	96.1	47.0	43.7
2.0	21.2	94.4	90.0	47.4	52.0

bonds in the biomass are hydrolysed (Tan et al. 2019), which can be verified by the differences in the total organic carbon values measured in the solid fraction of untreated and treated materials (Table 3).

Obviously, treated material has degraded, which can be visually observed in its structure changes. The material is lighter in colour, more fragile and with non-sharply bounded edges of the solids, which also indicates its degradation. The lowest conversion of total organic carbon from the materials into the liquid fraction (4.2, 2.2 and 0.7%) was observed for pre-treatment in water. The highest conversion of TOC occurred in the pre-treatment of all materials in 5% NaOH, where TOC from wheat straw decreased

from 43% to 9.5% and in the case of maize waste was decrement similar.

According to the measured values (Table 3), the alkaline pre-treatment had a more significant effect on the degradation of the materials compared to the acid methods. For the substrates tested, pre-treatment with 0.5% NaOH seems to be comparable to pre-treatment with 5% H₂SO₄. By maize waste, the effect of 0.5% H₂SO₄, 5% H₂SO₄ and 0.5% NaOH was comparable with respect to the conversion of carbon to the liquid fraction. Maize waste treated in 0.5% H₂SO₄ showed a 20.7% TOC loss, which is about 14% more than other materials treated this way. The most pronounced effect of thermo-chemical

Table 3. Percentage of TOC/TS_{untreated materials} and TOC (% in parentheses) in dried materials after pre-treatment

Pre-treatment method	Rapeseed straw		Wheat straw		Maize waste	
Untreated	44.1	(44.1)	43.0	(43.0)	41.6	(41.6)
H ₂ O	39.9	(43.5)	40.8	(48.2)	40.9	(48.0)
0.5% H ₂ SO ₄	36.1	(45.5)	36.1	(48.9)	20.9	(36.1)
5% H ₂ SO ₄	23.7	(35.3)	22.5	(41.5)	16.3	(28.6)
0.5% NaOH	28.4	(45.4)	18.6	(44.9)	22.7	(42.9)
5% NaOH	18.1	(36.0)	9.5	(38.6)	8.0	(37.8)

TOC – total organic carbon; TS – total solids

pre-treatment on material degradation was observed, except for one case (0.5% NaOH), for maize waste. It appears this material is more accessible to structural changes in pre-treatment processes.

Due to the degree of solubilisation and decomposition, also to the percentage of sugars released into the liquid portion of the pre-treatment solution, it could be said that treated materials in 0.5% NaOH have the greatest biogas potential. Since the degrees of solubilisation are higher, at the same time the proportions of TOC in the solid component appear to be sufficiently high and the values of sugars released into the liquid solution are low. It could be assumed that there has been sufficient disturbance of the material structure and at the same time, there was no great loss of cellulose and hemicellulose, which could break down into sugars and thus be reflected in higher values of total sugars in the liquid fraction.

The high percentages of sugars in liquid fractions after acid pre-treatments indicate the high loss of sugars caused by the degradation of cellulose and hemicellulose, which means that a smaller proportion of these polysaccharides remains in the solid fraction of pre-treated materials. High degrees of

solubilisation and high TOC losses in materials treated in 5% NaOH indicate the fact that in a solid proportion of materials remained small amounts of solids, which could be processed in a subsequent anaerobic process with a high biogas yield. To fully evaluate the efficiencies of the individual types of pre-treatments, solid parts of treated materials as well as liquid fractions after pre-treatment were used as substrates for biogas potential tests.

Effect of pre-treatments on the biogas production of lignocellulosic materials. The course of specific biogas productions from the solid parts of treated materials are reported in Figure 2. Obtained specific productions of methane are reported in Table 4. The most effective pre-treatment method in enhancing the specific biogas production compared to untreated material (by 159% for rapeseed straw, 240% for wheat straw and 59% for maize waste) was pre-treatment by 0.5% NaOH. The second most effective method was the pre-treatment with 5% NaOH, which increased the specific biogas production compared to untreated material by 41% for rapeseed straw, 140% for wheat straw and 42% for maize waste. In alkaline pre-treatments, the struc-

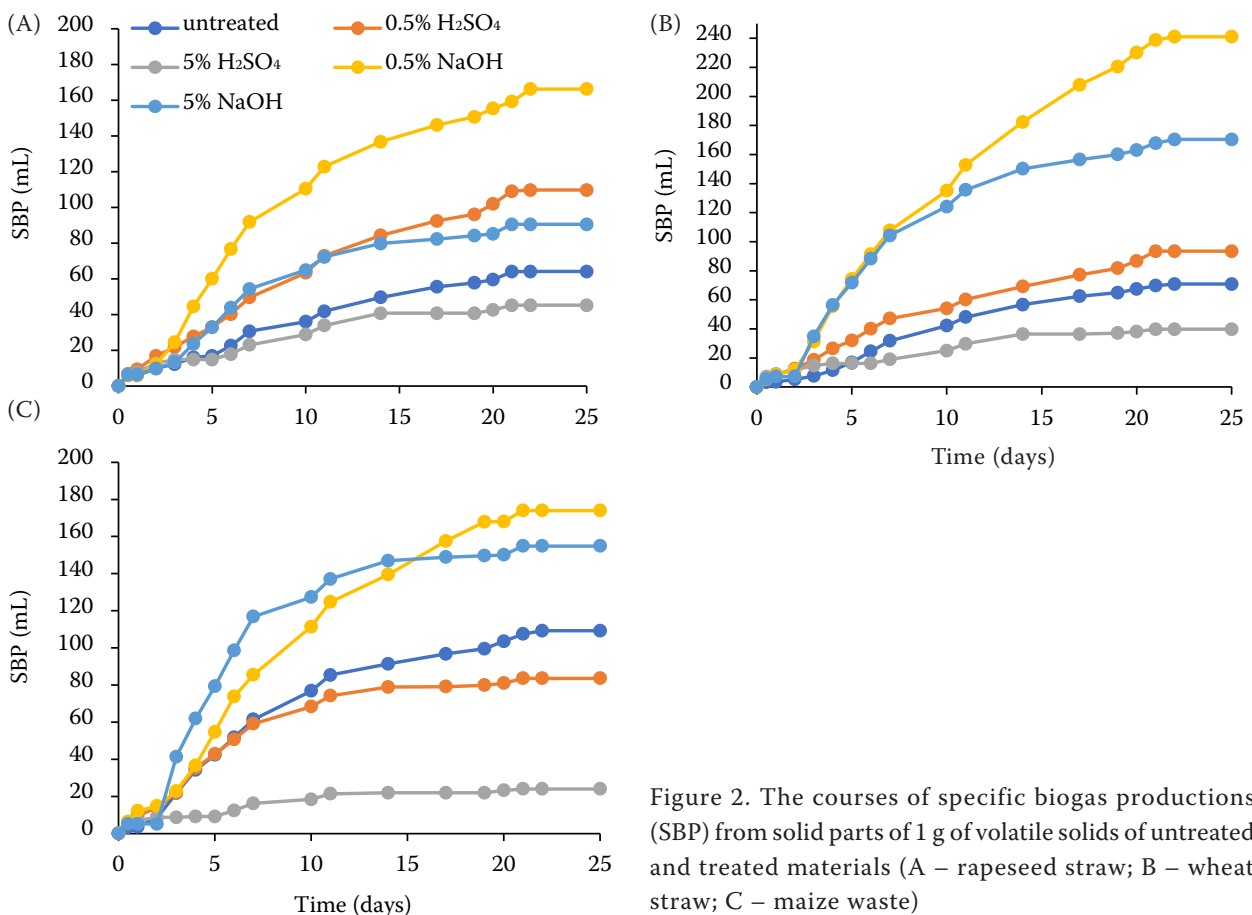


Figure 2. The courses of specific biogas productions (SBP) from solid parts of 1 g of volatile solids of untreated and treated materials (A – rapeseed straw; B – wheat straw; C – maize waste)

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Table 4. Specific methane productions (mL) from the solid parts of 1 g of volatile solids of treated materials

	Untreated	0.5% H ₂ SO ₄	5% H ₂ SO ₄	0.5% NaOH	5% NaOH
Rapeseed straw	32.2	61.9	25.6	103.1	53.6
Wheat straw	32.6	47.5	19.1	139.4	94.9
Maize waste	51.3	45.3	12.6	103.6	87.5

ture of the materials was disrupted but at the same time remained rich in cellulose content, which is a positive fact for possible enzymatic hydrolysis and subsequent anaerobic treatment of these materials.

The fact that there was a higher increase in biogas production at a lower concentration of NaOH compared to a higher concentration of NaOH, could be due to a higher residual organic content presented by TOC in the materials after pre-treatment by 0.5% NaOH, which is presented in Table 3.

In several works, it is noted that in alkaline methods, saponification reactions of the intermolecular ester bond, which crosslinks xylan and lignin, take place and thus lead to the destruction of the structural bonds between lignin and carbohydrates. Alkaline pre-treatment methods cause swelling of the biomass, which leads to a decrease in the degree of polymerisation and crystallinity and an increase in the internal surface area. There is also partial solvation of hemicellulose and disruption of the lignin structure by disruption of its glycosidic ether bond (Behera et al. 2014, Lee et al. 2014).

Pre-treatment by 0.5% H₂SO₄ increased the specific biogas production of solid parts of rapeseed straw (by 71%) and wheat straw (by 32%), but the specific biogas production of maize waste decreased by 23.5% compared to untreated material using this method. The 5% H₂SO₄ pre-treatment method did not prove effective for any material as there was a 29% decrease in specific biogas production for rapeseed straw, 44% for wheat straw and 78% for maize waste compared to untreated material. These values also confirm our assumptions from previous analyses. The cellulose of the individual materials decomposed in the acid pre-treatments to such an extent that these substrates are depleted of this main source of nutrition for anaerobic microorganisms.

Mirmohamadsadeghi et al. (2021) similarly reported a decrease in methane yield after acid pre-treatment using H₂SO₄ (1% w/w, at 160 °C, 5 min) also with discarding the pre-treatment liquer, which contained considerable amounts of sugars and volatile fatty acids released from hemicellulose degradation. They stated

that the acidic pre-treatments (causing hemicellulose removal) are not appropriate for biogas production from solid residues, as hemicellulose content was found to have a higher correlation with methane yield than cellulose content. For the reduced methane yield using H₂SO₄ could be responsible also sulphate ion inhibition as reported by Kim et al. (2018), when using the most dilute acid treatment (0.01% H₂SO₄, at 121 °C for 1 h) achieved the highest methane yield and for the most concentrated acid treatment (2% H₂SO₄, at 121 °C for 1 h) they achieved the lowest methane yield. Another reason why a higher concentration of H₂SO₄ has proven to be more inappropriate compared to a lower concentration is that at higher concentrations can occur generation of components such as furfural and HMF (5-hydroxymethylfurfural) because of dehydration of xylose, galactose, mannose, and glucose (Hendricks and Zeeman 2009).

As a higher release of sugars into the liquid fraction was observed in the acid pre-treatments, higher biogas production from the liquid fraction could be expected after the acid hydrolysis. But the results of specific biogas production (Figure 3) and specific methane production (Table 5) from the liquid fractions after acid and alkaline pre-treatments are not different from each other. Also, the values of biogas and methane productions are very low due to the measured COD and total sugars in the pre-treatment solutions, which indicates that the composition of organic matter and sugars released into liquid fractions during pre-treatment was not satisfactory for microorganisms of the anaerobic process. For comparison, in Figure 3 there are also values of specific biogas productions from the liquid fraction if the soaking medium was water – so the variant without the addition of any chemical agent. Table 6 shows the total biogas productions after adding the biogas productions from the solid and liquid fractions. Results show that approximately a quarter of the total biogas production from materials treated by alkaline hydrolysis is biogas produced from organic substances that have been released into the liquid fraction during hydrolysis. In acid hydrolysis, the

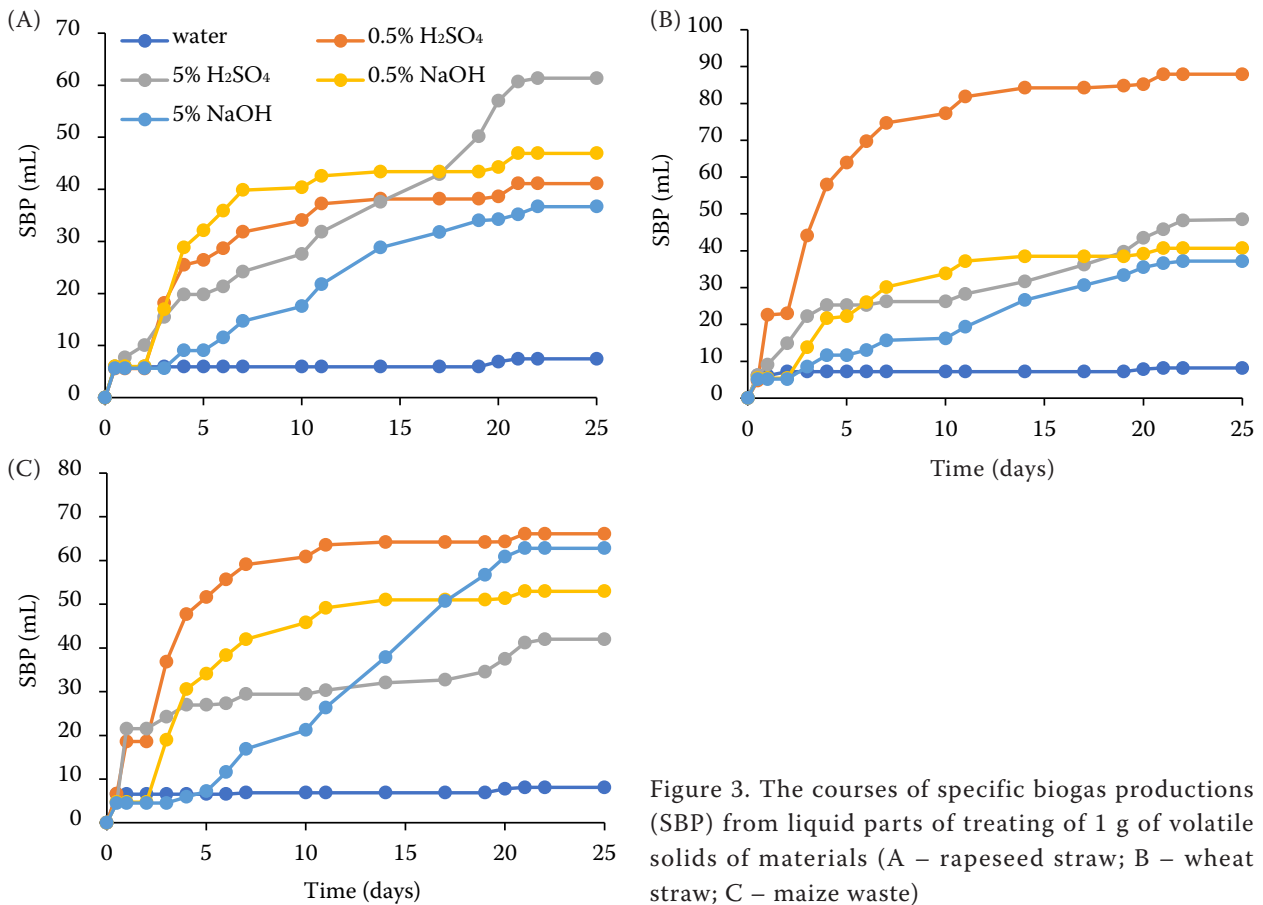


Figure 3. The courses of specific biogas productions (SBP) from liquid parts of treating of 1 g of volatile solids of materials (A – rapeseed straw; B – wheat straw; C – maize waste)

biogas produced from the liquid fractions represents approximately half of the total biogas production.

Considering the amount of neutralising agents used to neutralise the substrates before the start of the biogas potential test, it is also possible to consider the possible inhibitory effect of high salinity caused by higher neutralising reagents addition, especially in the case of 5% reagents. But as described in the Materials and Methods section, by adding water to obtain the final concentration of sludge 1 g/L VS, the liquid fraction was diluted about three times and the solid fraction even more. Thus, it can be said that the inhibitory effect of salinity has decreased. Our results show that all materials have higher biogas production using 0.5% NaOH. Differences between 0.5% NaOH and 5% NaOH may be due to higher salinity, as the

theoretical NaCl formation is 73 g/L when using 5% NaOH. However, after 3-fold dilution in the kinetic test, it is 24.3 g/L NaCl. Such a concentration can have a significant effect on the anaerobic degradation of lignocellulosic biomass, as confirmed by the work of Liu and Boone (1991) and Aslan and Şekerdağ (2015). Nevertheless, no significant differences were observed in the production of biogas from liquid fractions between the use of 0.5% NaOH and 5% NaOH. Even using 0.5% NaOH, the presumed NaCl concentration after neutralisation is 2.4 g/L, which is not the concentration at which inhibition should occur.

However, when using more concentrated H₂SO₄, not only the effect of salinity but also the effect of the presence of sulfates could have a negative effect. In the presence of sulfates, methanogenic microorgan-

Table 5. Specific methane productions (mL) from the liquid fractions of treating of 1 g of volatile solids materials

	0.5% H ₂ SO ₄	5% H ₂ SO ₄	0.5% NaOH	5% NaOH
Rapeseed straw	21.4	30.7	27.6	20.6
Wheat straw	40.6	23.1	23.0	21.5
Maize waste	34.2	21.2	29.8	33.4

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Table 6. Total specific biogas productions (mL) from 1 g of volatile solids of treated materials (solid and liquid parts)

	0.5% H ₂ SO ₄	5% H ₂ SO ₄	0.5% NaOH	5% NaOH
Rapeseed straw	150.8	106.5	213.2	127.2
Wheat straw	181.4	88.3	281.9	207.6
Maize waste	149.7	66.0	226.9	217.7

isms compete with sulfate-reducing microorganisms, which use sulfates as the final electron acceptor in the oxidation of organic substances. In addition, sulfate reduction produces sulfides that can cause sulfide inhibition of anaerobic processes (Chen et al. 2008).

The methane content in biogas for individual lignocellulosic materials was on average 56% for rapeseed straw, 52% for wheat straw and 53% for maize waste. A special feature of biogas from rapeseed straw was the high content of hydrogen sulphide – even above 5 000 ppm.

Comparing the achieved total specific biogas production of our investigated lignocellulosic materials pre-treated by 0.5% NaOH in the range 213–282 L/kg VS with other materials regularly used for biogas production as maize silage with specific biogas productions (SBP) 457 L/kg VS achieved in kinetic tests and 655 L/kg VS achieved during long-term operation of the reactor (Hutňan et al. 2010), dairy manure with SBP in the range 366–436 L/kg VS (El-Mashad and Zhang 2010), waste activated sludge with SBP of 396 L/kg VS (Xu et al. 2014), the achieved SBP may seem relatively lower. However, if we take into account that this would be a simple pre-treatment method that can be used in practice and the substrates being processed are agricultural waste, from which e.g. maize waste has no other application in agriculture than ploughing as an organic fertiliser, the SBP values achieved in this way can convince us of their suitability for use as sustainable substrates or co-substrates to conventional substrates for biogas production at real biogas plants. The methane content was 52–56%, which is a common value for carbohydrate substrates. Maize silage has a methane content of about 55% (Hutňan et al. 2010). Also, these results are values from a batch kinetic test. During long-term processing and adaptation of anaerobic microorganisms, it can be expected that the specific biogas production as well as the methane content from the investigated lignocellulosic materials will be higher.

These results show that the pre-treatment method that proved to have the greatest potential for use in practice is the hydrolysis in 0.5% NaOH, which would also represent a simple method with a low

environmental and economic impact, as it would involve the use of alkaline at low concentration. Since the degrees of solubilisation are higher, at the same time the proportions of TOC in the solid component appear to be sufficiently high, it could be assumed that there has been sufficient disturbance of the material structure. This statement was also confirmed by a test of the biogas potential, where this method proved with the highest biogas production compared to other methods.

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