EFFECT OF DILUTE ACID PRETREATMENT OF DIFFERENT ENERGY CROPS ON THEIR ENZYMATIC HYDROLYSIS

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Summary

In this work the efficiency of lignocellulosic material degradation under the influence of dilute acid hydrolysis was studied. Three species of energetic crops were used in the investigation: *Miscanthus giganteus*, *Sorghum Moensch* and *Sida hermaphrodita*. The aim of this work was to determine the influence of chemical hydrolysis of energetic plants on the efficiency of enzymatic hydrolysis and biogas production in anaerobic digestion process. The results obtained during the research indicate that the level of degradation of high-molecular structures of the plants, due to chemical hydrolysis, is quite high. The best results were achieved for biomass pretreatment with 3% (v/v) sulfuric acid with a reaction time of 30 min at 121°C, which leads to releasing high quantities of VFA and monomeric sugars into the supernatants. The maximum concentration of glucose and xylose obtained after dilute acid hydrolysis was 2.4 and 10.5 g/dm^3, respectively. The maximum concentration of VFA (volatile fatty acids) was equal to 3384 mgCH_3COOH/dm^3. Despite the high efficiency in degradation of hemicellulosic structures, this kind of chemical pretreatment was not

strong enough for delignification of biomass: no phenolic compounds were observed in hydrolyzates after process. Combined chemical and enzymatic hydrolysis leads to high concentrations of glucose: a sugar that is metabolized by methanogenic bacteria in anaerobic digestion process. This fact indicates that dilute acid hydrolysis is a promising method for adaptation in anaerobic degradation processes for production of gas biofuels under industrial conditions.

Introduction

The last decades of XX and XXI centuries can be called the decades of searching for renewable energy sources. Increasing consumption of fuels in many branches of industry makes science face the challenge, which undoubtedly is the necessity of searching for new, innovative methods of processing alternative, ecological and inexhaustible sources, before natural sources would not be able to satisfy human and economic needs.

One of the most frequently mentioned non-conventional energy sources, which can be used in many production and municipal processes, is green biomass [8]. Its chemical structure and relatively high energy value are the great potential for liquid and gas fuels production (bioethanol, biogas). Nowadays it is applied successfully in processes such as gasification, combustion, anaerobic digestion or composting for energy production. Huge plantations of selected, high-energy plants are formed in many areas satisfying local energy needs.

The technology most commonly used in practice is the process of anaerobic degradation of green biomass [1]. The advantages of this method are low adverse environmental effects, high efficiency of biogas production and wide feasibility of raw material selection [5]. The drawback is the time of the entire process, especially biological hydrolysis step, which can last even 6 months. In order to avoid this problem

and to eliminate an expensive step of enzymatic hydrolysis, which support the process, new methods of chemical and termochemical pretreatment are used [2,12]. They can lead to the transformation of high-molecular, polymeric structures of lignocellulosic materials into products easily biodegradable in anaerobic conditions [13].

One of the most popular and effective method of pretreatment is dilute acid hydrolysis. It is usually carried out under mild conditions: 0.5-10% [v/v] H_2SO_4 or HCl and 80-180°C. The times of reaction range from 15 minutes to several hours [6,9]. During this process the hemicellulose fraction is depolymerized at a lower temperature than the cellulose fraction, so the next enzymatic step is necessary to release the fermentable sugars without increasing costs. This method of pretreatment is relatively inexpensive. It allows to produce high hemicellulose recoveries and makes the cellulose digestible [3,9].

It is necessary to mention, that this kind of pretreatment provides the inhibitors releasing into supernatants. The compounds like hydroxymethylfurfural and furfural, which disturb the fermentation process, are the results of hydrolysis of acetyl groups linked to the sugars [11]. Therefore the possible low temperature of pretreatment and low concentration of acid should be used; the higher they are, the higher the production of the inhibitors is [9].

The aim of this work was to determine the influence of dilute acid hydrolysis of three different plant sources on the efficiency of enzymatic hydrolysis. The results obtained in the investigation may point which products of this pretreatment are responsible for the highest methane yield in biogas production with the use of acid hydrolysis.

Materials and Methods

Preparation of the raw plant material

Three species of energetic crops in the form of dry, cut grass have been used in this study: *Miscanthus giganteus*, *Sorghum Moench* and *Sida hermaphrodita*. The raw plant material was broken up in mechanical mill to obtain particles with dimensions of 0.1-1mm. In order to remove chlorophil, which disturbs the spectrophotometric measurements, the milled material was placed in the Soxhlet's apparatus and subjected to extraction with 96% ethanol, according to Polish Standard Method PN-92/P-50092. Extracted biomass was then rinsed by distilled water until the pH of filtrate is neutral. The material prepared in this way was dried in an oven at constant temperature of 45°C.

Dilute acid hydrolysis

The amount of 2.5g of weighed portion of biomass prepared according to the procedure mentioned above was suspended in 50 cm^3 of sulfuric acid solution and intensively mixed. The concentrations of acid were ranged from 0.25 to 3% (v/v). The sample was placed in the autoclave at 121°C for 30 min. and after this period of time it was cooled in water-bath. In the last step of the process the supernatant was separated from the mixture by means of centrifuge.

Enzymatic hydrolysis

The amount of 2.5 g of weighed portion of chemically hydrolysed plant biomass was suspended in 50 cm^3 of 50 mM citrate buffer solution at pH=4.8. Two types of enzymes: cellulase (Celluclast 1.5L) and cellobiase (Novozyme 188), were added to the sample in quantities allowing to reach 100- and 300-fold dilution, respectively. The flask with the sample was placed in a shaker and it was hydrolysed at constant temperature of 50°C for 24 hours. The obtained hydrolysate with pretreated biomass was then used as a feedstock in the process of methane fermentation carried out in shaken cultures.

Analytical Methods

In the raw plant material the following parameters were determined: hemicellulose and cellulose content (HPLC Method, Waters chromatograph equipped with Bio-rad Aminex HPX-87H column), lignin content (Polish Standard Method PN-92/P-50092), Total Solids (TS, drying at constant temperature of 105°C), Volatile Solids (VS, mineralization in an oven at constant temperature of 550°C), Ash (subtraction TS-VS), Chemical Oxygen Demand (COD, Polish Standard Method PN – 74/C–0457), elemental content (C, H, N, S; Elemental Analyzer NA 2500, CE Instruments).

The following parameters were determined in hydrolysates obtained after the process: volatile fatty acids (VFA, distillation with steam in BÜCHI apparatus), Chemical Oxygen Demand (COD, mineralization in HACH-LANGE apparatus, spectrophotometric analysis in DR 5000 apparatus), Total Organic Carbon (TOC, HACH-LANGE apparatus), General Nitrogen (Ng, HACH-LANGE apparatus), Total Phenolic Content (TPC, Follin Ciocalteou's Method, UV-VIS T80+ PG Instruments Limited spectrophotometer, λ=765nm), Glucose and Xylose Concentration (HPLC Method, Waters chromatograph equipped with Bio-rad Aminex HPX-87H column).

Results and Discussion

Chemical hydrolysis

The physicochemical characteristics of the three plant species subjected to chemical hydrolysis are shown in Table 1. Elemental content of biomass is given in Table 2.

Table 1. Characteristics of the raw materials

Parameter	***Miscanthus***	***Sida***	***Sorghum***
Hemicellulose content [%]	**24.4**	**21.5**	**25.9**
Cellulose content [%]	**26.5**	**25.1**	**23.0**
Lignin content [%]	**28.8**	**19.1**	**27.9**
Total Solids [%]	**94.1**	**95.6**	**95.7**
Volatile Solids [%]	**89.8**	**92.0**	**86.3**
Ash [%]	**4.3**	**3.6**	**9.4**
COD [mgO_2/g]	**52632**	**57843**	**50971**

Table 2: Elemental content of treated energy plants

Chemical composition	***Miscanthus***	***Sida***	***Sorghum***
Carbon [%]	**46.5**	**45.9**	**44.9**
Hydrogen [%]	**6.0**	**6.1**	**5.8**
Nitrogen [%]	**0.5**	**0.3**	**0.7**
Sulphur [%]	**0.0**	**0.0**	**0.0**

The concentrations of basic indicators (COD, VFA, TOC, Ng, TPC, glucose and xylose) were determined in supernatants obtained after reaction. As a leading parameter that testified of the highest level of biomass degradation, the concentration of COD after chemical pretreatment was chosen. Simultaneously, the other parameters were also taken into account.

As it is shown in Figure 1, the best results were obtained for the highest concentration of sulfuric acid in the sample. For all three plant species both COD and

VFA values determined in supernatants after chemical hydrolysis were the largest for 3% [v/v] of H_2SO_4, and their maximum concentrations were equal to 24400 mgO_2/dm^3 (for *Sorghum*) and 3384 mg CH_3COOH/dm^3 (for *Sida*), respectively. This data is in agreement with the literature [4,7]. The concentrations of particular indicators are the function of the concentration of acid used in pretreatment step, and they increase with that concentration. The same dependence was observed for other parameters: TOC, Ng, glucose and xylose. Figure 2 shows the dependence of monomeric sugars concentration on the sulfuric acid concentration for different species of energy plants, with the best results for *Miscanthus*. Our previous study on the kinetics of dilute acid hydrolysis of *Miscanthus giganteus* showed analogous trends [10]. This method of pretreatment strongly depends on both temperature and acid concentration.

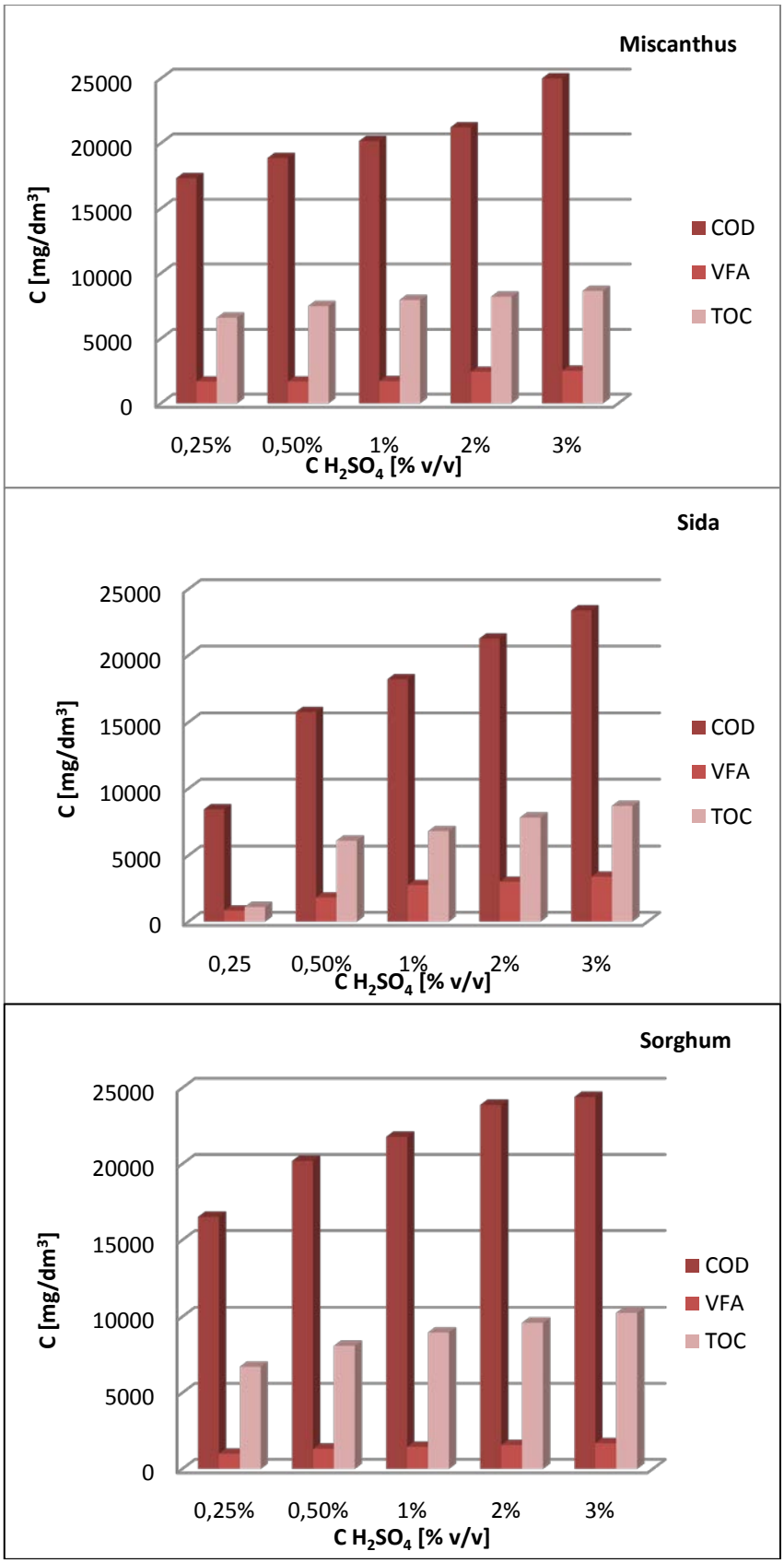

Figure 1: The concentrations of main indicators characterizing supernatants after dilute acid hydrolysis under optimal conditions for *Miscanthus*, *Sida* and *Sorghum*.

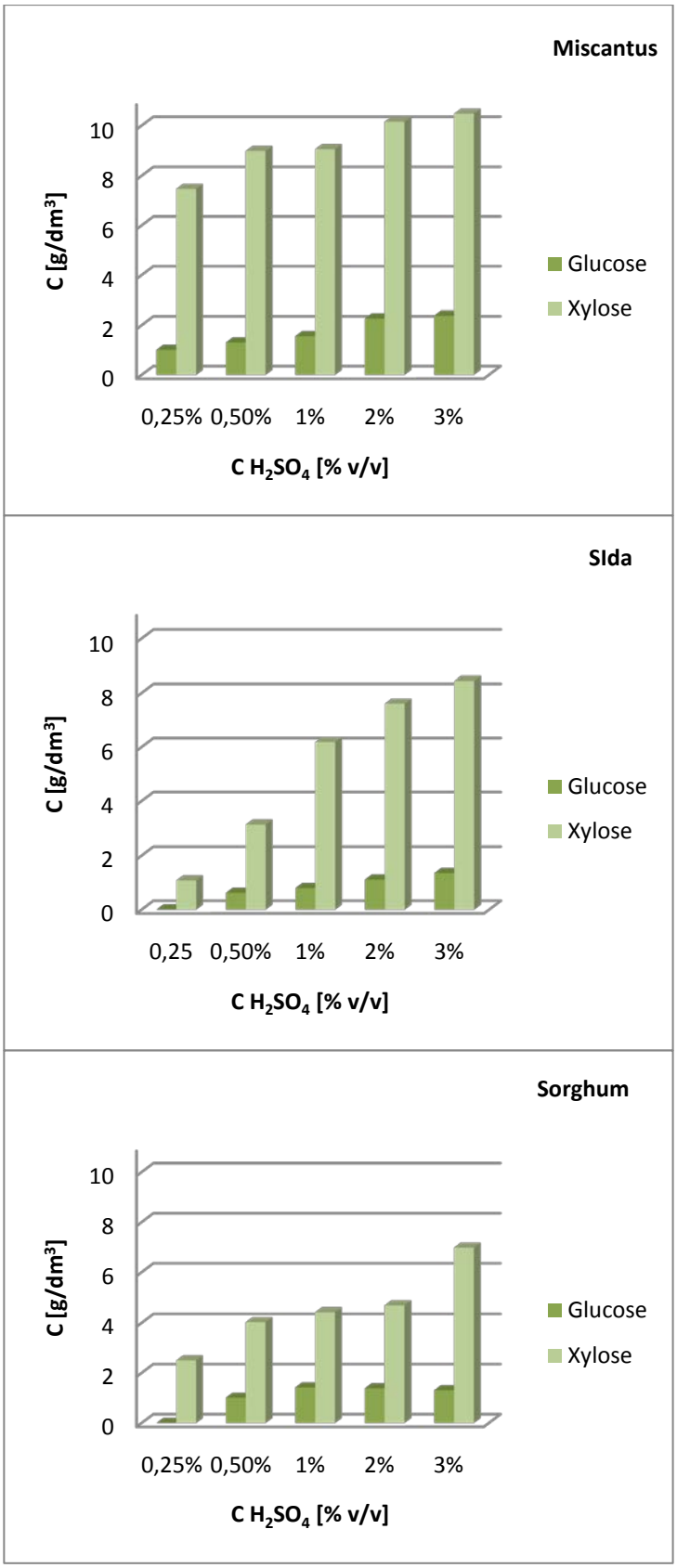

Figure 2: The concentrations of monomeric sugars released into the supernatants after dilute acid hydrolysis for *Miscanthus*, *Sida* and *Sorghum*.

The data achieved after dilute acid hydrolysis prove that this kind of pretreatment influence the degradation of hemi- and cellulosic structures. What is characteristic – the lack of phenolic compounds in the supernatants was observed. This fact testifies that no delignification took place. Nevertheless, the level of degradation of hemicellulose was high enough for further cellulose decomposition by enzymes.

Enzymatic hydrolysis

The two-step process consisting of termochemical and enzymatic hydrolysis was investigated. The aim of this study was the further degradation of the cellulosic structures. In obtained supernatants the glucose concentration was determined. The results are presented in Figure 3.

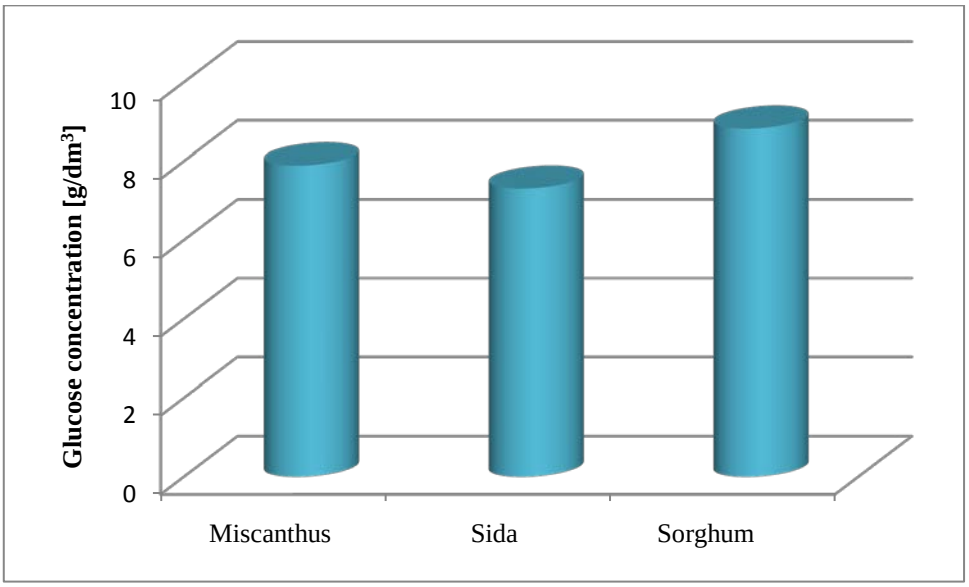

Figure 3: Effects of enzymatic hydrolysis on degradation of cellulose after dilute acid pretreatment for different plant sources.

The highest concentration of glucose was achieved for *Sorghum Moensch* and it was equal to 8.82 g/dm^3. For *Sida hermaphrodita* and *Miscanthus giganteus* these values were a bit lower (7.31 and 7.89 g/dm^3, respectively). In relation to cellulose content determined earlier in studied plants, these results testified of a sufficient degree of lignocellulosic biomass degradation with the use of sulfuric acid. Although the lignin

was not destroyed in chemical pretreatment this fact did not limit the availability of cellulose for an effective enzyme action. Hence, the possible biogas production through anaerobic digestion may result mainly from metabolism of monomeric sugars and then of other organic compounds, mostly VFA, by methanogenic microorganisms.

In order to confirm the possitive role of dilute acid hydrolysis in two-step degradation of lignocellulosic materials, the enzymatic hydrolysis of pure, crystalline cellulose was conducted. For this investigation the same types and quantities of enzymes were used. In the received hydrolysate the concentration of glucose was determined, and it was equal to 26.9 g/dm^3. That result was compared with the concentration of glucose after chemical and enzymatic treatment of plant materials. Because the percentage of cellulose in treated biomass is almost four-fold lower, these results seemed to be even better for two-step hydrolysis. It indicated a quite high level of cellulose degradation after dilute acid pretreatment and very high degree of hemicellulose destruction.

Conclusions

The results obtained in the investigation indicated a high ability of dilute acid hydrolysis to the chemical degradation of lignocellulosic structures. Although no delignification of biomass took place, the chemical degradation of other structures was high enough and the monomeric sugars were presented in supernatants. The data achieved after chemical pretreatment showed the dependence on both temperature and concentration of acid used for hydrolysis. Two-step chemical and enzymatic process turned out to be a very perspective method of lignocellulosic materials degradation for biofuels production, with the best results for *Sorghum Moensch*.

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