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INFLUENCE OF INITIAL ALKALINITY OF LIGNOCELLULOSIC WASTE ON THEIR ENZYMATIC DEGRADATION

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Keywords: Lignocellulosic wastes, enzymatic saccharification, alkaline hydrolysis, obtaining of the organic medium.

Abstract: The presented results of research on the effectiveness of enzymatic hydrolysis of lignocellulosic waste, depending on their initial depolymerisation in alkaline medium were considered in the context of the possibility of their further use in the fermentation media focused on the recovery of energy in the form of molecular hydrogen. The aim of this study was to determine the appropriate dose and concentration of a chemical reagent, whose efficiency would be high enough to cause decomposition of the complex, but without an excessive production of by-products which could adversely affect the progress and effectiveness of the enzymatic hydrolysis and fermentation. The effect of treatment on physical-chemical changes of homogenates' properties such as pH, COD, the concentration of monosaccharide and total sugars and the concentration of total suspended solids and volatile suspended solids was determined. The enzymatic decomposition of lignocellulosic complex was repeatedly more efficient if the sample homogenates were subjected to an initial exposure to NaOH. The degree of conversion of complex sugars into simple sugars during enzymatic hydrolysis of homogenates pre-alkalized to pH 11.5 and 12.0 was 83.3 and 84.2% respectively, which should be sufficient for efficient hydrogen fermentation process.

INTRODUCTION

Processes in which one can convert cellulosic biomass into useful products such as methanol, ethanol, methane and hydrogen, are of interest of many researchers. In comparison with alcohols and methane, hydrogen is being considered as more attractive energy source because of its high potential energy (122 kJ/g), which is the highest of all known fuels. Therefore, it is believed that cellulosic biomass conversion to hydrogen should play an important role in solving problems related to environmental pollution and constant increase in energy demand [1]. Among many processes that recover the energy in the form of molecular hydrogen, more and more attention is focused on biological processes, mainly fermentation, which require, however, provision of readily biodegradable substrate. Many authors suggest that in order to carry out the

hydrogen recovery by fermentation of lignocellulosic waste effectively, substrate must be first hydrolyzed to the level of free cellobiose molecules [2–8]. The possibility of using these wastes to produce hydrogen is therefore determined by depolymerization processes of lignocellulosic substrate (often preceded by a chemical enzymatic treatment) [9]. The subject is, however, little recognized and requires further research in this direction.

Lignocellulosic wastes are characterized by high resistance to biological degradation. However, in nature there are microorganisms, including bacteria and fungi, representing capability of efficient degradation of polymeric structures of hemicellulose and cellulose [10, 11]. These include bacteria from genera *Clostridium*, *Cellulomonas*, *Bacillus*, *Thermomonospora*, *Ruminococcus*, *Bacteriodes*, *Erwinia*, *Acetovibrio*, *Microbispora* and *Streptomyces*, and fungi such as: *Trichoderma reesei*, *Trichoderma koningii*, *Penicillium funiculosum*, *Myrothecium Verrucaria*, *Sporotrichum pulverulentum* and *Aspergillus niger*. Many researchers believe that the enzymatic methods should be preceded by pre-treatment of the material, causing the degradation of lignin [12, 13]. In addition, enzymes produced by bacteria, in contrast to the enzymatic system of fungi [14], in most cases are unable to degrade crystalline cellulose [15, 16]. To reduce the crystallinity of cellulose and to transform it in the amorphous form, a pre-treatment is required, which is adjusted to the type of mass fraction of individual components of the complex lignocellulose (lignin, hemicellulose and cellulose).

There is not much information on the effect of alkalization on lignocellulosic waste in the available science literature [10, 17–19]. Most of them concern the alkaline treatment of sewage and the impact of this process on their physical-chemical properties and performance in obtaining hydrogen. Many authors agree that the effectiveness of the chosen method of treatment depends primarily on the nature and composition of the processed waste material delignification [20, 21]. Therefore, research on the influence of the physical-chemical pre-treatment of lignocllulosic wastes in the process of the enzymatic hydrolysis of polymers (cellulose, hemicellulose), whose efficiency is crucial in the subsequent processes for energy recovery, is considered reasonable.

MATERIALS AND METHODS

Characterization of lignocellulosic waste

The study was carried out with use of sludge from paper mills. In these factories, in order to obtain a cellulosic mass for paper production, 80% of wood pulp and 20% of recycled waste paper are used. Sludges from washing out some machines grinding pulp were collected as random samples of a mechanical paper pulp dewatering.

Mechanical treatment of sludge

Wet sludges were dried to obtain an air dry substrate and then placed in the oven at 105°C. After drying to constant weight sludges were introduced into distilled 1% (g/V) H_2O and a 10 minutes homogenization at 2500 rev/min was performed. In the homogenates a general and organic suspension was determined by direct gravimetric method (by PB/26 ed.1, 08.17.2006). The filtrates obtained after filtration of homogenates through medium filter paper were characterized – pH by potentiometry method (PN-EN 12880), COD by

dichromates (PN-74/C-04578/03), monosaccharides and other sugars by the colorimetric method with Antron (PN-C-04628/02).

Alkaline Hydrolysis

Alkaline hydrolysis was performed for samples of homogenates, in which the appropriate pH was obtained by using drop dosage of 4M NaOH. The adjustment of pH was carried out until the pH of the samples tested successively reached 11.5, 12.0, 12.5, 13.0. Changes in pH were monitored with a pH meter. Alkalinization of a crude homogenates was carried out for 30 minutes in 2 dm^3 open reactors placed on magnetic stirrers. The stirring speed was 150 rev/min.

The process was evaluated by marking pH, COD and monosaccharides in the filtered samples and the SS concentration, VSS and total sugars in the non-filtered samples.

Heat treatment of homogenates previously treated with hydrolyzed

In order to check the possibility of intensification of the chemical homogenates hydrolysis (in alkaline media), the samples were thermally conditioned at reflux for another 30 minutes. The process was carried out in a 1 L round bottom flasks connected to a reflux condenser (to eliminate losses due to evaporation). The process was evaluated using the same analyses as for the alkaline hydrolysis.

Bacterial-enzymatical biopreparation

To carry out an enzymolysis of modified homogenate samples bacterial-enzymatical inoculant was used. The inoculant (biopreparation) contained bacterial granules containing lactic acid bacteria *Lactobacillus plantarum* KKP/788/p, *Lactobacillus plantarum* KKP/593/p, *Lactobacillus brevis* KKP/839/p, *Lactobacillus buchnerii* KKP/907/p and liquid concentrate of cellulolytic enzymes. The composition of the enzyme complex contained endo-1,4-β-glucanase, exo-1,4-β-glucanase (celobiohydrolase), β-glucosidase (celobiase) and endoksylanase.

The MRS medium

For the activation of enzymes and bacteria present in the biopreparation peptone-glucose MRS medium was used composed of 5 mg K_2HPO_4, 2 mg of di-ammonium hydrogen citrate, CH_3COONa 5 mg, 0.58 mg of $MgSO_4 \cdot 7H_2O$, 0.28 $MnSO_4 \cdot 4H_2O$, 10 mg of peptone K, 10 mg yeast extract and 20 mg of glucose. Ingredients were dissolved in L of distilled water.

Enzymatic Hydrolysis

The enzymatic hydrolysis of homogenates was preceded by activation of biopreparation. The enzymatic hydrolysis was performed for the following homogenates:

- raw
- raw after a thermal pretreatment
- raw after chemical and thermal pretreatment

Homogenates were mixed with an activated biopreparation. Research work has shown that the optimum volume of the bacterial-enzymatic inoculum (biopreparation) used for the efficient degradation of cellulose to sugars was 10% (V/V). The process of saccharification of polysaccharides was carried out in bioreactors placed on magnetic

stirrers. Constant temperature (45°C) was assured by placing the samples in a laboratory incubator. The process was carried out under anaerobic conditions (pH 5.5) for 54 hours and the concentration of monosaccharides and polysaccharides was being checked every 6 hours. The anaerobic conditions were created by blowing a gaseous nitrogen through the samples for 1 minute. The process was controlled by determination of pH, COD and monosaccharides in the filtered samples and the concentration of SS, VSS and total sugars in the non-filtered samples before and after the process.

The effectiveness of enzymatic hydrolysis was determined by the ratios shown by the equation:

$$\%EH = \frac{\Delta M_{EH}}{C} \cdot 0{,}9 \cdot 100 \ [\%]$$

where: ΔM_{EH} – the increase of concentration of monosaccharides in the process of enzymatic hydrolysis, g/L, C – concentration of sugars, g/L, 0.9 – correction factor for efficiency of the process.

RESULTS AND DISCUSSION

Evaluation of chemical-thermal pretreatment of homogenates

The average colloid concentration of homogenates used in the study was 10 g TSS/L. The organic mass was 92% of dry residue. The sample was slightly alkaline with pH 7.8. The concentration of monosaccharides in the filtrate was set at 16 mg $C_6H_{12}O_6$/L, COD was 113 mg O_2/L. Non-filtered sample had a concentration of total sugars at the level of 4914 mg/L.

Pretreatment processes of lignocellulosic material are intended to break down the compact complex by removing the lignin in the first place, then reducing the crystallinity of cellulose and hemicellulose. The study showed a stimulating effect of alkalinization on the liquefaction of organic solids homogenate (Table 1).

Table 1. Selected physical and chemical properties of homogenates of thermally conditioned at different pH values

symbols	Treatment applied		Initial pH	Final pH	COD mg O_2/L	Total sugar mg/L	Monosaccharides mg/L	TSS g TSS/L	VSS g VSS/L
	chemical	thermal							
A	–	–	7,8	7,8	113	4 914	16	10,0	9,1
B	–	+	7,8	7,1	321	4 877	129	9,6	8,7
C	NaOH	+	11,5	8,4	600	4 861	113	9,7	8,7
D		+	12,0	10,7	808	4 902	121	9,7	8,6
E		+	12,5	11,3	1 065	4 890	249	9,6	8,6
F		+	13,0	12,2	1 175	4 908	198	9,6	8,5

It was found that the lowering of the concentration of suspended organic matter during the alkalization from 9.1 to 8.7–8.5 g VSS/L was mainly caused by heating up. The obtained results were similar to those for the concentration of suspended organic matter (8.7 g VSS/L – Table 1) in a sample of the homogenate that undergone thermal treatment only. Liquefaction of organic suspensions caused a reduction of total suspended solids concentrations from 10 to 9.6–9.7 g TSS/L. These results correlate well with the results of monosaccharide concentrations in fluids of homogenates (Table 1). All conditioned samples revealed a significant increase in the concentration of monosaccharides from 16 to 129–249 mg/L. In samples treated with NaOH, an increase in the concentration of monosaccharides varied between 113–249 mg/L. As in the case of liquefaction of the suspensions, the increase was mainly caused by heating of homogenates when the initial pH was 11.5 or 12.0. In the case of the homogenates which were conditioned at pH 12.5 and 13.0, the monosaccharide concentration increased respectively by 85 or 136 mg/L. It was not a big increase, although still noticeable, and it probably was caused by partial decomposition of hemicellulose. The concentration of total sugars oscillate at the same level (4861 to 4908 mg/L) like at the control sample (4914 mg/L). Only monosaccharides underwent a change, from 16 to 249 mg/L in the total sugars.

It was found that the chemical-thermal treatment of homogenates carried out under alkaline conditions caused the lowering of pH (Table 1). This indicated the production of acidic organic compounds takes place during only the chemical and thermal conditioning. It was shown that with greater participation of monosaccharides and acidic organic compounds in liquid homogenates there was a significant increase in the concentration of organic compounds expressed by COD. In samples treated in alkaline conditions the increase of the value of COD was up to 600–1175 mg COD/L.

In the samples conditioned with NaOH an increase of COD was associated with a partial liquidation of the organic mass and acidic organic compounds.

Efficiency of enzymatic hydrolysis of pre-conditioned homogenates in alkaline conditions

In subsequent studies an enzymatic hydrolysis process for homogenates previously treated with NaOH (samples C to F) was carried out. Efficiency of hydrolysis was calculated by the increase in the concentration of monosaccharides and was compared to the results obtained with homogenates of untreated chemicals (samples A and B). Enzymatic hydrolysis was carried out under anaerobic conditions for 54 hours.

The aim of this study was to determine the reaction time necessary to achieve the highest concentration of monosaccharides in liquid homogenates. It was shown that for all tested samples the highest concentration of monosaccharides was observed after 36 h (Fig. 1).

Homogenates, which were previously subjected to an alkaline conditioning at initial pH 11.5, 12.0, 12.5 and 13.0, were characterized by a concentration of monosaccharides respectively: 4612, 4705, 3871 and 3400 mg/L. It was found that the time of exposure of homogenates to the enzymes over 36 h resulted in only slight changes in the concentration of these sugars.

It was stated that a higher concentration of monosaccharides was observed in samples conditioned at initial pH 11.5 and 12. In the sample which was subjected to thermal--chemical treatment at pH 13.0, the concentration of monosaccharides was the lowest and

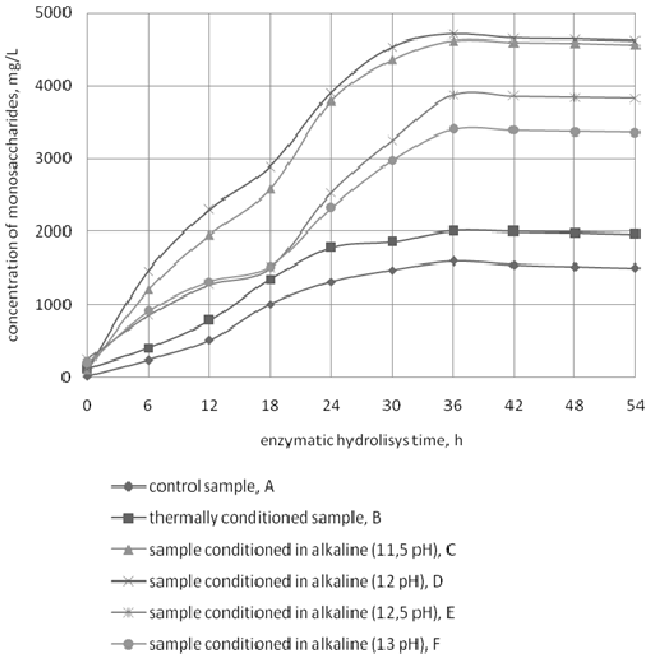

Fig. 1. Changes in the concentration of monosaccharides during enzymatic hydrolysis of homogenates

471 mg/L higher than the concentration of monosaccharides in the sample conditioned at pH 12.5. It can be assumed that the conditioning of homogenates at extremely high pH values, during pre-treatment, led to the production of substances that cause a partial inhibition of the enzymes responsible for hydrolysis of cellulose and hemicellulose. This was confirmed by the analysis of the curves showing the intensity of the saccharification of homogenates (Fig. 1), which shows the stagnation of the process between 12 and 18 hours.

Enzymatic hydrolysis preceded by alkalinization of the initial homogenates pH to 11.5 or 12.0 contribute to increased efficiency of liquefaction complex sugars from 28.9% corresponding to the control sample (A) and 34.9% of the sample conditioned thermally (B) to 83.3% and 84.2%, respectively, observed in the samples thermally conditioned at pH 11.5 (C) and pH 12.0 (D) (Fig. 2).

The alkaline conditioning at pH 11.5 and 12.0 was considered favorable. The reduction of concentrations of these organic suspensions was noted with successively 4.5 and 4.6 g TSS/L, which corresponds to the lowering by 51.7% and 53.5% comparing to the concentrations of organic suspensions in the samples before the process. For comparison, the loss of organic suspended solids concentrations in samples not subjected to the initial alkalinization was only 17.6% and 21.8%, respectively. Liquefaction of organic suspensions caused an increase of the COD from 2551 mg COD/L to 5875 and 6212 mg COD/L (Table 2). During the enzymatic hydrolysis the pH decreased from 5.5 to 5.2 (Table 2), showing that some acidic substances (by-products) were also produced.

It was shown that for samples that were pre-conditioned at higher pH values, i.e. 12.5 and 13.0 (samples E and F) homogenates saccharification efficiency was 66.7 and 58.7%,

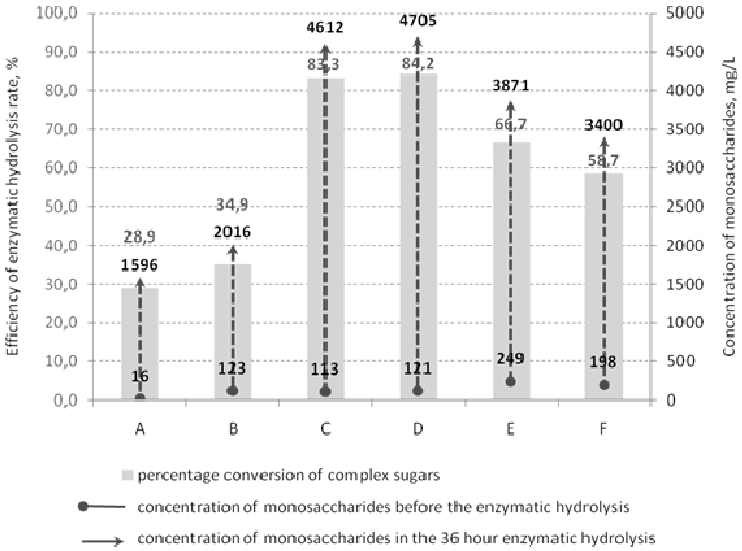

Fig. 2. The efficiency of the enzymatic hydrolysis and the increase in the concentration of monosaccharides during the 36 hours of the process – explanations are given in the text

respectively. Also, the effect of liquefaction of organic suspensions was lower – 43 and 37.6%, respectively. Changes in other physical-chemical indicators for homogenates E and F that underwent the enzymatic hydrolysis revealed themselves in the same way as for samples C and D. This applies to the decrease of pH from 5.5 to 5.3 and increase in COD to 5400 or 5043 mg COD/L.

Similar results concerning the influence of pre-conditioning of other lignocellulosic substrates under alkaline conditions to an enzymatic delignification of the complex were obtained by other authors [15, 21–28]. The use of the enzymatic hydrolysis had also a positive impact on the performance of hydrogen production in the fermentation process [15, 22, 28]. For example, Xiao and Liu [23] using the initial acidification (pH 2.0) and alkalization (pH 12.0) of sewage sludge have shown that as a result of these processes, there were liquefaction of organic substrate resulting in increasing the participation of dissolved carbohydrates and increasing the value of the COD in the liquid sludge in relation to the sample not subjected to conditioning at the extremes of pH. Chemical treatment of the substrate contributed from 2 to about 10 times more to hydrogen production during fermentation of hydrolysates, the better results were obtained for pre-alkalized substrates.

Kim and Shin [28] by 24 hour long chemical conditioning of food waste at pH 12.5 showed a low production of hydrogen, 63 mL H_2/g VSS, while Shin and Youn [29] without the chemical pre-treatment received higher amount of hydrogen, 125 mL H_2/g VSS. Also other authors [30, 31] confirmed the low efficiency of food waste pre-treatment carried out at pH>12. Researchers who did not support the enzymatic hydrolysis of the substrate by the chemical treatment, obtained the comparable amount of hydrogen of 65 mL H_2/g VSS [30] and 77 mL H_2/g VSS [31].

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Table 2. Selected physical-chemical parameters of homogenates after enzymatic hydrolysis

Symbols	Treatment applied		Treatment applied	Initial pH	Final pH	COD (36 h) Mg O_2/L	Total su gar (54 h) mg/L	Monosaccharides mg/L			TSS g TSS/L	VSS g VSS/L
	chemical	thermal						0 h	36 h	54 h		
A	–	–	+	5,5	5,3	1 950	4 803	16	1596	1503	8,4	7,5
B	–	+	+	5,5	5,3	2 551	4 760	129	2016	1969	7,7	6,8
C	NaOH	+	+	5,5	5,2	5 875	4 755	113	4612	4562	5,2	4,2
D		+	+	5,5	5,2	6 212	4 771	121	4705	4615	5,1	4,0
E		+	+	5,5	5,3	5 400	4 785	249	3871	3821	6,0	4,9
F		+	+	5,5	5,3	5 043	4 766	198	3400	3358	6,4	5,3

SUMMARY AND CONCLUSIONS

The study showed a stimulating effect of the alkalinization on the liquefaction of homogenate organic solids in the next stage of research involving enzymolysis. The enzymatic digestion of lignocellulose complex was significantly more efficient if the sample homogenate was subjected to an initial exposure to chemical reagents. The degree of conversion of complex sugars into simple sugars during enzymolysis of pre-alkalized homogenates to pH 11.5 and 12.0 was 83.3 and 84.2%, respectively. The lower efficiency of saccharification was also noted for homogenates pre-conditioned at higher pH values, i.e. 12.5 and 13.0, giving 66.7% and 58.7% respectively. Also, the effect of liquefaction of organic suspensions was lower, 43 and 37.6%, respectively. It was found that the liquefaction of organic suspensions was not a true measure of the studies, but it was well correlated with the increase of monosaccharides in the liquid homogenate and could become an indirect indicator of the effectiveness of enzymolysis.

The results led to the following conclusions:

1. The treatment process using alkali at pH 12.5 and 13 contributed to the lower degradation of complex sugars in comparison to the process carried out in milder conditions.
2. Thermal treatment of sludge increased an impact of chemical substances (NaOH), leading to a more efficient decomposition of the lignocellulosic complex.
3. The pre-treatment (thermal-chemical hydrolysis) of lignocellulosic waste caused the breakdown and aeration of lignocellulosic complex and reduced its crystallinity. This was conducive to an increased susceptibility of waste to the sacchrifying enzymes.

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WPŁYW WSTĘPNEJ ALKALIZACJI ODPADÓW LIGNOCELULOZOWYCH NA ICH ROZKŁAD ENZYMATYCZNY

Przedstawiane wyniki badań nad efektywnością hydrolizy enzymatycznej odpadów lignocelulozowych w zależności od ich wstępnej depolimeryzacji w środowisku alkalicznym rozpatrywano w kontekście możliwości ich dalszego wykorzystania w procesie fermentacji, ukierunkowanej na odzysk nośnika energii w postaci wodo-

ru cząsteczkowego. Celem badań było ustalenie odpowiedniej dawki i stężenia reagenta chemicznego, którego skuteczność byłaby na tyle duża by powodować dekompozycję kompleksu bez nadmiernego wytwarzania produktów ubocznych, mogących niekorzystnie wpływać na przebieg i efektywność hydrolizy enzymatycznej oraz samej fermentacji. Określano wpływ obróbki fizyczno-chemicznej na zmiany takich właściwości homogenatów jak pH, ChZT, stężenie monosacharydów i cukrów ogólnych oraz stężenie zawiesin ogólnych i organicznych. Proces enzymatycznego rozkładu kompleksu lignocelulozowego był wielokrotnie efektywniejszy w przypadku, gdy próbki homogenatów poddawano wstępnej ekspozycji na działanie NaOH. Stopień konwersji cukrów złożonych do cukrów prostych podczas enzymolizy homogenatów wstępnie alkalizowanych do pH 11,5 i 12,0 wynosił odpowiednio 83,3 i 84,2 %.