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Characteristics of plant biomass and their effect on enzymatic saccharification

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Abstract : Enzymatic hydrolysis of a large number of not-treated and pretreated plant materials of various origins containing different amounts of cellulose, hemicelluloses and lignin has been studied. To disclose the effect of chemical composition and structural characteristics on yield of glucose, a correlation analysis was performed and squared correlation coefficients (R^2) were calculated. This analysis showed that hemicelluloses have a negligible impact on enzymatic digestion of the cellulosic component. Content of lignin affected negatively on enzymatic cleavage of cellulose, and namely an inversely proportional regression between content of lignin in the samples and yield of glucose was observed ($R^2 = 0.67$). Conversely, increased cellulose content in the biomass affects positively on enzymatic digestion and glucose output ($R^2 = 0.84$). To improve the correlation, an effect of crystallinity degree of cellulose on hydrolysability should be taken into consideration. As a result, the best correlation with maximum squared coefficient ($R^2 = 0.98$) was found for the dependence of glucose yield (Y) on the combined parameter Z, which includes the content and crystallinity of cellulose, as well as the content of lignin in the investigated biomass samples. As follows from the regression equation Y=f(Z), an increase the content of cellulose, reducing of its crystallinity and decreasing of the lignin content in the samples promotes enzymatic cleavage of the cellulosic component. The discovered correlation Y=f(Z) permits prediction the saccharification degree of pretreated biomass and can be used for choice the best pretreatment method. In particular, the nitric acid/alkaline pretreatment of herbaceous plants provides obtaining the delignified biomass enriched with low-crystalline cellulose that shows an excellent enzymatic digestibility.

Keywords : Biomass; Chemical composition; Structural characteristics; Enzymatic hydrolysis; Yield of glucose; Correlation analysis.

INTRODUCTION

Current industrial technologies of biofuels and biochemicals are based on the hydrolysis of natural carbohydrates (sugar and starch) into glucose, with the subsequent biological or chemical transformation of the monosaccharide into various bioproducts[1, 2]. Since natural carbohydrates are required by the

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food industry, their use for manufacturing of non-food bioproducts is limited. Alternative way to obtain valuable bioproducts without competing with food and feed industry is the use of non-edible plant materials (biomasses) as feedstocks. These biomass types involve residues of agricultural plants (e.g. stalks, husks, cobs, etc.), forest residues (e.g. sawdust, twigs, shrubs, etc.), waste of wood, textile, pulp, paper and cities, as well as some plant species (e.g. Miscanthus, Switchgrass, Bermuda grass, etc.). Agriculture, forestry, pulp and paper industry, as well as cities create vast amounts of lignocellulosic residues. Moreover, huge amounts of algae are not utilized yet and can be used as appropriate feedstock for productions energy or chemicals. The not-edible plant raw materials are attributed to abundant, renewable and inexpensive biomass types. The total amount of such biomass that accumulates annually in the world is estimated in 10 billion tons at least. Only in USA annual accumulation of the lignocellulosic biomass is of around 1 billion tons[3].

Any plant material comprises three main polymeric components – cellulose, hemicelluloses and lignin. Natural lignocellulosic biomass can contain 25 to 50% cellulose, 20 to 40% hemicelluloses, and 10 to 35 % lignin[4-7]. In plant cell walls, the cellulosic nanofibrils are glued by hemicelluloses into fibrillar bundles that are surrounded by lignin layers[5, 7].

Cellulose is a linear, stereo-regular semi-crystalline polysaccharide that is built of repeated D-glucopyranosyl units linked by 1, 4-β-glycosidic bonds[8, 9]. The cellulose chains form thin and long nanofibrils consisting of statistically alternated crystallites and non-crystalline amorphous domains. The crystallites are stable constituents, whereas the amorphous domains are weak points of the cellulose structure. Therefore, the high crystallinity of cellulose is a main factor limiting enzymatic cleavage of this polysaccharide[10, 11].

Hemicelluloses are hydrophilic amorphous heteropolymers. The macromolecules of hemicelluloses consist of acetylated links of pentoses or hexoses. In addition to physical bonding of cellulose, hemicelluloses also form ester bonds with lignin. Thus, in the cell walls of plant fibers, hemicelluloses fulfill a function of intermediate binder between hydrophilic cellulose fibrils and hydrophobic lignin layers[7, 9].

Lignin is a rigid aromatic, amorphous and hydrophobic polymer stable to some chemical reagents and cellulolytic enzymes[6, 8]. Lignin is a complex polymer of phenylpropane units, which are cross-linked to each other with a variety of different chemical bonds. In the plant cell walls, lignin layers surrounding hydrophilic cellulose fibrils and hemicelluloses and protect them from the enzymatic attack[7, 12-14].

The common technology for obtaining bioproducts from non-edible biomass involves three main steps[2]:

1. Pretreatment of the initial biomass;
2. Enzymatic hydrolysis of the pretreated biomass in order to convert the cellulosic component into glucose;
3. Transformation of glucose into final bioproducts.

As known, initial biomasses are highly recalcitrant to enzymatic hydrolysis due to dense inaccessible structure, increased crystallinity and low content of cellulose, barrier properties of lignin and non-productive sorption of enzymes by non-cellulosic components. Therefore, some kind of pretreatment is usually applied to make the cellulosic component more accessible to enzymes[7, 9, 15]. Various pretreatment methods have been proposed, including steam explosion, acidic treatment, alkaline extraction, ammonia treatment, oxidation and some others. Pretreatment methods and conditions determine the structure, chemical composition and hydrolysis degree of pretreated biomass samples. The effective pretreatment should be inexpensive and must provide an obtaining of the accessible cellulose-rich biomass with high enzymatic digestibility.

It is believed that the main reason for the low enzymatic cleavage of cellulose in initial biomass is the presence of increased amounts of non-cellulosic components, lignin and hemicelluloses, hindering access of enzyme molecules to cellulose fibrils[16, 17]. As known, in the plant cell walls lignin layers form a physical barrier to cellulolytic enzymes. Non-productive sorption of cellulolytic enzymes by lignin is also regarded as an important factor hin-

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dering the enzymatic hydrolysis of cellulose[17]. Furthermore, denaturation and inactivation of the bound enzymes on the lignin surface was observed at the hydrolysis conditions[18]. Removal of lignin from plant materials breaks the barrier layers and reduces the non-productive absorption of the enzymes, thus improves enzymatic hydrolysis of the cellulose constituent[7, 17-20].

Several studies have discussed the impact of hemicelluloses on enzymatic hydrolysis of cellulose in biomass samples. It has been shown that enzymatic digestion of cellulose can be significantly improved after removal of hemicelluloses, thereby suggesting that also hemicelluloses form a barrier to cellulolytic enzymes[17, 21]. Moreover, acetyl groups of natural hemicelluloses can inhibit the enzymes[22]. However, other studies don't support a negative effect of hemicelluloses on enzymatic hydrolysis of the cellulosic component[23, 24]. Simultaneous lignin alteration or removal during various pretreatments can confound the role of hemicelluloses. In particular, the extraction of hemicelluloses under alkaline pretreatments of biomass is accompanied always by the removal of lignin, thereby indirectly improves the enzymatic hydrolysis.

The effect of the most important constituent - cellulose, on the enzymatic hydrolysis has been studied insufficiently, although it is known that increase the content of low-crystalline cellulose in the pretreated biomass is probably the main factor that may raise the yield of glucose, while a high crystallinity degree of cellulose hinders the enzymatic digestibility[7].

Thus, despite abundant investigations a role of polymeric components and structural features of the plant biomass in enzymatic hydrolysis is not enough clear. The promising approach to clarify this problem is application correlation analysis that is widespread in scientific researches[25, 26]. In this paper a quantitative correlation analysis was carried out in order to find the best concordance between main features of various biomass samples, on the one hand, and the yield of glucose produced by enzymatic hydrolysis, on the other hand. This approach can be used for prediction the saccharification degree and help for choice the suitable pretreatment method.

EXPERIMENTAL

Materials

The various plant materials – wood chips of pine, spruce and poplar; flax fibers; cotton linters; bagasse of sugar cane; switchgrass; corn stover and corn cobs; wheat and rice straw, were used as initial biomass samples. The initial samples were cut, knife-milled and screened through a sieve to obtain the fraction of 2-3 mm.

Pretreatments

The initial plant materials were pretreated in one or two steps by different methods at various conditions (TABLES 1 and 2).

The pretreated biomass samples were washed up to neutral pH and squeezed on vacuum glass-filter up to a final solids content of 20-30 wt.%. As a result, about eighty pretreated samples were prepared.

Analysis of chemical composition

The chemical composition of initial and pretreatment biomass samples was determined by conventional methods of chemical analysis[5, 7, 27]. The content of holocellulose was measured after complete selective delignification of the biomass with sodium chlorite. The obtained holocellulose sample was hydrolyzed with boiling 1.5% hydrochloric acid for

TABLE 1 : Methods and conditions for one-step pretreatment of initial plant materials

Method	Reagent	LSR*	Temperature, ^{o}C	Time, min
Steam explosion (SE)	1% H_2SO_4	5	165	5
Acidic treatment (AC)	3% H_2SO_4	10	100	60
Alkaline extraction (AL)	2% NaOH	10	100	60
Non-selective oxidation (NSO)	10% NaClO	10	25	60
Selective oxidation (SO)	1% $NaClO_2$	20	100	90

Note: LSR is liquid to solid ratio

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TABLE 2 : Methods and conditions for two-steps pretreatment of initial plant materials

Method	Steps	Reagents	LSR	Temperature, °C	Time, min
AH	(1). Alkaline	2% NaOH	10	100	30
	(2). Hypochlorite	6% NaClO	10	25	30
AB	(1). Alkaline	2% NaOH	10	100	30
	(2). Bleaching	0.5% $NaClO_2$	10	100	30
NA	(1). Nitric acid	5% HNO_3	5	110	30
	(2). Alkaline	1% NaOH	10	100	30

2 h. The content of cellulose was calculated from the dry residue remained after hydrolysis of the holocellulose, while the content of hemicelluloses was measured from weight loss of the hydrolyzed holocellulose sample. Content of lignin Klason was analyzed according to TAPPI standard T222. Three of the same samples were tested to calculate an average value and standard deviation. The standard deviation (SD) at determination of the percentage of components was ± 1 %.

The percentage of lignin in highly delignified samples was evaluated by Kappa number (K) in accordance with TAPPI standard T236.

$$L\ (\%) = 0.13\ K \quad (1)$$

In this case, SD at determination of the lignin content was ± 0.3 %.

Determination of cellulose crystallinity

Initial and pretreated plant materials were converted into holocelluloses, which were hydrolyzed by dilute HCl to remove hemicelluloses and isolate the celluloses (see section 2.3). Crystallinity of the isolated cellulose samples was determined by WAXS-method. A Rigaku-Ultima Plus diffractometer (CuK_α – radiation, λ=0.15418 nm) was used for X-ray investigations. Diffractograms were recorded in the $\varphi=2\Theta$ angle range from 5 to 80°. After recording of the diffractograms, the background was separated, and selected X-ray patterns were corrected and normalized. Then diffraction intensities from crystalline and non-crystalline regions were separated by a computerized method. The degree of crystallinity (*X*), i.e. weight part of crystalline domains in cellulose, was calculated according to equation:

$$X = \int J_c\, d\varphi \,/\, \int J_o\, d\varphi \quad (2)$$

where J_c and J_o are the corrected and normalized diffraction intensities for crystalline regions and sample respectively. Three of the same cellulose samples were tested. The standard deviation at determination of degree of crystallinity was ± 0.02.

Enzymatic hydrolysis

The biomass samples were hydrolyzed with a mixture of commercial cellulolytic enzyme Accelerase-1500 (DuPont Ind. Biosciences, Wilmington, DE, USA) and β–glucosidase Novozyme-188 (Novozymes A/S, Bagsvaerd, Denmark). The loading of Accelerase was 15 FPU per 1 g of solid sample and of β–glucosidase was 7 CBU per 1 g of solid sample. Enzymatic hydrolysis of the samples was carried out in 50-mL polypropylene tubes. The samples containing 1 g of the solid matter and 10 ml of 50 mM acetate buffer (pH=4.8) were put into the tubes, and then the enzyme cocktail was added. Further, an additional volume of the buffer was supplemented to achieve final concentration of the biomass sample 50 g/L (C_b). The tubes closed with covers were placed in a shaker incubator at 50°C and shaken at 150 rpm for 24 h. Finally, the tubes were centrifuged in order to separate the sugar solution (hydrolyzate) from the residual biomass.

Concentration of the glucose (C_g, g/L) and other sugars in the hydrolyzate was determined by the by HPLC-apparatus of Agilent Technologies 1200 Infinity Series. The Amines HPX-87H column was used. Main conditions of the analysis were: temperature 45°C; mobile phase 0.005 M sulfuric acid; flow rate 0.6 ml/min. The sample of hydrolyzate was preliminary filtered through 0.45 μm Nylon filter and degassed. Yield of glucose after enzymatic hydrolysis of the biomass sample was calculated by the equation:

$$Y\ (\%) = 100\%\ (C_g/C_b) \quad (3)$$

Three samples of the same biomass type were hydrolyzed simultaneously to obtain accurate results.

The standard deviation at determination of the glucose yield was ± 2%.

RESULTS AND DISCUSSION

The content of three main polymeric components, i.e. cellulose, hemicelluloses, and lignin, in the initial plant materials is shown in TABLE 3. The other components of the initial biomass samples were extractives, proteins and mineral admixtures (ash). The investigated biomass samples contained 35 to 90% cellulose, 5 to 38% of hemicelluloses and 1 to 28 % of Klason lignin. The high content of cellulose (90%) contained cotton linters. Flax fibers had increased content of cellulose (73%), but low content of lignin (4%). Wood samples had lower content of cellulose (45-48%), but high content of lignin (23-28%). Bagasse, switchgrass, corn stover and wheat straw contained an intermediate content of cellulose (37-38%) and lignin (17-20%). Corn cobs and rice straw samples contained 35-36% cellulose and 10-12% lignin. Besides, the corn cobs had the highest content of hemicelluloses, 38%.

Various pretreatments of the initial biomasses cause essential changes in the chemical composition. The effect of single and double pretreatments on the chemical composition can be illustrated on the example of corn stover biomass (Figure 1, 2). The distinctive feature of the steam explosion (SE) and acidic pretreatment (AC) is the removal of the main part of hemicelluloses and forming cellolignin with increased content of cellulose and lignin. In contrast to SE and AC-pretreatment, the alkaline pretreatment or non-selective oxidation leads to extraction both of hemicelluloses and lignin, but appreciable amounts of these components remain yet in the pretreated biomass. The removal of non-cellulosic components is accompanied by increasing of cellulose content in the pretreated biomass (Figure 1).

TABLE 3 : Chemical composition of initial biomass samples

Biomass	Cellulose, %	Hemicelluloses, %	Lignin, %
Pine	48	20	28
Spruce	47	22	27
Poplar	45	25	23
Bagasse	38	27	20
Switchgrass	37	28	18
Corn stover	37	26	19
Corn cobs	35	38	12
Wheat straw	37	27	17
Rice straw	36	25	10
Flax fibers	73	12	4
Cotton linters	90	5	1

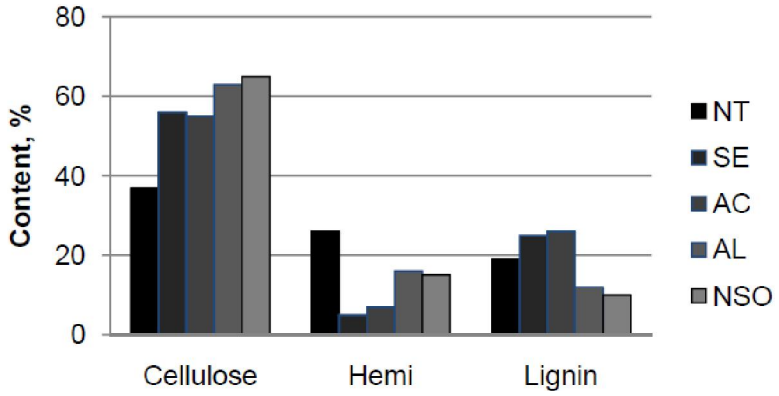

Figure 1 : Percentage of cellulose, hemicelluloses (Hemi) and lignin in corn stover biomass: non-treated (NT), stream-exploded (SE), acid pretreated (AC), alkali pretreated (AL) and non-selective oxidized (NSO)

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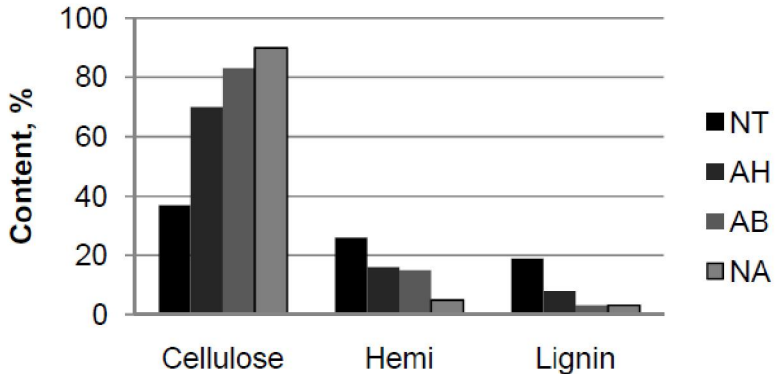

Figure 2 : Percentage of cellulose, hemicelluloses (Hemi) and lignin in corn stover biomass: non-treated (NT) and double pretreated by AH, AB and NA methods (see TABLE 2)

TABLE 4 : Crystallinity degree of cellulose in biomass of various origins

Biomass	Crystallinity degree
Flax fibers	0.67-0.69
Cotton linter	0.68-0.72
Pine	0.63-0.65
Spruce	0.63-0.65
Poplar	0.62-0.63
Bagasse	0.54-0.56
Switchgrass	0.52-0.54
Corn stover	0.51-0.53
Wheat straw	0.50-0.52
Rice straw	0.49-0.51
Corn cobs	0.48-0.50

Selective oxidation of lignin in the plant material with sodium chlorite gives holocellulose containing only polysaccharides – cellulose and hemicelluloses. The double pretreatment methods permit obtaining cellulose-rich biomass samples with a small content of non-cellulosic ingredients (Figure 2).

After AL, AH or AB pretreatments of flax fibers and cotton fibers, practically pure celluloses can be isolated.

The X-ray investigations showed that crystallinity degree of cellulose component depended mainly on origin of the biomass, and lesser on the pretreatment method (TABLE 4).

Chemical composition and structural characteristics of pretreated plant biomass are crucial for the subsequent enzymatic hydrolysis. So, removal of hemicelluloses and simultaneous increase of the percentage of cellulose and lignin after SE or AC-pretreatment has an ambiguous effect on enzymatic digestibility. On the one hand, increasing of cellulose content in the pretreated biomass and reducing of crystallinity degree can promote enzymatic hydrolysis; but on the other hand, increasing of lignin content should hinder the hydrolysis. Besides, as a result of decomposition of hemicelluloses at increased temperatures so called pseudolignin can be formed, increasing the total lignin content in the SE or AC biomass[28]. The final result showed some improvement of the enzymatic digestibility of the pretreated biomass in comparison with the non-treated sample. However, the yield of glucose after enzymatic hydrolysis of the SE or AC-pretreated biomass was relative low, 25-30% only (Figure 3). The alkaline pretreatment or non-specific oxidation leads to removal of non-cellulosic component and considerable increase in the content of cellulose, which can contribute to the enzymatic digestibility of the pretreated biomass.

Indeed, the experiments have shown that, for example, the alkali pretreated biomass after enzymatic hydrolysis gives an increased yield of the sugar, about 64%. The cellulose-rich, AH, AB and NA biomasses show the highest digestibility with the glucose yield of 78-85% (Figure 3). The similar results were established also for other biomass types pretreated by the same methods.

Highly crystalline cellulose samples of flax and cotton linters exhibited lower enzymatic digestibility than decrystallized celluloses of herbaceous (TABLE 5).

For a detailed study of the effect of individual polymeric components on the enzymatic digestion, a

TABLE 5 : Crystallinity degree of cellulose and yield of glucose for AB-pretreated biomass

Biomass	Crystallinity degree	Yield, %
Rice straw	0.50	91
Wheat straw	0.52	84
Corn stover	0.53	80
Flax fibers	0.68	62
Cotton linter	0.70	60

correlation analysis was performed. Moreover, regression equations and squared correlation coefficients (R^2) were calculated. For this purpose, about various untreated and pretreated biomass samples with different chemical compositions were investigated.

As follows from the analysis, hemicelluloses have a negligible impact on enzymatic hydrolysis of the cellulosic component of the samples (Figure 4). The squared correlation coefficient for the yield of glucose as a function of the hemicelluloses content was slight, $R^2 = 0.16$ (TABLE 6); this means there is no correlation. The obtained results don't confirm the supposition about barrier properties of hemicelluloses obstructing the access of enzymes to the cellulosic constituent of biomass[21, 22].

The special experiments showed that the used cellulolytic enzymes can partly hydrolyze also xylan and turn it into xylose[29]. Thus, hemicelluloses, in particular xylan, are not serious barrier for the used cellulolytic enzyme preparation. Improvement of the enzymatic digestion after removal of hemicelluloses from the biomass is probably a side effect caused by simultaneous extraction of lignin and increase of the cellulose content.

Lignin has an evident negative effect on the en-

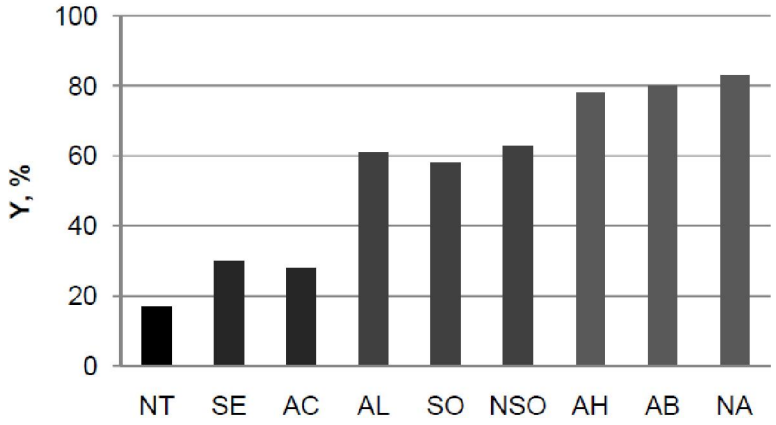

Figure 3 : Yield glucose after enzymatic hydrolysis of corn stover biomass: non-treated (NT), stream-exploded (SE), acid pretreated (AC), alkali pretreated (AL), selective oxidized (SO), non-selective oxidized (NSO) and double pretreated by AH, AB and NA methods

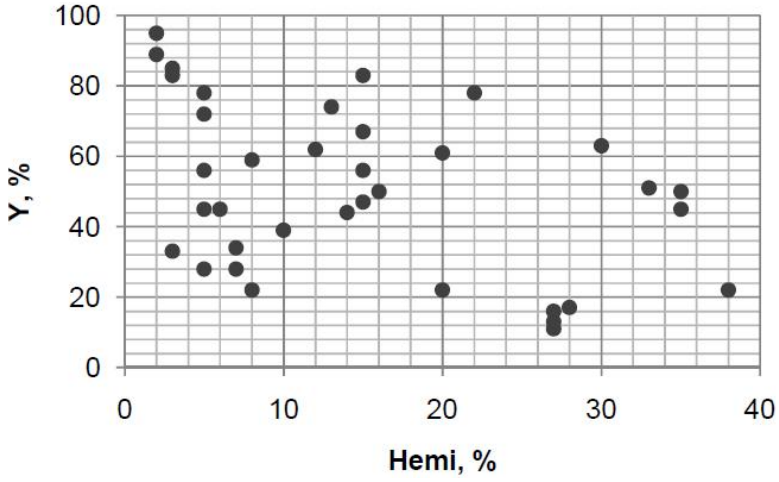

Figure 4 : Yield of glucose after enzymatic hydrolysis of various biomass samples having different content of hemicelluloses (Hemi)

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TABLE 6 : Squared correlation coefficients for regression Y=f(Parameters)

Parameters, %	Regression equation	R^2
Content of hemicelluloses (HC, %)	Y= -0.88 HC + 64	0.16
Content of lignin (L, %)	Y= -2.07 L + 76	0.67
Content of cellulose (C, %)	Y = 1.18 C - 26	0.84
Content and crystallinity (X) of cellulose	Y= 2.58 C(1-X) - 27	0.90
Combined parameter Z = 2C(1-X) - L	Y= 0.98 Z + 3	0.98

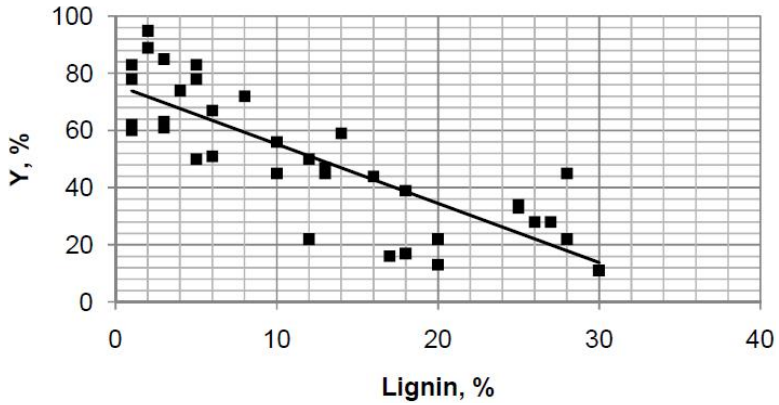

Figure 5 : Correlation between content of lignin and yield of glucose after enzymatic hydrolysis of various biomass samples

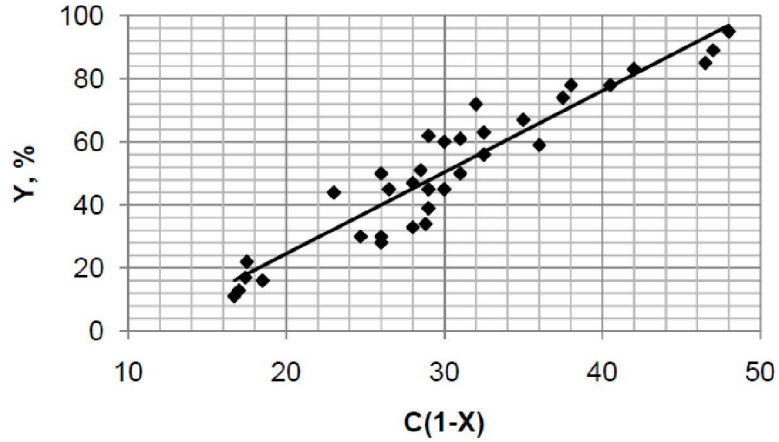

Figure 6 : Yield of glucose after enzymatic hydrolysis of various biomass samples as a function of content of cellulose (C) and its crystallinity degree (X)

zymatic hydrolysis of the cellulosic constituent of biomass samples, and namely an inversely proportional regression was observed between lignin content in the samples and yield of glucose (Figure 5). This result conforms to data of other researchers[17-20]. The correlation coefficient for the yield of glucose as a function of the lignin content in biomass has a moderate value, $R^2 = 0.67$ (TABLE 6); it means there is a sufficient correlation. It is important to note that the barrier mechanism of lignin is apparently valid only for the untreated strongly lignified biomass samples. Pretreatments of the biomass cause a disruption of the barrier layers of lignin. However, the non-productive sorption of cellulolytic enzymes by residual lignin remains and acts as a factor impeding the enzymatic hydrolysis of cellulose. The decrease content of lignin in biomass reduces the non-productive sorption of enzymes and promotes enzymatic cleavage of cellulosic component of biomass.

The correlation for the yield of glucose as a function of the cellulose content (C, %) in biomass was satisfactory (TABLE 6). The increased cellulose content in the pretreated biomass is a significant fac-

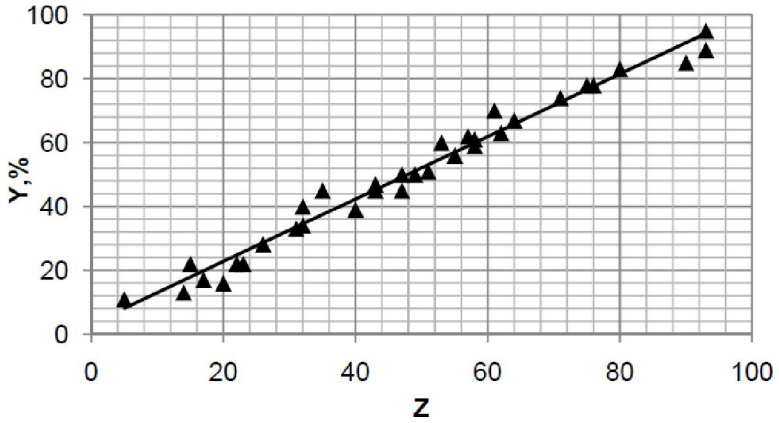

Figure 7 : Dependence of glucose yield on the combined parameter Z

tor that promotes rise the yield of glucose. Nevertheless, the variances of experimental points from average values were relatively great. To reduce variances, an effect of crystallinity degree of cellulose (X) on hydrolysability should be taken into consideration. The obtained dependence Y=f(C, X) (Figure 6) had a high correlation coefficient $R^2 = 0.90$ (TABLE 6).

Finally, the best correlation with maximum squared coefficient $R^2 = 0.98$ was found for the dependence of glucose yield on the combined parameter Z = 2C(1-X) - L (Figure 7); where X is crystallinity degree of cellulose, C is percentage of cellulose and L is percentage of lignin in the investigated biomass samples. This correlation can be expressed by the following regression equation:

$$Y = 0.98\ Z + 3 \qquad (4)$$

Since the correlation coefficient is very high, $R^2 = 0.98$ (TABLE 6), the regression (4) is resemble the exact functional dependence. Thus, increasing of the content of cellulose, reduction of its crystallinity and decreasing of the content of lignin in the biomass samples contribute to enhance the enzymatic digestion of the cellulosic component.

Applying the regression equation (4), the enzymatic reactivity of the pretreated biomass with determined features can be predicted. This equation can be also a basis for choice the best pretreatment method. For example, the alkaline pretreatment (AL) provides obtaining the pretreated corn cobs with Z = 64; therefore, the AL- method is preferred than, for example, the acidic pretreatment method with Z = 40. Another example is the NA-pretreatment of corn stover that provides obtaining the pretreated biomass with increased value of Z = 81; therefore, after enzymatic hydrolysis this biomass gives the high yield of glucose, about 83%.

CONCLUSIONS

The enzymatic hydrolysis of a large number of not-treated and pretreated biomass samples of various origins containing different amounts of cellulose, hemicelluloses and lignin has been investigated. The study of initial plant materials bears out that non-treated biomass samples are highly recalcitrant to enzymatic hydrolysis and give a slight glucose output, 20-30% only. This can be explained by dense inaccessible structure, increased crystallinity and low content of cellulose, barrier properties of lignin and sorption of enzymes by non-cellulosic components that hinder enzymatic hydrolysis of the cellulosic component. Various pretreatment methods of the initial biomasses are applied in order to remove non-cellulosic components, increase the content of cellulose and ultimately improve the enzymatic cleavage. However, the final chemical composition of the pretreated biomass and its enzymatic digestibility depends on the particular method and conditions of the pretreatment, as well as on the biomass origin. For example the distinctive feature of the acidic pretreatment is the removal of the main part of hemicelluloses and forming cellolignin with increased content of cellulose and lignin. In contrast to acidic pretreatments, the alkaline pretreatment of the biomass leads to reduction in the content both of hemicelluloses and lignin; besides the removal of

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non-cellulosic components is accompanied by increase of the cellulose content in the pretreated biomass.

To disclose the effect of individual polymeric components of the biomass, i.e. cellulose, hemicelluloses and lignin, on the glucose yield, a correlation analysis was performed and squared correlation coefficients (R^2) were calculated. As follows from the investigations, the dependence of glucose output on the content of hemicelluloses in the biomass samples has a very low correlation coefficient. Thus, hemicelluloses have no appreciable effect on enzymatic digestibility of the cellulosic component of the biomass. This can be explained by fact that enzyme preparations have also xylanase activity and can cleave the amorphous hemicelluloses. Enzymatic hydrolysis of holocellulose samples yields a mixture of glucose and xylose. Moreover, molecules of enzyme can easily diffuse through the swollen layers of amorphous hemicelluloses. The final conclusion that hemicelluloses are not serious barrier for cellulolytic enzymes and don't prevent cleavage of the cellulosic component.

Lignin is a rigid aromatic, amorphous and hydrophobic polymer stable to cellulolytic enzymes. Lignin layers surrounding cellulose fibrils protect them from the enzymatic attack. Non-productive sorption of cellulolytic enzymes by lignin is also regarded as an important factor hindering the enzymatic hydrolysis of cellulose. It is important to note that the barrier mechanism of lignin is apparently valid only for the untreated strongly lignified biomass samples. Pretreatments of the biomass cause a disruption of the barrier layers of lignin. However, the non-productive sorption of cellulolytic enzymes by residual lignin remains and acts as a factor impeding the enzymatic hydrolysis of cellulose. The decrease content of lignin reduces the non-productive sorption of enzymes and promotes enzymatic cleavage of cellulosic component of biomasses. Therefore, an inversely proportional regression is observed between lignin content in the biomass and enzymatic digestibility of the cellulosic component.

As known, cellulose is a semi-crystalline polymer; moreover the high crystallinity is a main factor limiting complete enzymatic cleavage of this polymer. The experiments showed that decreasing of cellulose crystallinity and increasing of cellulose content in the biomasses gave a positive effect on the enzymatic digestion and yield of glucose.

Finally, the best correlation with maximum squared coefficient ($R^2 = 0.98$) was found for the dependence Y=f(Z); where combined parameter Z =[2C(1-X) – L]; X is crystallinity degree of cellulose, C is percentage of cellulose and L is percentage of lignin in the investigated biomass samples. Applying the regression equation: Y= 0.98 Z + 3, the enzymatic digestibility of the pretreated biomass with determined chemical composition and crystallinity degree can be predicted. This equation can be also a basis for choice the most suitable pretreatment method providing the high reactivity of the pretreated biomass for enzymatic conversion.

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