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Effect of chemical composition of plant biomass on enzymatic hydrolysis

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Abstract : 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 studied. To disclose the effect of individual polymeric components 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 ($R^2 = 0.68$). Conversely, increased cellulose content in the biomass affects positively on enzymatic digestion and glucose output ($R^2 = 0.90$). The best correlation ($R^2 = 0.98$) was found for the dependence of glucose yield (Y) on difference between contents of cellulose and lignin (X) in the investigated biomass samples. Thus, increasing the content of cellulose and decreasing the content of lignin in the samples promotes enzymatic cleavage of the cellulosic component. The discovered correlation Y=F(X) is a basis of choice the best pretreatment method. In particular, the mild alkaline pretreatment supplemented with bleaching can be a quite efficient method since it provides obtaining the delignified biomass enriched with cellulose that shows an excellent enzymatic digestibility.

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

INTRODUCTION

Current industrial technologies of biofuels and biochemicals are based on the hydrolysis of carbohydrates to monosaccharides, mainly to glucose, with the subsequent biological or chemical transformation of the monosaccharides into bioproducts[1]. At present, the main sources of carbohydrates are juices of sugarcane and sugar beet, as well as starches of corn, wheat, potatoes and some others agricultural plants. In Brazil about 100 percents of the bioethanol are produced from juice of sugar cane. In USA approximately 85 percents of the total ethanol production relies on the corn grains. Since carbohydrates are required by the food industry, their use for production of biofuels or biochemicals is limited. Moreover, further expansion of the production to higher volumes of the bioproducts can cause a shortage of land areas, soil exhaustion, lack of food and feed products and raise their prices. Federal office of Germany for the environment said that in the world is not enough agricultural land for cultivation specially

the energy crops.

Alternative way to obtain biofuels and valuable biochemicals without competing with food and feed industry is the use of non-edible biomass as a feedstock. This biomass type involves 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[2].

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[3-6].

Cellulose is a linear, stereo-regular semi-crystalline polysaccharide that is built of repeated D-glucopyranosyl units linked by 1, 4-β-glycosidic bonds[7,8]. 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. As known, the high crystallinity of cellulose is a main factor limiting complete enzymatic cleavage of this polymer. However, only a few cellulose samples such as cotton cellulose, microcrystalline cellulose and some others, have a high crystallinity degree (70-80 %). The crystallinity degree of natural cellulose in the non-edible biomass of agricultural and herbaceous plants, energy crops, forest residues and others is relative low (50-55%) and is not an obstacle to enzymatic degradation[1]. In plant cell walls, the cellulosic nanofibrils are glued by hemicelluloses into fibrillar bundles that are surrounded by lignin layers[4,6].

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[6,8].

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 hemicelluloses and cellulose fibrils protect them from the enzymatic attack[6,9-11].

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

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. Therefore, some kind of pretreatment is usually applied to make the cellulosic component more accessible to enzymes[6,8,12]. 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 and cellulose-rich biomass with high enzymatic digestibility.

It is believed that the main reason for the low accessibility of cellulose in initial biomass is the presence of increased amounts of non-cellulosic components, lignin and hemicelluloses[13,14]. As known, in the plant cell walls lignin layers form a physical barrier to cellulolytic enzymes that hinders hydrolysis of cellulose. Non-productive adsorption of cellulolytic enzymes by lignin is also regarded as an important factor preventing access of the enzymes to cellulose[14]. Furthermore, denaturation and inactivation of the bound enzymes on the lignin surface was observed at the hydrolysis conditions[15].

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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[6,14-17].

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[14,18]. Moreover, acetyl groups of natural hemicelluloses can inhibit the enzymes[19]. However, other studies don't support a negative effect of hemicelluloses on enzymatic hydrolysis of the cellulosic component[20,21]. 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 component - cellulose, on enzymatic hydrolysis has been studied insufficiently, although it is known that increasing the content of cellulose in the pretreated biomass is probably the main factor that may increase the yield of glucose[6].

Thus, despite abundant investigations a role of each polymeric component of the plant biomass in enzymatic hydrolysis is not enough clear. To clarify the problem, in this paper a quantitative correlation analysis was carried out in order to find the best concordance between the content of cellulose, hemicelluloses and lignin, on the one hand, and the yield of glucose produced by enzymatic hydrolysis of various biomass samples, on the other hand.

EXPERIMENTAL

Materials

The seven plant materials – poplar, bagasse of sugar cane, switchgrass, corn stover, 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 (TABLE 1, 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 fifty various samples were prepared.

Chemical analysis

The chemical composition of initial and pretreatment biomass samples was determined by conventional methods of chemical analysis[4,6,22]. The content of

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

Method	Reagent	LSR*	Temperature, °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

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

Method	Steps	Reagent	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	1% $NaClO_2$	10	100	30
NA	(1). Nitric acid	5% HNO_3	5	110	30
	(2). Alkaline	1% NaOH	10	100	30

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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 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.

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

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

Enzymatic hydrolysis

The biomass samples were hydrolyzed with a mixture of commercial cellulolytic enzyme GC-220 (DuPont Ind. Biosciences, Wilmington, DE, USA) and β–glucosidase Novozyme-188 (Novozymes A/S, Bagsvaerd, Denmark). The loading of GC-220 was 15 FPU (or 12.5 mg) 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. 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 55°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:

$$Y\ (\%) = 2\ C_g \qquad (2)$$

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 investigated biomass samples contained 35 to 45% cellulose, 25 to 38% of hemicelluloses and 10 to 23 % of Klason lignin. The higher content of cellulose (45%) and lignin (23%) was observed for the initial poplar biomass. Bagasse, switchgrass, corn stover and wheat straw contained an intermediate content of cellulose (37-38%) and lignin (17-20%). The low lignified corn cobs and rice straw 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.

TABLE 3 : Chemical composition of initial biomass samples

Biomass	Cellulose, %	Hemicelluloses, %	Lignin, %
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

In contrast to SE and AC-pretreatment, the alkaline pretreatment or non-selective oxidation leads to extraction both of hemicelluloses and lignin, but appre-

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ciable 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). Selective oxidation of lignin in the plant material (e.g. corn stover) with sodium chlorite gives holocellulose containing only polysaccharides – cellulose (about 56%) and hemicelluloses (about 44%). The double pretreatment methods permit obtaining cellulose-rich biomass samples with a small content of non-cellulosic ingredients (Figure 2).

Changes in the chemical composition of plant biomass caused by pretreatment 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, increased cellulose content in the pretreated biomass can promote enzymatic hydrolysis, but on the other hand, increased lignin content should hinder the hydrolysis. 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 AB and NA biomasses show the highest digestibility with the glucose yield of 80-85% (Figure 3). The similar results were established also for other biomass types pretreated by the same methods.

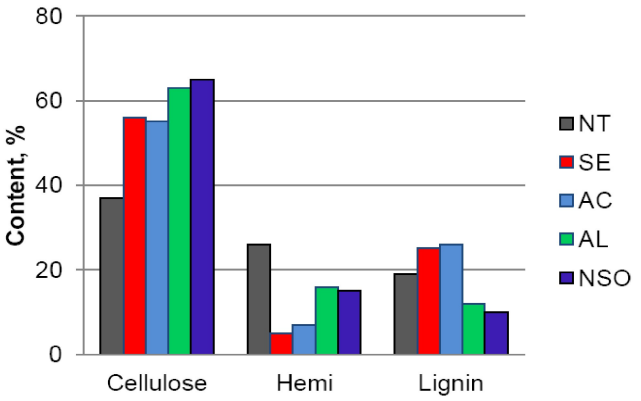

Figure 1 : Percentage of cellulose, hemicelluloses (Hemi) and lignin in corn stover biomass: Non-treated (NT), stream-exploded (SE), acid pretreated (AC), alkali pretreated (AL) and non-selective oxidized (NSO)

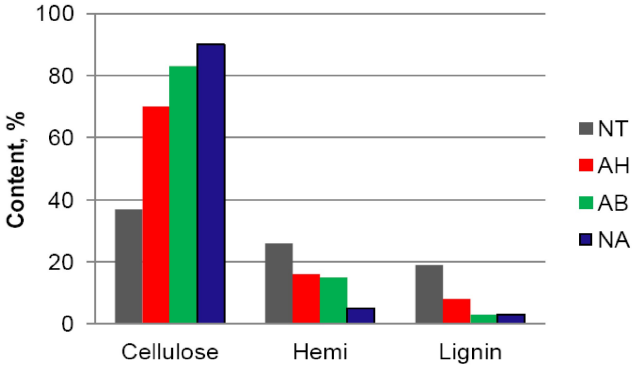

Figure 2 : Percentage of cellulose, hemicelluloses (Hemi) and lignin in corn stover biomass: Non-treated (NT) and double pretreated by AH, AB and NA methods (see TABLE 2)

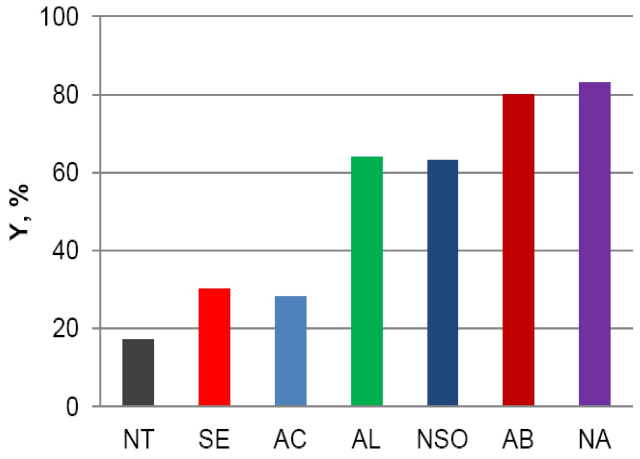

Figure 3 : Yield glucose after enzymatic hydrolysis of corn stover biomass: Non-treated (NT), stream-exploded (SE), acid pretreated (AC), alkali pretreated (AL), non-selective oxidized (NSO) and double pretreated by AB and NA methods

For a detailed study of the effect of individual polymeric components on the enzymatic digestion, a correlation analysis was performed. Moreover, regression equations and squared correlation coefficients (R^2) were calculated. For this purpose, about fifty 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 con-

tent was slight, $R^2 = 0.16$ (TABLE 4); 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 component of biomass[18,19]. 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.

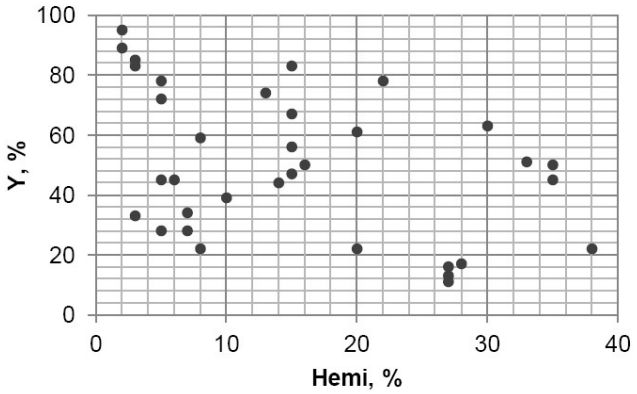

Figure 4 : Yield of glucose after enzymatic hydrolysis of various biomass samples having different content of hemicelluloses (Hemi)

Lignin has an evident negative effect on the enzymatic hydrolysis of the cellulosic component of biomass samples, and namely an inversely proportional regression was observed between lignin content in the samples and yield of glucose (Figure 5). The correlation coefficient for the yield of glucose as a function of the lignin content has a moderate value, $R^2 = 0.68$ (TABLE 4); it means there is a sufficient correlation.

TABLE 4 : Squared correlation coefficients for regression Y=F(X)

X, %	Regression equation	R^2
Hemicelluloses	Y= -0.9 X + 64	0.16
Lignin (L)	Y= -2.2 X + 78	0.68
Cellulose (C)	Y = 1.3 X - 33	0.90
Difference: C-L	Y = 0.98 X + 0.6	0.98

Note: X is percentage of the component or of the difference C-L

The correlation coefficient for the yield of glucose as a function of the cellulose content has a high value, $R^2 = 0.90$ (TABLE 4). There is a good correlation between cellulose content and yield of glucose after enzymatic hydrolysis of various biomass samples (Figure 6). So, increased cellulose content in the pretreated biomass is the main factor that promotes rise the yield of glucose.

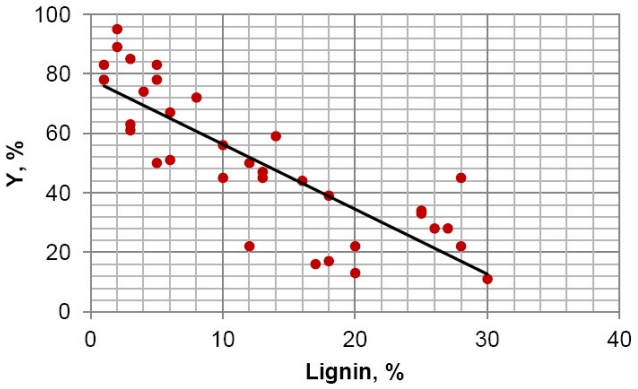

Figure 5 : Correlation between content of lignin and yield of glucose after enzymatic hydrolysis of various biomass samples

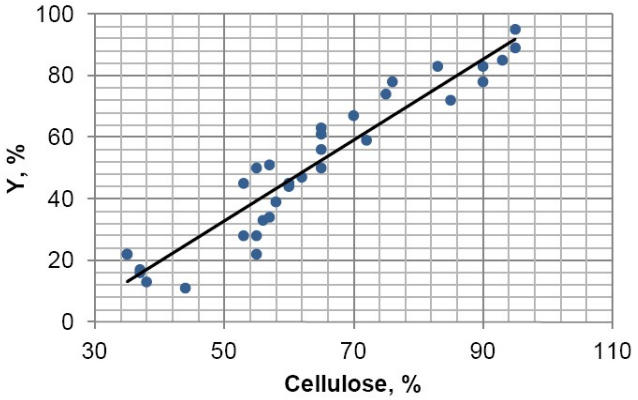

Figure 6 : Correlation between content of cellulose and yield of glucose after enzymatic hydrolysis of various biomass samples

The best correlation was found for the dependence of glucose yield on the difference (X) between percentage contents of cellulose (C) and lignin (L) in the investigated biomass samples (Figure 7). This correlation can be expressed by the following regression equation:

$$\mathbf{Y = 0.98\ X + 0.6} \qquad \mathbf{(3)}$$

where X (%) = C – L

Since the correlation coefficient is very high, $R^2 = 0.98$, the regression (3) is resemble the exact functional dependence. This evidences that just the content of cellulose and lignin in the biomass rather than structural details of these components determines the enzymatic cleavage and sugar output. Thus, increase the content of cellulose and decrease the content of lignin in the biomass samples contributes to enhance the enzymatic

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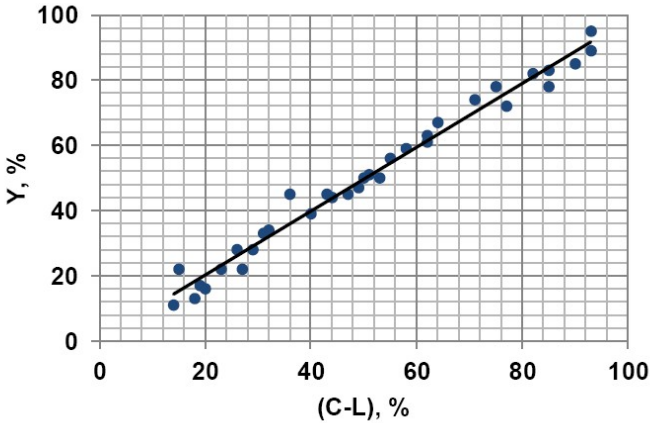

Figure 7 : Dependence of glucose yield on difference between contents of cellulose (C) and lignin (L) in the pretreated biomass samples

digestion of the cellulosic component.

Using the regression equation (3), the enzymatic reactivity of the pretreated biomass with determined chemical composition can be predicted. This equation can be also a basis for choice the best pretreatment method. As it follows from the results, when the difference (X) between percentage content of cellulose and lignin in the pretreated biomass exceeds the boundary value (X_b = 60%), the pretreated biomass will be suitable for enzymatic hydrolysis, since the most part of the feedstock transforms enzymatically into glucose. Vice versa, if $X < X_b$, it can be argued that the pretreated biomass will be poorly suitable for enzymatic hydrolysis.

For example, the alkaline pretreatment (AL) provides obtaining the pretreated corn cobs with X = 64% > X_b. Therefore, the AL-pretreatment method is preferred than, for example, the acidic pretreatment method with X = 40% < X_b. Another example is the AB-pretreatment of corn stover that provides obtaining the pretreated biomass with increased value of X = 82%; therefore, after enzymatic hydrolysis this biomass gives the high yield of glucose, about 80%.

CONCLUSIONS

The enzymatic hydrolysis of a large number of not-treated and pretreated biomass samples of various origins (poplar, bagasse, switchgrass, corn stover, corn cobs, wheat and rice straw) 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 a low content of cellulose and presence in the non-treated materials more 50 percents of non-cellulose 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 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 low correlation coefficient, R^2=0.16 only. 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. For example, the experiments revealed that the used enzyme preparation hydrolyzes the birch xylan and turns it into xylose. 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 a barrier to 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. 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. However, the crystallinity degree of natural cellulose in non-edible biomass of various origins is relative low and is not an obstacle to enzymatic degradation. The experiments showed that the increased cellulose content in the biomasses has a positive effect on the enzymatic digestion and glucose output. As a result, the directly proportional regression between cellulose content and enzymatic digestibility is observed.

Increasing the content of cellulose and decreasing the content of lignin in the samples promotes enzymatic cleavage of the cellulosic component. Therefore, the best correlation was found for the dependence of glucose yield (Y) on the difference (X) between percentage contents of cellulose and lignin in the investigated biomass samples. Using the regression equation: $Y = 0.98 X + 0.6$, the enzymatic reactivity of the pretreated biomass with determined chemical composition can be predicted. This equation can be also a basis for choice the best pretreatment method. For this purpose, the boundary value, $X_b = 60\%$, was found. If $X > X_b$, the pretreated biomass will be suitable for enzymatic hydrolysis, since the most part of the feedstock can be enzymatically converted into glucose. Vice versa, if $X < X_b$, it can be argued that the pretreated biomass will be poorly suitable for enzymatic hydrolysis.

REFERENCES

[1] M.Ioelovich, E.Morag; BioResources, **6**, 2818-2834 **(2011)**.

[2] R.D.Perlack, L.L.Wright, A.F.Turhollow, R.L.Graham, B.J.Stokes, D.C.Erbach; Report of DOE & USDA, http://www1.eere.energy.gov/biomass/pdfs/final_billionton_vision_report2.pdf; **(2005)**.

[3] D.Fengel, G.Wegener; 'Wood Chemistry, Ultrastructure, Reactions', Walter de Gruyter, Berlin/ New York, **(1984)**.

[4] D.Fengel; J.Polym.Sci., **36C**, 383-392 **(1971)**.

[5] B.Saha; J.Ind.Microbiol.Biotechnol., **30**, 279-291 **(2003)**.

[6] M.Ioelovich, E.Morag; BioResources, **7**, 1040-1052 **(2012)**.

[7] M.Ioelovich; SITA, **1**, 68-77 **(1999)**.

[8] S.J.Horn, G.Vaaje-Kolstad, B.Westereng, V.G.Eijsink; Biotechn.Biofuel., **5**, 45-57 **(2012)**.

[9] C.A.Mooney, S.D.Mansfield, M.G.Touhy, J.N.Saddler; Biores.Technol., **64**, 113-119 **(1998)**.

[10] X.Pan, D.Xie, N.Gilkes, D.J.Gregg, J.N.Saddler; Appl.Biochem.Biotechnol., **121/124**, 1069-1079 **(2005)**.

[11] L.Zhu; Fundamental study of structural features affecting enzymatic hydrolysis of lignocellulosic biomass. Dissertation, Texas University, **(2005)**.

[12] R.Chandra, R.Bura, W.Mabee, A.Berlin, X.Pan, J.Saddler; Adv.Biochem.Eng.Biotechnol., **108**, 67-93 **(2007)**.

[13] S.I.Mussatto, M.Fernandes, A.M.Milagres, I.C.Roberto; Enz.Microb.Technol., **43**, 124-129 **(2008)**.

[14] B.Yang, Z.Dai, S.Ding, C.E.Wyman; Biofuels, **2**, 421-450 **(2011)**.

[15] J.Rahikainen, S.Mikander, K.Marjamaa, T.Tamminen, A.Lappas, L.Viikari, K.Kruus; Biotechnol.Bioeng., **108**, 2823-2834 **(2011)**.

[16] P.Binod, K.U.Janu, R.Sindhu, A.Pandey; Hydrolysis of lignocellulosic biomass for bioethanol production. In: 'Biofuels', A.Pandey, (Ed); Academic Press, Burlington, **(2011)**.

[17] R.Sun, X.Song, R.Sun, J.Jiung; BioResources, **6**, 317-328 **(2011)**.

[18] K.Grohmann, R.Torget, M.E.Himmel; Biotechnol. Bioeng., **15**, 59-80 **(1985)**.

[19] K.Grohmann, D.J.Mitchell, M.E.Himmel, B.E.Dale, H.A.Schroeder; Appl.Biochem.Biotechnol., **20/21**, 45-61 **(1989)**.

[20] L.T.Fan, Y.-H.Lee, M.M.Gharpuray; Adv.Biochem. Eng., **23**, 157-187 **(1982)**.

[21] R.Torget, P.Werdene, M.Himmel, K.Grohmann; Appl.Biochem.Biotechnol., **24/25**, 115-126 **(1990)**.

[22] R.M.Rowell; 'Handbook of Wood Chemistry and Wood Composites', CRC Press, Boca Raton, **(2005)**.