Romanian Biotechnological Letters *Vol. 18, No. 5, 2013*

ORIGINAL PAPER

Pretreatments applied for second generation ethanol production from agricultural lignocellulosic residues

Received for publication, April 15, 2013
Accepted, August 21, 2013

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Abstract

In this work we applied mechanical and physicochemical pretreatment methods to convert agricultural lignocellulosic residual biomass (wheat straw and corn stalks) to ethanol by enzymatic hydrolysis and fermentation. We determined total sugars and glucose released after hydrolysis and concentration of ethanol in the fermentation media. The highest yield of total sugars in wheat straw was observed after physicochemical pretreatment: 35.6 g total sugar/100 g biomass, followed by mechanical pretreated wheat straw: 20.8 g total sugar/100 g biomass, physicochemical pretreated corn stalks: 13.23 g total sugar/100 g biomass and mechanical pretreated corn stalks: 12.73 g total sugar/100 g biomass. The percentages of glucose from total reducing sugar released after hydrolysis of pretreated biomass are: 17.88% in the physicochemical pretreated corn stalks, 15.09% in the mechanical pretreated wheat straw and 20.5% in the physicochemical pretreated wheat straw. The concentrations of ethanol obtained in fermentation media were: 4.08% in physicochemical pretreated wheat straw, 3.2% in mechanical pretreated wheat straw, 3.10% in mechanical pretreated corn stalks and 2.80% in physicochemical pretreated corn stalks. Mechanical pretreatment can be an alternative to physicochemical pretreatment in corn stalks, while in wheat straw, applying physicochemical pretreatment results in significantly higher yields.

Keywords: agricultural biomass, ethanol, lignocelluloses, fermentation, pretreatment

Introduction

The fossil fuel resources we use today are not going to be available to the human consumption forever. Demographic explosion, the need of human comfort and the fact that fossil fuels are about to exhaust, forces countries with economic potential to find alternatives to fossil fuels.

For this purpose, the EU Commission presented a Communication on alternative fuels for road transportation and a set of measures to promote the use of biofuels.

The *EU Biofuels Directive* has set the goal of obtaining a target of 20 % energy from renewable sources in overall community energy consumption by 2020 and a mandatory 10 % minimum target to be achieved by all member states for the share of biofuels in transport petrol and diesel consumption by 2020 (DIRECTIVE 2009/28/EC**[1]**).

By this legal frame, the followings will be achieved: the CO_2 emissions shall be reduced, the security of energy supply shall improve, and new opportunities for sustainable rural development shall be generated. Each member state must set national indicative targets in line with the reference percentages of the *Biofuel Directive*. (DIRECTIVE 2009/28/EC **[1]**).

To fulfill the obligations imposed by the EU Biofuels Directive, Romania establishes the following national goals: fuel providers put on market only gasoline and diesel with a biofuels content as follows:

- From the date of entry into force of this Governmental Decision, ***diesel*** must contain not less than 5% biofuel by volume; starting from 1 January 2013 – 6% and from 1 January 2015 – 7%.

- From the date of entry into force of this Governmental Decision, ***gasoline*** must contain not less than 4.5% biofuel by volume; starting from 1 January 2013 – 6%; from 1 January 2015 – 8%; from 1 January 2017 – 9% and from 1 January 2019 – 10% (MONITORUL OFICIAL AL ROMANIEI Nr. 716/11.10.2011 **[2])**.

Many different types of energy crop have been proposed for enzymatic hydrolysis of cellulosic biomass and bioethanol production, but by using grain as raw material a major disadvantage occurs, that is the increase in price for food and feed grain. A solution for this problem is the second generation of biofuels, which means the use of non-food and non-feed materials in order to not interfere in the food price regulation (T. VINTILĂ & S. NEO **[3]**). An alternative to the use of grain as raw material could be the biodegradable agricultural wastes and numerous reviews and scientific papers have been published on the subject of ethanol production from lignocellulosic biomass (J.P. LANGE **[4]**; L.R. LYND, **[5]** LEE, **[6]**; LIN & TANAKA, **[7]**).

Regarding the hydrolysis step, C. Diguţa & al (2007) investigated the efficiency of several commercial enzymes on the conversion of energy crops to fermentable sugars and shows that an increase from 0.5 % to 1 % of enzyme concentration is not economically relevant, because it determined an increase with only 8 % of reducing sugars concentration (C. DIGUŢA & al. **[8]**). Ş. Jurcoane & al. (2009) tested several pretreatment methods applied to maize straw (physical, chemical in combination with enzymatic hydrolysis) regarding their efficiency on cellulose and hemicellulose degradation to reducing sugars. Best results were obtained for maize whole crop when was used as pretreatment 3% H_2SO_4 / 30 minutes at 121^oC followed to enzymatic hydrolysis (Ş. JURCOANE & al. **[9])**. K. Belkacemi, S. Hamoudi (2003) studied enzymatic hydrolysis of corn stalk hemicellulose at 30^oC and pH 5. Saccharification was 90% and sugar was released after 10 h (K. BELKACEMI & S. HAMOUDI **[10];** N. SARKAR & al. **[11]**). Chen et al. (2008) studied enzymatic hydrolysis of maize straw using cellulase from *T. reesei* and cellobiase from *A. niger*. And proved that by addition of 5 g/L Tween 80, the hydrolysis yield has improved by 7.5% (M. CHEN & al. **[12];** N. SARKAR & al. **[11])**. Alkaline peroxide pretreated wheat straw showed 96.75% yield after enzymatic hydrolysis whereas atmospheric autocatalytic organosolv pretreated wet wheat straw gave above 75% yield (B.C. SAHA & M.A. COTTA [13]; N. SARKAR & al. **[11]**). Although there are many studies in the domain is important that research to be continued for finding new ways to reduce costs in ethanol industry, find new resources of raw material, improve pretreatment methods depending on the available raw material and increase bioethanol competitiveness related to gasoline (O. J. SANCHEZ & C. A. CARDONA **[14];** N. SARKAR & al. **[11]**).

In this research we applied and studied the effect of mechanical and physicochemical pretreatment methods of lignocellulosic biomass on the subsequent hydrolysis and fermentation steps for second generation ethanol production. The lignocellulose used as raw material in this study is agricultural residual biomass: corn stalks and wheat straw. The main purpose was to compare the efficiency of pretreatment methods in terms of amount of glucose related to total sugars released by using these pretreatment methods on corn stalks and wheat straw.

Materials and methods

1. Raw materials and experimental pattern

The raw material used in this study is: corn stalks and wheat straw resulted after harvesting these crops in the stage of biological maturity from the didactic farm of University of Agricultural Science and Veterinary Medicine from Timişoara. To evaluate the influence of pretreatment methods on the productivity of ethanol from the lignocellulosic biomass, we applied in this work the whole process of conversion of lignocellulose to ethanol (Figure 1). The efficiency of the applied pretreatment methods is determined in this study by analyzing the concentration of water soluble sugars and glucose after hydrolysis step and the concentration of ethanol obtained after fermentation of the hydrolysates.

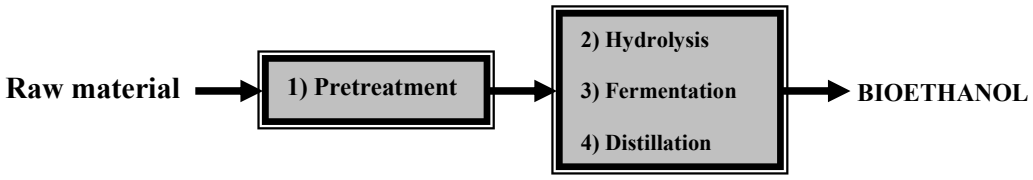

Figure 1. The main steps to obtain ethanol from lignocellulose

2. Pretreatment of the raw material

We used two types of pretreatment. One is a combination of physical and chemical pretreatments indicated by previous research (J. MOHAMAD & al. **[15]** T. VINTILĂ & al **[16]**) to be optimal for this kind of raw materials (steam combined with 2% NaOH). This method generates pollutants, uses chemicals and consumes energy for steam generation. For this reason, we applied an alternative method of pretreatment, which applies the mechanical disruption of lignocellulosic complex by milling to obtain fine particles. This method uses energy, but does not use chemicals, consequently it is less polluting. These pretreatments were done in order to release cellulose and hemicelluloses from the lignocellulosic complex and thereby increase the rate of hydrolysis of these polymers into fermentable sugars and subsequently increase ethanol yields.

By combining these pretreatment methods and types of biomass, we constructed four experimental batches. In batches **I** and **III**, the corn stalks and the wheat straw were milled by using a Cyclotec^{TM} 1093 (Foss Tecator AB, Sweden) mill with 2.0 mm mesh. In batches **II** and **IV**, the corn stalks and the wheat straw chopped in 2-3 cm size particles were subjected to a combination of thermal and alkaline pretreatment: the biomass soaked in 2% NaOH was autoclaved 30 minutes at 1 bar (121^{o}C). After this treatment, the biomass was washed with 10% H_2SO_4 until pH 6.5 and with 12 equivalent volumes of water in order to remove the inhibitors resulted during pretreatments. For each type of biomass, a quantity of 136 grams fresh biomass was pretreated. After pretreatment and washing, the biomass absorbed water and the following quantities of soaked biomass were obtained: 287.06 grams of whet pretreated wheat straw and 313.45 grams of whet pretreated corn stalks. The main pretreatment goal is to increase the enzyme accessibility and to improve the digestibility of cellulose.

The four types of pretreated biomass were introduced in four glass flasks (capacity 1.000 ml) through a glass funnel, according to the scheme presented in Table 1. We used glass flasks having special openings for attaching sensors to measure CO_2 and ethanol concentration in the fermentation phase. In this stage, the openings are covered with plastic covers and rubber

gaskets. The biomass was suspended in citrate buffer pH 5.0 to create the optimal condition for the cellulase complex during hydrolysis. The pH 5.0 is indicated by de cellulase provider (Novozymes) to be optimal for the cellulase complex used in this experiment. To provide nutritional support for the yeast multiplication during the fermentation phase we add yeast extract 1% and peptone 2% in citrate buffer. The final volumes of the biomass / citrate buffer mixtures were 900 ml in each flask.

The four glass flasks were autoclaved (30 minutes at 121^{o}C) to ensure sterile conditions and prevent contamination of the content.

3. Enzymatic hydrolysis of the raw material

Pretreated biomass was used as substrate in enzymatic hydrolysis stage. The hydrolysis of pretreated biomass was performed as follow: after autoclaving, the flasks were left to cool to 45^{o}C. Commercial enzymes purchased from *Novozyme*^{TM} were added to pretreated biomass. We used the NS22086 cellulase complex, part of a *Novozymes* cellulosic ethanol enzyme kit. NS22086 contains cellulase, and xylanase (endo -1,4-) and catalyzes the breakdown of cellulose material into glucose, cellobiose, and glucose polymers. Enzyme activity: 1000 BHU (Biomass Hydrolysis Unit)/g. *Novozymes* recommends an enzyme dosage of 1-5% w/w total solids. After addition of enzymes (5 g enzyme complex/100 g cellulose), the four flasks were incubated for 48 hours at 50^{o}C. The incubation time (48 hours) was established based on the results obtained in previous experiments, where we analyzed the conversion of a variety of cellulosic feedstock by using NS22086 cellulase complex (T. VINTILĂ & al. **[17]**). The flasks content was subjected to stirring for 5 minutes every 24 hours. Stirring of the flasks content disperse the biosolids for a better contact with the enzyme, reduces scum build-up, dilutes level of inhibitors, retains inorganic material in suspension and reduces thermal stratification (R.H. ZHANG & Z.Q.ZHANG **[18]**).

After 48 hours of hydrolysis, flasks were removed from the incubator and 2 ml liquid samples/batch was harvested to determine pH, glucose and total sugars resulted after the hydrolysis of cellulose and hemicelluloses from the pretreated biomass. At the end of this stage we determined the concentration of total sugar released in hydrolysis medium. It is important to know not only the amount of total sugars, but also the amount of glucose released after hydrolysis because the yeasts are able to ferment hexozes (such as the glucose released from cellulose) to ethanol, but not pentoses (such as xylose and arabinose released from hemicelluloses).

4. Fermentation

The hydrolysis media containing fermentable sugars obtained in the previous step were fermented by yeasts to produce ethanol and CO_2. The yeast strain used in this phase was *Saccharomyces cerevisiae CMIT* 2.21 from the collection of Industrial Microorganisms of the Laboratory of Applied Microbiology from University of Agricultural Science and Veterinary Medicine of Timisoara. We inoculated the fermentation media with 2.5 g (humid biomass) of yeast for each 100 ml. The fermentation of the 4 batches was done 4 days in water bath incubator at 32^{o}C. The batch bottles were subjected to stirring for 10 minutes each 24 hours, in order to disperse the media components for better contact with the yeasts (O.A. OSUNKOZA & N.J. OKWUDINKA **[19]**).

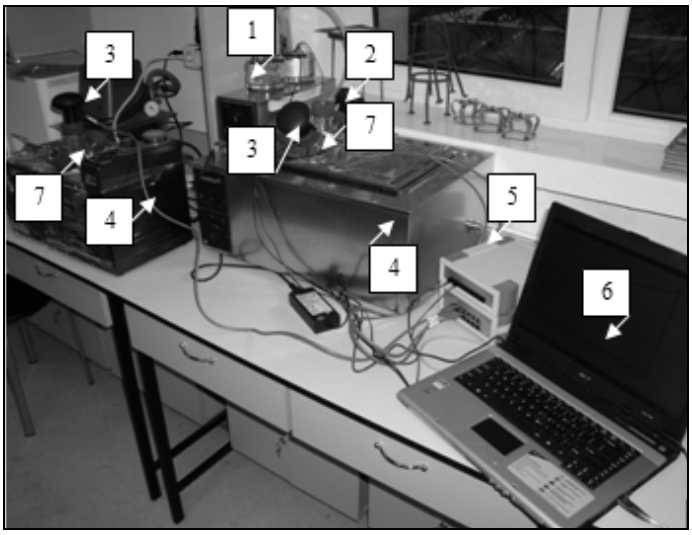

Figure 2. Data recording with BlueSens equipment
(1-mili gas counters; 2-ethanol sensor ; 3-CO_2 sensor;
4-water bath; 5- function box for gas counters and sensors;
6-data acquiring computer, 7- batch flasks)

5. Analysis

We used the following methods of analysis:

The **ethanol production** measurement was possible by using BlueSens^{TM} fermentation equipment provided with infrared ethanol and CO_2 sensors, and gas-counters (Ritter, Germany). The ethanol and CO_2 sensors have been attached to the glass flasks which have been underwent through the previous pretreatment and hydrolysis steps.

The gas released during fermentation (CO_2) is transported to volumeters (*milligascounter – BlueSens^{TM}*), where the volume of gas produced is measured (Figure 2). Data provided by sensors and volumeters are transferred to a function box. From the function box all the data are transported to the computer where the on-line measurements are stored by the software called *BACVis* (Figure 2). The sensors are automatically recognized by means of their identification number and the data are recorded on ACSI-format during intervals, which can be set from 10 seconds to 120 minutes.

To determine the **total sugars**, the 2 ml liquid samples harvested from hydrolysys and fermentation media were centrifuged 10 minutes at 9.000 rpm and supernatant was analyzed by DNS method (T.K. GHOSE **[20]**). The concentration of reducing sugars released during the enzymatic hydrolysis was indirectly determined measuring the absorbance at 540 nm of the color reaction.

To measure the concentration of **glucose** we used the Multiparametric analyzer, which uses enzymatic reactions to determine exclusively glucose with the following reagents: Phosphate buffer, phenol, GOD, POD, Amino-4-antipirine. The glucose was converted into a red quinonic complex; the absorption was read at 500 nm.

A Consort C932 pH meter (Consort, Belgium) was used to determine the **pH values** during the experimental period.

Dry matter (DM %) of the biomass and fermentation residues was determined by oven-drying at 105^{o}C.

Research data were analyzed by a non–parametric method (Mann–Whitney U criteria test) for data comparison. The Mann-Whitney U test is used to determine if there is any difference between independent variables in the experimental batch, in our case between total sugars and

glucose released in the process of hydrolysis of the raw material. In order to do this each one of the four experimental batches was compared to all the other three batches.

Results and discussion

The pretreatment methods applied have generated four types of raw materials for hydrolysis stage. Applying physicochemical pretreatment**,** two types of wet biomass were obtained (some soluble components from the raw biomass have been removed by chemical hydrolysis and washing processes). Applying mechanical pretreatment two types of dry biomass were obtained (the losses of components during milling are insignificant).

Table 1. Total sugars and glucose after enzymatic hydrolysis of the raw material

	CORN STALKS				WHEAT STRAW			
	BATCH I		BATCH II		BATCH III		BATCH IV	
	Total sugars	Glucose	Total sugars	Glucose	Total sugars	Glucose	Total sugars	Glucose
	(g/100 g biomass)		(g/100 g biomass)		(g/100 g biomass)		(g/100 g biomass)	
x± sx	**12.73**± 0.26	**2.09**± 0.06	**13.23**± 0.25	**2.26**± 0.04	**20.77**± 0.53	**3.13**± 0.07	**35.60**± 1.52	**7.30**± 0.21
s	0.59	0.13	0.56	0.09	1.19	0.17	3.41	0.48
CV	4.45	6.17	4.46	4.02	5.72	5.32	6.13	4.44

x – *mean*
S – *standard deviation*
Sx – *standard error of the mean*
Cv – *coefficient of variation*

Table 2. P–value of total sugars after enzymatic hydrolysis of the raw material (Mann-Whitney Test)

Total sugars from:		CORN STALKS		WHEAT STRAW	
		BATCH I	BATCH II	BATCH III	BATCH IV
CORN STALKS	BATCH I	-	0.117^{is}	0.009**	0.009**
	BATCH II		-	0.009**	0.009**
WHEAT STRAW	BATCH III			-	0.009**
	BATCH IV				-

is – p > 0.05
**** - p < 0.01**

Table 3. P–value of glucose after enzymatic hydrolysis of the raw material (Mann-Whitney Test)

Glucose from:		CORN STALKS		WHEAT STRAW	
		BATCH I	BATCH II	BATCH III	BATCH IV
CORN STALKS	BATCH I	-	0.94^{is}	0.009**	0.009**
	BATCH II		-	0.009**	0.009**
WHEAT STRAW	BATCH III			-	0.009**
	BATCH IV	-	-	-	-

is – p > 0.05
**** - p < 0.01**

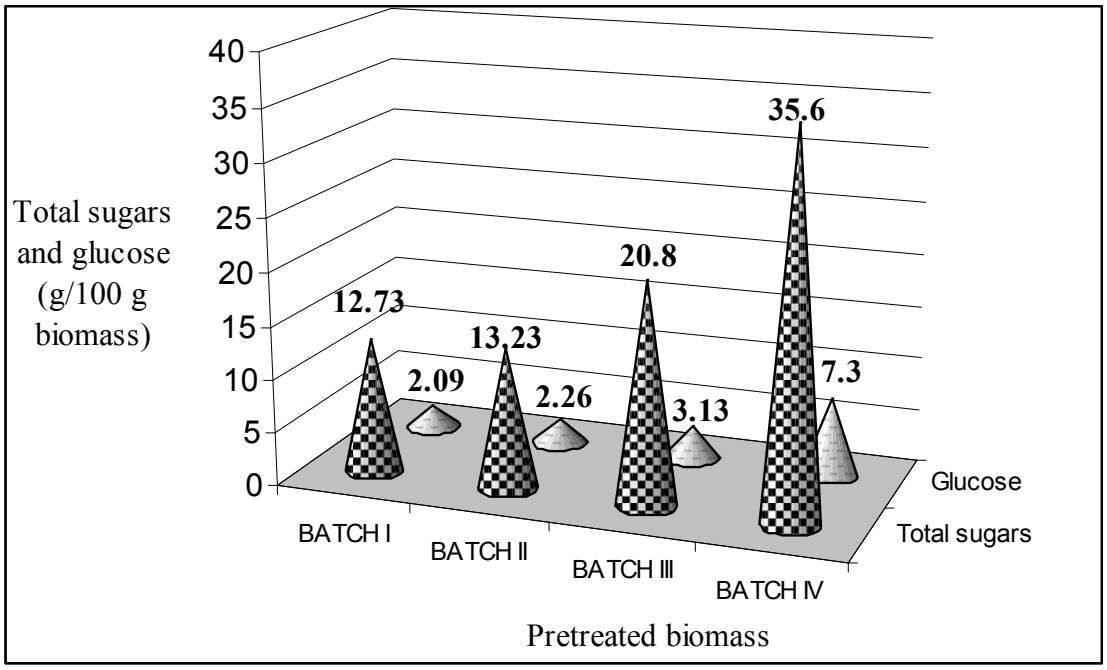

Figure 3. Total sugars and glucose released after enzymatic hydrolysis of the pretreated biomass

The highest yield of total sugars was observed in physicochemical pretreated wheat straw: 35.6 g total sugar/100 g biomass, followed by mechanical pretreated wheat straw: 20.8 g total sugar/100 g biomass, physicochemical pretreated corn stalks: 13.23 g total sugar/100 g biomass and mechanical pretreated corn stalks: 12.73 g total sugar/100 g biomass.

In case of mechanical pretreated corn stalks: from the amount of total sugars (12.73 g), only 2.09 g is represented by glucose, which means that only 16.41% of total sugars is glucose. The percentages of glucose from total reducing sugar are: 17.88% in the physicochemical pretreated corn stalks, 15.09% in the mechanical pretreated wheat straw and 20.5% in the physicochemical pretreated wheat straw.

By using different pretreatment methods and enzymatic hydrolysis no significant difference ($p>0.05$) regarding the total sugars was found between batch I (mechanical pretreated corn stalks) and batch II (physicochemical pretreated corn stalks). Regarding the amount of total sugars resulting from hydrolysis for all the other experimental batches (Table 3), the statistic differences between the groups are distinctively significant ($p<0.01$).

Physicochemical pretreated wheat straw generates more total sugars and glucose than the mechanical pretreated wheat straw. Thus, we recommend the application physicochemical pretreatment for ethanol production from wheat straw.

Our results are comparable with those reported by C.L. Hsu & al. (2011) who obtained 27.1 g total sugars/100g DM in case of treating the corncob with 0.25-0.5 % sulfuric acid/20 minutes(C. L. HSU & al. [22]). In sulfuric acid pretreatment method substances as furfural and 5-hydroxyfurfural are generated, reason why the fermentation process is inhibited (B. P. ADRADOS & al. [21]).

After hydrolysis the pH values were 5.0 in mechanical pretreated corn stalks, 4.9 in physicochemical pretreated corn stalks, 4.4 in mechanical pretreated wheat straw and 4.0 in physicochemical pretreated wheat straw. The pH was adjusted to 5.5 in all cases before fermentation.

During the fermentation process yeast can ferment only compounds such as glucose, fructose, maltose, cellobiose, galactose, sucrose, manitose which were released during the hydrolysis stage. Xylose, arabinose and other pentoses found in the fermentation medium will not be fermented by yeasts. At the end of the fermentation process all these compounds will be detected as residual sugars.

The results regarding the residual sugars detected after yeasts fermented the raw material are shown in Table 4 and Figure 4.

Table 4. Residual sugars after fermentation of hydrolysates

	CORN STALKS		WHEAT STRAW	
	BATCH I	**BATCH II**	**BATCH III**	**BATCH IV**
	(g/100g biomass)	(g/100g biomass)	(g/100g biomass)	(g/100g biomass)
x± sx	**5.25**±0.10	**7.30**±0.27	**13.35**±0.33	**24.65**±0.34
s	0.23	0.59	0.74	0.75
CV	4.33	5.26	4.25	5.94

x – *mean*
S – *standard deviation*
Sx – *standard error of the mean*
Cv – *coefficient of variation*

Table 5. P–value of residual sugars after raw material fermentation (Mann-Whitney Test)

		CORN STALKS		WHEAT STRAW	
		BACH I	**BACH II**	**BACH III**	**BACH IV**
CORN STALKS	**BATCH I**	-	0.009**	0.009**	0.009**
	BATCH II		-	0.009**	0.028*
WHEAT STRAW	**BATCH III**			-	0.009**
	BATCH IV				-

*** - p < 0.05**
****- p < 0.01**

The highest concentration of residual sugars was found in physicochemical pretreated wheat straw: 24.65 g residual sugar/100 g biomass, followed by mechanical pretreated wheat straw: 13.35 g residual sugar/100 g biomass, physicochemical pretreated corn stalks: 7.3 g residual sugar/100 g biomass and mechanical pretreated corn stalks: 5.25 g residual sugar/100 g biomass.

Significant difference ($p < 0.05$) regarding the residual sugars was recorded between these two batches: physicochemical pretreated corn stalks (Batch II) and physicochemical pretreated wheat straw (Batch IV). As far as the amount of residual sugars resulting from fermentation for all the other experimental batches (Table 7) is concerned, the statistic differences between the groups are distinctively significant ($p<0.01$).

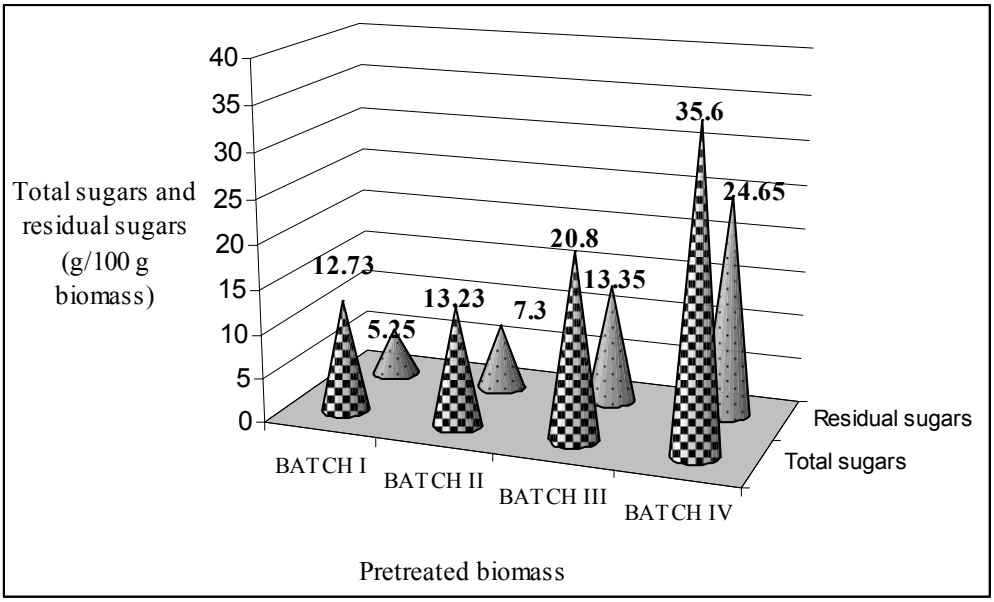

Figure 4. Concentration of total sugars released after hydrolysis and the residual sugars after fermentation (the difference between them represents fermentable sugars)

The amounts of total sugars presented in figure 4 were generated after enzymatic hydrolysis, but only a fraction of this amount can be used by the yeast during fermentation stage and converted to ethanol. By subtracting the residual sugars from the total sugars obtained after hydrolysis, we can find the ratio of carbohydrates fermented by yeasts. Consequently, the following values can be generated: in batch I 7.48 g fermentable carbohydrate from 12.73 total sugar; in batch II 5.93 g fermentable carbohydrate from 13.23 g total sugar; in batch III 7.45 g of fermentable carbohydrate from 20.8 g of total sugars; in batch IV 10.95 g from 35.6 g of total sugars.

The fraction of unconverted sugars (xylose, arabinose, other pentoses) in our experiment is relatively high. This high amount of unfermentable sugar is generated either by the enzymatic system we applied, which includes a wide range of enzymes capable to hydrolyze different carbohydrate polymers, or the biomass hydrolysis occurred only partially with production of large quantities of oligoglucides, which cannot be fermented by yeast to ethanol. To solve this problem we can use GM yeasts able to ferment pentoses and long-chain carbohydrates, or to use other species of microorganisms (yeast like: *Pichia stipitis*, *Candida shehatae* and *Pachysolen tannophilus* can assimilate pentoses but their ethanol production rate from glucose is at least five times less than that observed for *S. cerevisiae*) (O. J. SANCHEZ & C. A. CARDONA **[14],** N. SARKAR & al. **[11]**). But, this topic does not represent the subject of the accomplished research and will be approach in a future experiments.

In each of the 4 batches the initial concentration of biomass was 136 g (DM). During the hydrolysis and fermentation process the biomass was decomposed, and at the end of the experiment we recovered the following quantities of fermentation residues (DM): 95.07 g mechanical pretreated corn stalks, 11.27 g physicochemical pretreated corn stalks, 17.48 g mechanical pretreated wheat straw and 20.44 g physicochemical pretreated wheat straw. This indicates different values of biomass hydrolysis and liquefaction rates (see Figure 5). Although in batches II and III the percentage of destructured biomass is high, the results

regarding the sugars released indicates that the liquefaction of lignocellulose involves numerous depolymerizations, others then carbohydrates hydrolysis (mainly delignification).

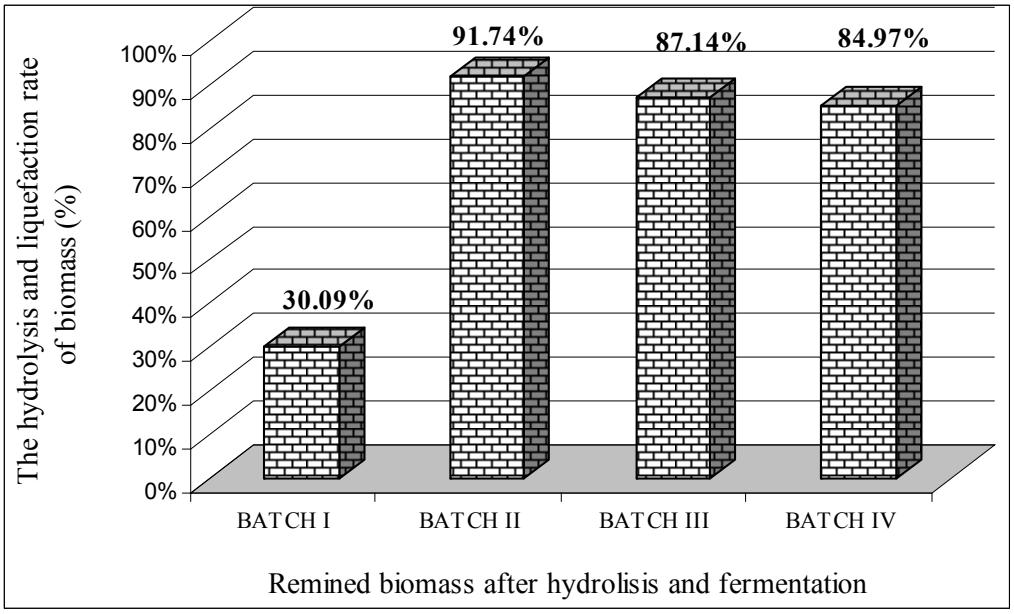

Figure 5. The hydrolysis and liquefaction rate of biomass

After fermentation, the pH values dropped to 5.0 in mechanical pretreated corn stalks, 4.8 in case of physicochemical pretreated corn stalks, 4.3 in mechanical pretreated wheat straw and 3.9 in physicochemical pretreated wheat straw.

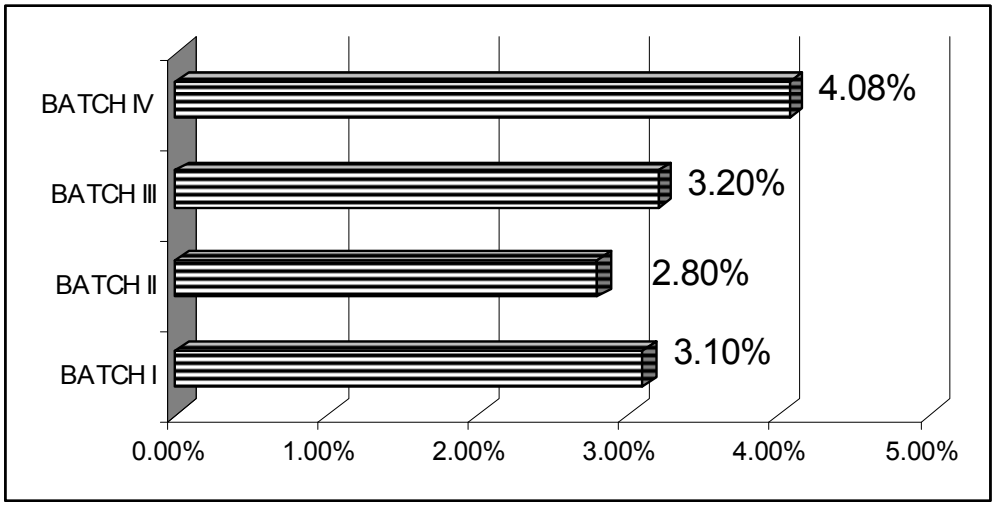

Figure 6. Final concentration of ethanol in fermentation media

In the batch bottles containing physicochemical pretreated wheat straw, after fermentation of 15% DM lignocellulose (136 g DM/900 ml hydrolysis medium), a maximum concentration of 4.08% ethanol was produced, while in the batch bottles containing mechanical pretreated wheat straw the maximum alcoholic concentration was 3.2% ethanol. After the fermentation of corn stalks hydrolysates, the maximum concentration of ethanol was 3.10% for mechanical pretreated corn stalks and 2.80% for physicochemical pretreated corn stalks. Calculating the

yields of ethanol reported to lignocellulose (DM), the calculations generated the following results: 0.272 g g^{-1} physicochemical pretreated wheat straw, 0.213 g g^{-1} mechanical pretreated wheat straw, 0.186 g g^{-1} for physicochemical pretreated corn stalks and 0.206 g g^{-1} mechanical pretreated corn stalks Our results are comparable with those reported by other researches, for example J. Szczodrak obtained 2.4% (w/v) ethanol after fermentation with *S. cerevisiae* of 10% (w/v) DM chemically treated wheat straw (ethanol/lignocellulose yield 0.24 g g^{-1}), while in the SSF, the production increased to 3% (ethanol/lignocellulose yield 0.3 g g^{-1}) (J. SZCZODRAK **[23]**). These results demonstrates that using *S. cerevisiae* as fermenting organism, the productivity in terms of ethanol/lignocellulose yield is comparable with processes using recombinant *E. coli* strains as fermenting organism, as in study reported by B. C. Saha and M. A. Cotta in 2007. In their research, the concentration of ethanol from lime pretreated enzyme saccharified wheat straw by recombinant *E. coli* strain FBR5 was 2.25%, with a yield of 0.29 g g^{-1} straw. In the case of SSF by the *E. coli* strain, the ethanol concentration was 2.06 % with a yield of 0.26 g g^{-1} straw (B. C. SAHA & M. A. COTTA **[24]**).

Conclusions

By analyzing the data presented above, we can conclude that in case of corn stalks the applied pretreatment methods led to similar results regarding the sugars released after hydrolysis and the ethanol obtained as well. In this case we recommend milling as mechanical pretreatment method, for reasons related to environment protection, as no polluting residues are generated (in fact, there are no residues at all). But it should be considered that this pretreatment method may involve high production costs as it requires initial investment for special mills acquisition, and implies high energy consumption for milling the raw material.

Comparing the two pretreatment methods applied to wheat straw, the high amount of total sugars was obtained after physicochemical pretreatment. We recommend the use this pretreatment method for high yields of ethanol from wheat straw. Some disadvantages have to be considered regarding physicochemical pretreatment: generates pollutants, uses chemicals and consumes energy for steam generation.

Acknowledgements

This paperwork was carried out during the project “Post-doctoral School of Agriculture and Veterinary Medicine POSDRU/89/1.5/S/62371”, co-financed by the European Social Fund through the Sectorial Operational Programme for the Human Resources Development 2007–2013.

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