STUDIES REGARDING THE ALKALINE AND ALKALINE- PEROXIDIC PRETREATMENT INFLUENCE UPON THE REDUCING SUGARS QUANTITY OBTAINED FROM THE WHEAT STRAWS

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Abstract: *The main goal of this study was the evaluation of the reducing sugars quantity obtained from the wheat straws using the enzymatic complex Accellerase 1500 from Genencor in different conditions of alkaline and alkaline-peroxidic pretreatment.*
In the first phase we observed the induced modifications for the lignocellulosic materials at the cellulasic attack by the application of some alkaline treatments upon them. At the same time, we also observed the way in which the concentration of the alkaline solution influences the results of the enzymatic attack applied afterwards to the lignocellulosic materials. We used NaOH solutions at concentrations of 0,5 %, 1,0 %, 2,0 % and 4 %. From the data obtained we can conclude that the best results have been seen in the case of high concentration NaOH solutions.
In the second phase we tried to obtain some results with the same efficiency as in the previous phase but by applying an alkaline pretreatment coupled with a peroxidic pretreatment. For this purpose we tested several hydrogen peroxide concentrations (of 0,5 %, 1,0 %, 1,5 % and 2 %) , keeping constant the concentration of the NaOH solution.
The reducing sugars production was evaluated at the enzymatic hydrolysis periods of 24 and 48 h by the DNS method.
The maximum content of reducing sugars from the analyzed raw material was obtained in the case of the combined alkaline-peroxidic pretreatment at 100°C for 2h using NaOH of 4 % concentration and H_2O_2 of 2,0 % concentration.

Keywords: *lignocellulosic materials, enzymatic hydrolysis, cellulase, DNS method.*

1. Introduction

Production of fuel ethanol from renewable lignocellulosic materials has been extensively studies in the last decades [1].
Lignocellulosic biomass is structurally complex, composed of crystalline cellulose (source of glucose) and amorphous hemicellulose (source of pentose such as xylose and arabinose; hexose such as glucose, galactose and mannose) as its major sugar polymers [2], [3].
Biochemical conversion of lignocellulosic biomass through saccharification and fermentation is a major pathway for bioethanol production [4], [5].
Lignocellulosic materials to be considered for ethanol production include wood, crops from annual plants, agricultural residues (cereal straw, rice hulls, cotton waste), waste paper and municipal solid wastes [6], [7]. Wheat straw is one of the most abundant crop residues in European countries with a production of 170 million tonnes and seems to be the cheapest and the most useful raw material for the ethanol production [8]. It is comprised mainly of cellulose (33-40%), hemicellulose (20-25%) and about 20%

lignin [9]. Bioethanol from these feedstocks could be an attractive alternative for disposal of these residues.
An initial pretreatment stage is needed to soften the material and break down its structure to make it more susceptible to an enzymatic attack before fermentation [10].
The ideal pretreatment consists on avoiding the need for reducing the size of biomass particles, preserves the pentose fraction, limits formation of degradation compounds that inhibit growth of fermentative microorganisms, minimise energy demands and reduce costs [10].
The main goal of this study was to evaluate the reducing sugars quantity obtained from the wheat straws in different conditions of alkaline and alkaline-peroxidic pretreatment.

2. Experimental

2.1. Raw material

The raw material used for obtaining the reducing sugars was represented by wheat straws obtained from the farmers from Vrancea area. The raw material was air dried and grinded in the hammer mill at dimensions of approximately 2 mm.

2.2. The wheat straws pretreatment

The alkaline pretreatment:
A given quantity of lignocellulosic material (4 g) was treated in the first phase with the same volume of NaOH solution (100 ml), at different concentrations. Thus there were used for the straws pretreatment NaOH solutions at concentrations of 0.5 %, 1 %, 2 %, and 4 %.
For to realize the experiment it was used a solid/ liquid ratio of 1:25. The samples were thermostated at a temperature of 100°C, the treatment period being of 2 h.
The alkaline- peroxidic pretreatment
In the second phase the lignocellulosic material (wheat straws) was simultaneously treated with NaOH and H_2O_2 solutions. The NaOH solution concentrations were the same as in the previous phase and the H_2O_2 solution concentration varied between 0.5 and 2 %. The samples were thermostated at a temperature of 100°C, for 2 h.
The pretreated straws were cleaned with water (approximately 12 volumes) for to eliminate the inhibitors resulted after the pretreatment (furfural, HMF, sodium hydroxide) and the resulting glucose, that inhibits the cellulase enzymatic activity.
The pretreated lignocellulosic material was suspended in buffer solution of Na_2HPO_4 – citric acid with a pH of 5 and it was subjected to the enzymatic hydrolysis.

2.3. The enzymatic hydrolysis of the pretreated wheat straws

For to check the measure in which the applied pretreatments influenced the susceptibility of the lignocellulosic materials at the enzymatic attack they were subjected to the enzymatic hydrolysis.
The enzymatic hydrolysis was done by using the enzymatic complex Accelerase 1500 the working method being the following: the pretreated straws were washed with distilled water until they reached a neutral pH and after they were suspended in 100 ml buffer solution of Na_2HPO_4 0.2M – citric acid 0.1M, at a pH between 4.6 and 5.0. Then the enzymatic solution was added in the dosage of 0.25 ml/g biomass. The samples were thermostated at the temperature of 50°C, and the reducing sugars content was determined after 24 and 48 h.
Accellerase 1500 is produced by a genetically modified strain of Trichoderma reesei and represents an enzymatic complex especially formed by exoglucanases, endoglucanases, hemicellulase and beta-glucosidase. Accellerase 1500 is able of efficiently hydrolyze the lignocellulosic biomass in fermentable monosaccharide. Accellerase 1500 contains a high level of beta-glucosidase activity in comparison with the

cellulases available on the market and thus it ensures almost completely the cellobiose conversion to glucose.
The benefits of the enzymatic complex Accellerase 1500 in comparison with the conventional cellulases are the following:
- an improved saccharification performance for a variety of raw materials;
- ability to operate in simultaneous saccharification and fermentation (SSF) processes, two step sequential hydrolysis and fermentation (SHF)
- processes a high beta-glucosidase activity that leads to higher saccharification and fermentation rates than for the ethanol;
- an improved formula for reducing the inhibition risk for the fermentation organisms.

2.4. Methods of analysis

The quantification of the reducing sugars after the pretreatment phases and the enzymatic hydrolysis was performed using the DNS method [11].
This method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. This involves the oxidation of the aldehyde functional group (eq. 1), simultaneously, 3.5-dinitrosalicylic acid (DNS) is reduced to 3-amino, 5-nitrosalicylic acid (eq. 2) under alkaline conditions, whose absorbance was measured with a spectrophotometer at 575 nm.

aldehyde group $\xrightarrow{\text{oxidation}}$ carboxyl group (1)

3.5-dinitrosalicylic acid $\xrightarrow{\text{reduction}}$ 3-amino, 5-nitrosalicylic acid (2)

With the aid of DNS method one can determine the concentration of all the reducing sugars in the hydrolysis environment not only for the glucose. It is important to be aware of the presence of all the reducing sugars for the yeast has the capacity to assimilate not only the glucose but also the saccharose, maltose and maltotriose.
In figure 1 it is presented the calibration curve for the reducing sugars determination by the DNS method.

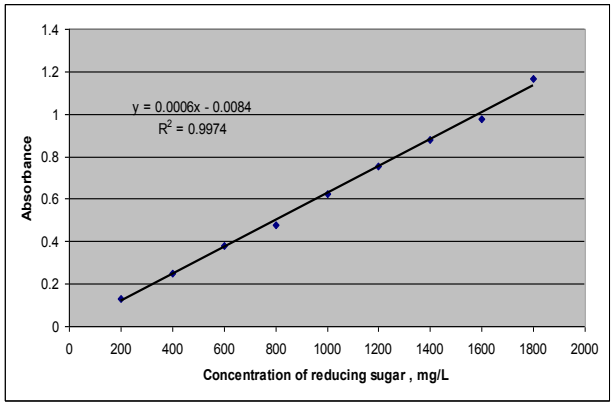

Figure 1. Calibration curve for determination of reducing sugar by DNS method

3. Results and discussion

3.1. The influence of the alkaline pretreatment upon the enzymatic degradation of the lignocellulosic materials

The variants used in this study are:
Variant *P1:* pretreated wheat straws with NaOH 0.5%;
Variant *P2:* pretreated wheat straws with NaOH 1%;
Variant *P3:* pretreated wheat straws with NaOH 2%;
Variant *P4:* pretreated wheat straws with NaOH 4%;
The alkaline pretreatment was led at a temperature of 100°C for 2 h, and the enzymatic hydrolysis was led at 50°C. The results obtained in the case of the alkaline pretreatment are presented in figure 2.
We can observe that the alkaline pretreatment induces clear modifications in the release of reducing sugars. The release of reducing sugars for the enzymatic hydrolysis increases along with the increasement of NaOH concentration.

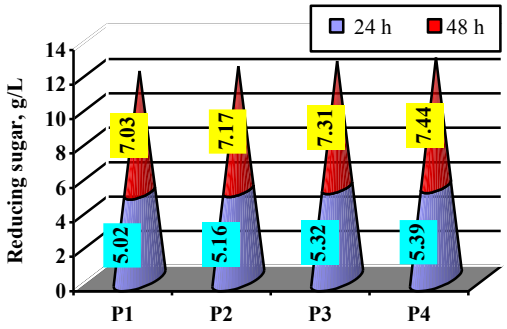

Figure 2. The alkaline pretreatment and the enzymatic hydrolysis at 50°C

The glucidic content is also highly influenced by the period of the enzymatic hydrolysis. In this way we can observe that in a period of 48 h of enzymatic hydrolysis the glucidic content increases with ≈ 2 g/L.

3.2. The influence of the alkaline-peroxidic pretreatment upon the enzymatic degradation of the lignocellulosic materials

From the data previously obtained we can say that the best results can be obtained in the case of the NaOH solutions of high concentrations. But this fact brings along besides the economic issues (duet o the high costs of NaOH and the using of large quantities of water for its elimination) ecological issues due to the release in the environment of the cleaning waters. In this situation we tried to obtain results with the same efficiency as for the alkaline pretreatment by applying an alkaline treatment coupled with a peroxidic one. For this purpose we tested several concentrations of hydrogen peroxide keeping constant the NaOH solution concentration.

3.2.1. Study 1:

The variants used in this study are:
Variant *P5:* pretreated wheat straws with NaOH 0.5% and H_2O_2 0.5 %;
Variant *P6:* pretreated wheat straws with NaOH 0.5% and H_2O_2 1.0 %;
Variant *P7:* pretreated wheat straws with NaOH 0.5% and H_2O_2 1.5 %;
Variant *P8:* pretreated wheat straws with NaOH 0.5% and H_2O_2 2.0 %;
The alkaline-peroxidic pretreatment was led at a temperature of 100°C for 2 h, and the enzymatic hydrolysis was led at 50°C. By the enzymatic hydrolysis of the wheat straws as presented above we obtained the results shown in figure 3.

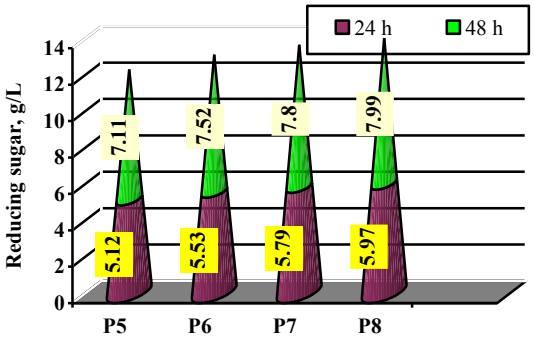

Figure 3. The alkaline-peroxidic pretreatment and the enzymatic hydrolysis at 50°C

We can observe that the adding of H_2O_2 leads to the increase of reducing sugars accumulation. By comparing the values registered for the alkaline-peroxidic treated variants as well as for the NaOH treated variants we observe significant increasements. The efficiency of the alkaline-peroxidic pretreatment is proportional with the H_2O_2 concentration.

3.2.2. Study 2:

The variants used in this study are:
Variant P9: pretreated wheat straws with NaOH 1 % and H_2O_2 0.5 %;
Variant P10: pretreated wheat straws with NaOH 1 % and H_2O_2 1.0 %;
Variant P11: pretreated wheat straws with NaOH 1 % and H_2O_2 1.5 %;
Variant P12: pretreated wheat straws with NaOH 1 % and H_2O_2 2.0 %;
The alkaline-peroxidic pretreatment was led at a temperature of 100°C for 2 h, and the enzymatic hydrolysis was led at 50°C.

By the enzymatic hydrolysis of the wheat straws as presented above we obtained the results shown in figure 4.

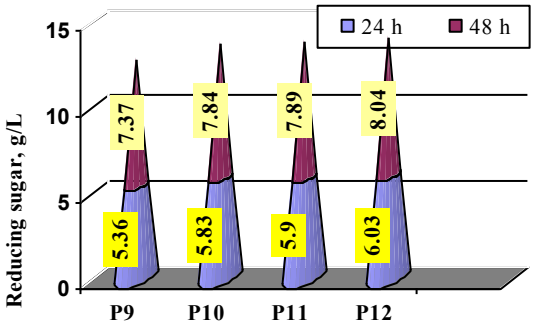

Figure 4. The alkaline-peroxidic pretreatment and the enzymatic hydrolysis at 50°C

The data obtained reveal an increasement of the reducing sugars accumulation when using NaOH of 1 % concentration. When using H_2O_2 for the wheat straws treatment the increasement is accentuated along with the H_2O_2 concentration increasement.

3.2.3. Study 3:

The variants used in this study are:
Variant P13: pretreated wheat straws with NaOH 2 % and H_2O_2 0.5 %;
Variant P14: pretreated wheat straws with NaOH 2 % and H_2O_2 1.0 %;
Variant P15: pretreated wheat straws with NaOH 2 % and H_2O_2 1.5 %;
Variant P16: pretreated wheat straws with NaOH 2 % and H_2O_2 2.0 %;
The alkaline-peroxidic pretreatment was led at a temperature of 100°C for 2 h, and the enzymatic hydrolysis was led at 50°C. By the enzymatic hydrolysis of the wheat straws as presented above we obtained the results shown in figure 5.

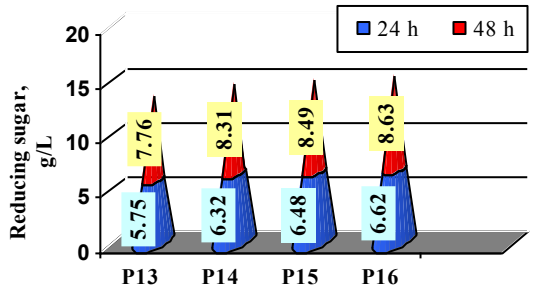

Figure 5. The alkaline-peroxidic pretreatment and the enzymatic hydrolysis at 50°C

The data obtained reveal an increasement of the reducing sugars accumulation when using NaOH of 2 % concentration. When using H_2O_2 for the wheat straws pretreatment the increasement is accentuated along with the H_2O_2 concentration increasement.

3.2.4. Study 4:

The variants used in this study are:
Variant P17: pretreated wheat straws with NaOH 4 % and H_2O_2 0.5 %;
Variant P18: pretreated wheat straws with NaOH 4 % and H_2O_2 1.0 %;
Variant P19: pretreated wheat straws with NaOH 4 % and H_2O_2 1.5 %;
Variant P20: pretreated wheat straws with NaOH 4 % and H_2O_2 2.0 %;
The alkaline-peroxidic pretreatment was led at a temperature of 100°C for 2 h, and the enzymatic hydrolysis was led at 50°C. By the enzymatic hydrolysis of the wheat straws as presented above we obtained the results shown in figure 6.

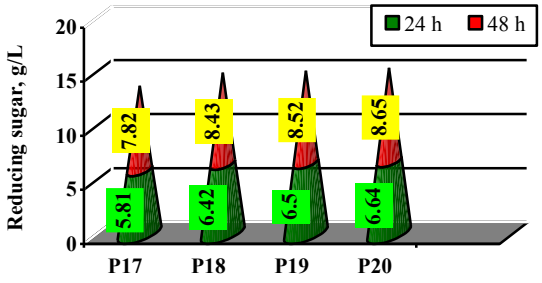

Figure 6. The alkaline-peroxidic pretreatment and the enzymatic hydrolysis at 50°C

It is observed that the H_2O_2 adding leads to the increasement of reducing sugars accumulation fact that also happens along with the NaOH solution concentration increasement.

4. Conclusion

After the alkaline pretreatment applied to the lignocellulosic materials (wheat straws) for 2 h at 100°C, from all the variants taken in consideration by this study the best results are obtained in the case of the NaOH solution of 4% concentration. It can be noticed a proportional increasement of the reducing sugars accumulation with the NaOH solution concentration used for the pretreatment. This is applied for a concentration of the solution up to 2%, for in the cases of higher concentrations the dependence is no longer linear. When applying the alkaline-peroxidic pretreatment for the same NaOH concentration the treatment's efficiency increases along with the used H_2O_2 concentration, being registered increasements of the reducing sugars accumulation ~35% higher than in the cases of the variants treated with NaOH of the same concentration. We can conclude from the experiments that the optimal method for treating the wheat straws is represented by the NaOH 4% and H_2O_2 2% variant. The enzymatic hydrolysis of the alkaline and alkaline-peroxidic pretreated wheat straws using the Accellerase 1500 solution for 48 h led at an increased efficiency in comparison with the 24 h hydrolysis. The lignocellulosic biomass has the potential of becoming the key element in the future increasement of the quantity of generated bioenergy. Its universal availability in high quantities as well as the fact that nowadays it is not used on a large scale are the main reasons for which the lignocellulosic biomass is considered to be one of the most promising resources for the future of bioenergy production.

The pretreatments can significantly improve the reducing sugars production and subsequently the bioethanol production and they must be seen as a key for the biochemical conversion of the lignocellulosic materials.

5. References

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