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BIO-FUELS FROM CELLULOSE BY MICROWAVE IRRADIATION

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ABSTRACT: *For increasing cellulose accessibility to the enzymatic attack, the pretreatment is a necessary step to alter some structural characteristics of cellulosic materials. Microwave pre-treatment of sugar beet processing waste were investigated and compared to conventional heating in glucose yield and ethanol production by Simultaneous Saccharification and Fermentation (SSF) and conventional process. The results shows that conventional heating during 8 min has better glucose yield than microwave heating at 500 W during 5 min, respectively 66.03 % (g glucose/g raw material) and 53.15 % at pH 12. Results show also that microwave and conventional heating combined with NaOH solution has better effect cellulose degradation than combined with HCl. Parameters such as microwave intensity and irradiation time have a significant effect on cellulose structure and in this way increase glucose yield. Furthermore results show that higher microwave power with shorter pre-treatment time and the lower microwave power with longer pretreatment time had almost the same effect on glucose yield and also in ethanol production. The highest ethanol yield was obtained with microwave/alkali pre-treatment at 250 W during 10 min (24.04 % g ethanol/g raw material), and do not differ with microwave/alkali 500 W during 5 min (21.94 %). These yields are higher than those obtained with conventional heating, 14.00 % for SSF.*
KEYWORDS: *bioethanol, microwave irradiation, simultaneous saccharification*

INTRODUCTION

The world is facing an energy crisis due to increasing concern related to fossil energy sources, such as environmental impact, climate change, finite availability and security of supply. Another issue is the increasing of the consumption. Today, the transportation sector worldwide is almost entirely dependent on petroleum-based fuels. It is responsible for 60% of the world oil consumption. Around the world, there were about 806 million cars and light trucks on the road in 2007. These numbers are projected to increase to 1.3 billion by 2030 and to over 2 billion vehicles by 2050 (Balat, 2011). This growth will affect the stability of ecosystems and global climate as well as global oil reserves. An alternative fuel must be technically feasible, economically competitive, environmentally acceptable, and readily available must be found (Eggeman et Elander, 2005). Biofuels production is one of solution which can solve these problems.

A key issue for processing of the lignocellulosic raw material for bioethanol production is the degradation of cell-wall polymers to liberate the monosaccharide (Jorgensen et al., 2007). The conversion of cellulose to ethanol includes mainly 3 steps: pre-treatment, saccharification and fermentation. The aim of the pre-treatment is to decrease the recalcitrant structure of cellulose such as cellulose cristallinity to increase enzyme accessibility (El-Zawawy et al., 2011). Pre-treatment greatly affect the efficiency of saccharification and the ethanol production cost as well. Over the years, a number of different methods such as steam explosion (Ballesteros et al., 2004), thermal methods (Liu and Wyman, 2005), acid and alkaline hydrolysis pre-treatment has been developed to enhance the cellulose degradation, remove hemicellulose and lignin and alter the structure of them.

There can be found study focus on the efficiency of Simultaneous Saccharification and Fermentation (SSF) technology and optimize the process parameter for it (Szép et al. 2009). SSF has many advantages over the conventional ethanol fermentation process because of enhanced ethanol yield due to the minimized end-product inhibition (Zhu et al., 2005 Beszedes et al., 2011). Furthermore the overall cost of technology can be reduced by eliminating the need for separated reactor for saccharification and fermentation.

Microwave heating is based on the ability of a particular substance such as a solvent or substrate to absorb microwave energy and effectively convert the electromagnetic energy to heat. Molecules with a dipole moment attempt to align themselves with the oscillating electric field of the microwave irradiation, leading to rotation. In liquid and solid phase, this rotation produces friction which results in an increase of the temperature. It is possible to achieve rapid and uniform heating of relatively thick materials. When microwave are directed towards a material, part of the energy is reflected, part is

transmitted through the surface and of this latter quantity, and part of it is absorbed.Although the use of microwave for cooking is widespread, the application of this technology to the processing materials is relatively new development. As many researchers have already stated in numerous published papers microwave irradiation (usually at the ISM –Industrial Scientific and Medical – frequency of 2.45 GHz) produces efficient internal heating for most chemical reactions, delivering energy exactly where it is needed, even under exothermic conditions. Another valuable advantage of using controlled microwave dielectric heating for chemical synthesis is the dramatic reduction in reaction times: from days and hours to minutes and seconds. These two properties are sufficient motivation to promote the use of microwaves in "greener chemical processes" (Leonelli et Mason, 2010).

The Aim of this study is to examine the effect of MW pre-treatment on the enzymatic hydrolysis of cellulose, to optimize the process parameter of MW pre-treatment and to investigate the efficiency of separated saccharification and fermentation (S&F) and the simultaneous saccharification and fermentation (SSF)

MATERIALS AND METHODS

Raw material. *Sugar beet processing waste was obtained from sugar industry in Serbia. After sugar extraction pulp were pressed and dried to obtained pellets. Then particle was reduces to 0.8 mm.*

Pre-treatments. *In this study two heating methods was compared. A Labotron 500 laboratory microwave with output power 250 W and 500 W was used in our experiments. Pre-treatment was carried out as follows: 5 g of sugar beet powder was first placed in a beaker with 100 mL of distilled water (pH 7), HCl solution (pH 2) or NaOH solution (pH 12). Two beakers were irradiated in the same time with continuous irradiation for 2 to 8 minutes in the microwave oven. Finally the volume was adjusted at 100 mL with distilled water if it's necessary.*

A hot plate was used for conventional heating pre-treatment, the sample was warmed up to 80 °C and this temperature was kept during the pre-treatment time.

Enzymatic hydrolysis. *After pre-treatment, pH was adjusted at 5.5 and two enzymes were added: 0.5 mL cellulose from Trichoderma reesei ATC 26921 (Sigma) and 0.5 mL β-glucosidase from Aspergillus niger (Sigma). Assays were carried out in conical flask at 40 °C on a stirrer plate (100 rpm) during 7 days.*

Samplings were carried out every day to follow glucose concentration. To stop enzyme reaction, samples were frozen and glucose concentration was measure 24 hours later.

Fermentation. *After 5 days saccharification, 600 mL of biomass and 0.6 g of Saccharomyces cerevisiae (LW 128-91, Hefix 100) were added to Minifors fermentor. Fermentations were carried out in non sterile conditions at 35 °C, during 7 days with pH regulation at 4.5 and 120 rpm. Glucose concentration was followed every days and ethanol concentration at 7 days.*

Simultaneous Saccharification and Fermentation. *Simultaneous saccharification and fermentation (SSF) reaction mixture contained 600 mL of pre-treated sugar beet (30 g sample in 600 mL distilled water or NaOH solution pH 12), 6 mL enzyme (3 mL cellulose and 3 mL β-glucosidase) and 0.6 g S. cerevisiae. Same parameters than fermentation were used: 120 rpm, 35 °C and pH at 4.5. SSF were carried out during 7 days.*

ANALYTICAL METHOD

Glucose yield. *The glucose yield was tested by the dinitrosalicylic acid (DNS) assay method. This is based on the color reaction of 3,5-dinitrosalicylic acid and reducing sugar in the alkaline solution and hot water.*

Glucose yield was calculated as follows:

$$Y_G\%(g_{glucose}/g_{raw\ material}) = \frac{[glucose]\times V_{saccharification}}{weigh\ raw\ material\ (5\,g)}\times 100$$

[glucose] : glucose concentration in g.L^{-1} and $V_{saccharification}$ in L.

Ethanol yield. *Ethanol production was measured after 7 days fermentation. 300 mL of biomass were distillated, then ethanol concentration was measured with refractometer Refracto 30PX, Mettler Toledo). Ethanol yield was calculated as follows:*

$$Y_{ethanol}(g_{ethanol}/g_{glucose}) = \frac{[ethanol]\times\frac{V_{distillate}\times 2}{100}\times\rho_{ethanol}}{raw\ material\ wieght\ (30\,g)}$$

where [ethanol] is in % (v/v), $V_{distillate}$ in mL, $\rho_{ethanol}$ in g.mL^{-1}.

RESULTS ANALYSIS

Each experiment was carried out once, and to know the variance one experiment was carried out 3 times: none heating at pH 7. After one week saccharification, glucose yields were calculated and the variance was 0.119. This value was used for statistical analysis, ANOVA one and two factors were carried out (at 5 %) to test the significance of our results.

RESULTS AND DISCUSSION. *Heating effect*

During preliminary experiment, two heating methods were compared: microwave irradiation (MW) and conventional heating (CH). This pre-treatment were carried out at three pH (2, 7 and 12) and compared to a control with none heating (None).

Figure 1 shows the maximum glucose yield obtained after 1 week saccharification for three pH and four pre-treatment.

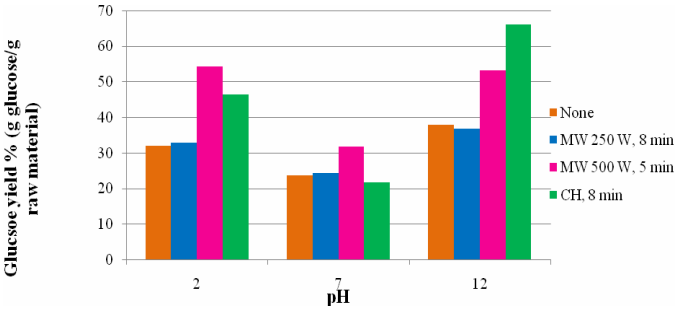

Figure 1 : Heating effect on glucose yield for four pre-treatments

First of all we could see that the highest glucose yield was obtained for microwave 500 W, 5 min and conventional heating 8 min: respectively 54.39 % and 46.53 % at pH 2 and 53.15 % and 66.03 % at pH 12. On the second hand, glucose yield for microwave 250 W, 8 min and the control (None) are not significantly different for each pH. For example at pH 2 glucose yield was 32.83 % and 32.05 % respectively for MW 250 W, 8 min and control. These parameters do not allow better cellulose degradation compared to the control.

In their study, Gabhane et al., (2011) showed that microwave heating could be efficient only for high temperature (> 200 °C). These results are also agreed with Hu et Wen (2008) study's where reducing sugar yield is higher with microwave pretreatment than conventional heating when temperature reached 190 °C. Budarin et al., (2010) shows in their study why microwave start to be efficient for temperature higher than 180 °C. This temperature has been identified as a turning point in the microwave degradation of cellulose. In fact this temperature corresponds to the glass transition in the polymer. Above this temperature, the specific microwave effect becomes pronounced and the rate of degradation of cellulose increases significantly. The enhanced molecular freedom within this region resulting from the glass transition allows improved interaction between the microwave energy and cellulose. Below 180 °C polar molecule in cellulose have less freedom so cannot rotate easily, resulting in poorer interaction.

During conventional heating energy came from the bottom of the sample, on the contrary the use of microwave allows energy diffusion in the entire sample. Polar molecule absorbs this energy and converts this one in heating. This heating allows a change in cellulose structure and increase the enzyme accessibility.

However it's quite difficult to compare these two methods. For conventional heating, temperature is reached at 80 °C during 8 min and before this step temperature increase. It means that the sample is warming for more than 8 min. During microwave pre-treatment two samples were both irradiated at 8 min. We can conclude that microwave can increase glucose yield compare to control, and in certain condition it might be more efficient than conventional heating (short duration time).

pH effect

In this part pH effect was investigated. Figure 2 shows the pH effect on 2 pre-treatments methods: MW 500 W during 5 min and conventional heating during 8 min.

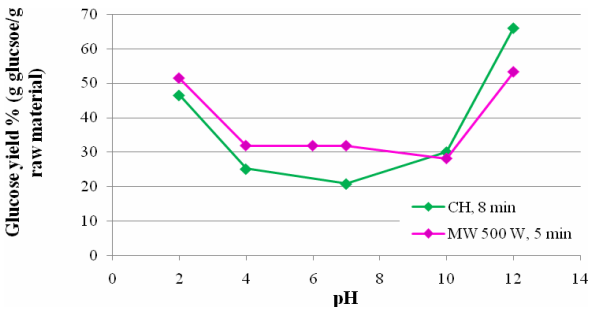

Figure 2 : pH effect on two pre-treatments, conventional heating and microwave 500 W

We have two typical pH curves with the best glucose yield at pH 2 and pH 12, and the minimum for pH 7. The optimum pH seems to be pH 12, with 66.03 % and 53.22 % respectively CH and MW heating. Moreover, these yields are significantly different

with pH 2. Pre-treament in alkaline solutions allows better cellulose degradation than acid solution. For our further experiments, pH 12 is considered as the optimum pH for the pre-treatment.

The lasts years, pre-treatment combined MW and alkaline were studied. This kind of pre-treatment depends on lignin concentration in raw material. Intra-molecular saponification between xylanes from hemicellulose and other compounds such as lignin could destabilize the raw material structure and its porosity. In this way during hydrolysis step, enzyme accessibility will be easier (Sun et Cheng, 2002).

Moreover dilute alkaline solution cause swelling which increase the exchange surface, reduce polymerisation degree and cristallinity. This perturbation causes lignin separation (Alvira et al., 2010).

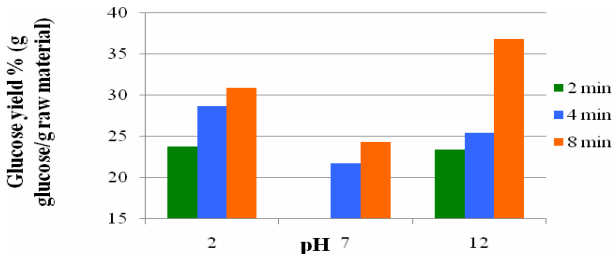

Figure 3 : Irradiation time effect on glucose yield for sample irradiated during 2, 4 and 8 minutes at 250 W.

Irradiation time effect

To observe irradiation time effect, three series of experiments were carried out at different pH. Samples are irradiated à 250 W during 2, 4 and 8 minutes. Figure 3 shows these results.

For each pH an increase of glucose yield can be seen when irradiation increase also. At pH 12 the yield start at 23.37 % with 2 minutes irradiation and then increase to 25.48 % and 36.68 % for 4 and 8 minutes, this increase is significant at 0.05 %. The same tendency is observed at pH 2 and 7.

During microwave irradiation, energy is transmitted to the sample. This energy excites the dipole molecule and causes a temperature increase. When the irradiation time increase collision between molecules also increase, and the hydrogen bond broke because of the alternative electromagnetic field.

However if the duration was too long the hydrogen bonds re-associated because of the large amount of intramolecular heat energy and sugar can be destroy, consequently affecting pretreatment efficiency (Gong et al., 2010).

Microwave intensity effect

In these experiments effect on microwave intensity was investigated. In some studies, it can be read that a short duration microwave irradiation with high intensity has the same effect than a longer duration with a lower power.

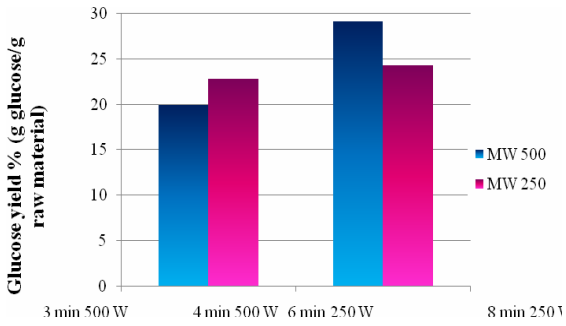

Figure 4 : Power effect on glucose yield

In this experiment two microwave intensity are compared: 250 W and 500 W. First 3 min at 500 W was compared to 6 min at 250 W, and 4 min at 500 W was also compared to 8 min at 250 W. Figure 4 shows the glucose yield after 7 days saccharification.

Even if yields are significantly different, it can be seen that the yield are close. However these results show that microwave power has a significant effect on glucose yield.

Same results are observed by Zhu et al., (2005) and Gong et al., (2010). In this one date shows that the power effect is not linear. In a first part when microwave intensity increase reducing sugar yield increase also, until reaches a maximum (in their study 540 W). After this pick, reducing sugar yield decrease. It is explained that for too high power the increase of the temperature is too quick and oxygen bonds between monomers breaks to create free radicals. In this time, heating activate condensation reaction to form more stable structure.

Bioethanol production

For SSF and S&F parameters with the best glucose yield (according to preliminary experiments) were carried out: pH 7, none heating as control, CH 4 min at pH 12, MW 500 W, 5 min at pH 12 and MW 250 W, 10 min at pH 12. Figure 5 shows glucose concentration evolution during SSF (A) and conventional process (B) process. It can be seen on Figure 5 (A) that during the first day glucose concentration increase because of enzyme hydrolysis and yeast adaptation phase. For the control, we have not the data for t = 1day, the dotted line show approximately glucose concentration we should have, according to other results. The highest glucose concentration was obtained after 1 day for MW 500 W and MW 250

W pre-treatments, respectively 2.41 % and 2.15 %. Then the yeast used the glucose as substrate, resulted in glucose concentration decrease to 0.74 % and 0.35 % for MW 500 W and MW 250 W.

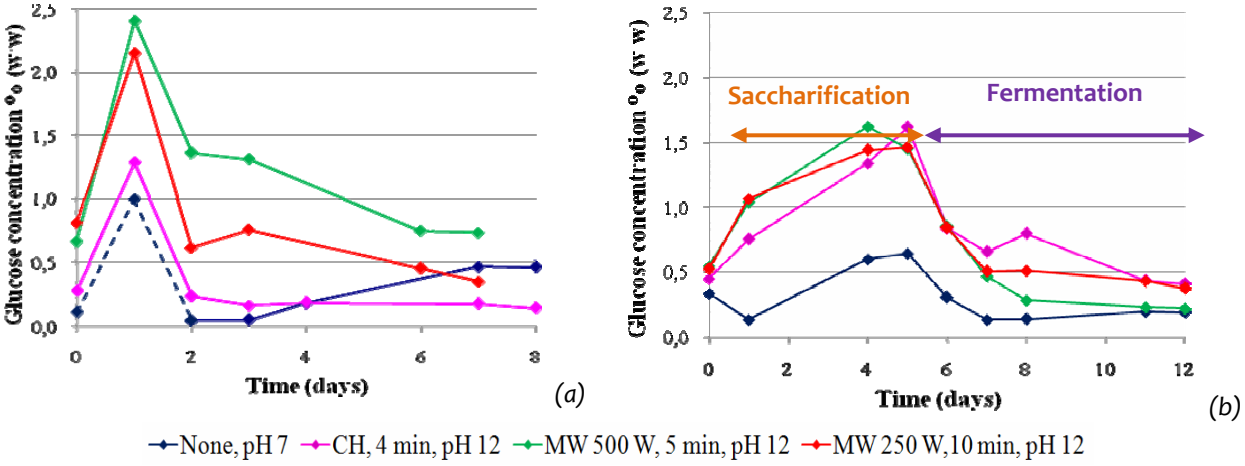

Figure 5 : Glucose concentration evolution during SSF process (A) and conventional process S&F (B) for four pre-treatments

Figure 5 (B) shows glucose concentration evolution when conventional process is carried out: during the five first days, that correspond to saccharification, glucose increase. Contrary to SSF, maximum glucose concentration was obtained for both MW pre-treatments and CH, respectively 1.62 % and 1.44 % for MW 500 W and 250 W, and 1.61 % for CH. Then during fermentation this concentration decrease until less 0.5 % for both pre-treatments.

Figure 6 shows ethanol yield after 7 days SSF and 12 days S&F. This Figure shows ethanol yield after 7 days fermentation for SSF and S&F. It can be seen that there is no significant difference between SSF and S&F for each pre-treatment. Moreover we can see that the best ethanol yield is for MW 250 W, 24.04 % with SSF process. This value is not significant different with MW 500 W, 21.94 %.

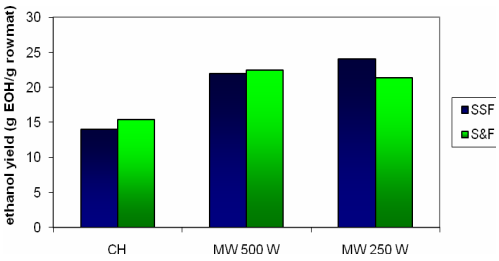

Figure 6: Comparison of ethanol yield for SSF and S&F process on pH12

Even if CH had the best glucose yield in preliminary experiments, ethanol yield is only around 15 % for both SSF and S&F. It shows that glucose concentration is not the only factor which influence ethanol yield. Furthermore Palmqvist et Hahn-Hägerdal (2000) and Ge et al., (2011) reported that during hydrolysis of lignocellulosic material, a wide range of compounds which are inhibitory the microorganism are formed or released. These inhibitors are divided in three groups: weak acids, furan derivates and phenolic compounds.

Even if the yield was not significant different between SSF and conventional process, it can be conclude that SSF is a better process than S&F because of less reaction time, 7 days for SSF, 12 days with S&F.

CONCLUSIONS

In this study the use of microwave to enhance bioethanol from sugar beet processing waste was investigated. Results shows that microwave could greatly enhance glucose yield during enzymatic hydrolysis compared to control. Furthermore it could be seen that pre-treatment with microwave combined to NaOH solution (pH 12) could be more efficient than microwave/acid pre-treatment. Irradiation time and microwave intensity affect glucose yield too and short time irradiation with high microwave intensity has approximated same effect than longer duration time at lower power.

Simultaneous Saccharification and Fermentation was investigated and compared to conventional process. The best ethanol yield was obtained with microwave/alkali pre-treatment at 250 W during 10 min (24.04 %), and do not differ with microwave/alkali 500 W during 5 min (21.94 %). These yields are higher than those obtained with conventional heating, 14.00 % for SSF. Even if ethanol yield was not significant different to conventional process, SSF could be a better process according to reaction time (7 days to 12 days) and cost decrease.

The microwave/alkali pre-treatment could be an efficient method of sugar beet processing waste for its ethanol production.

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