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ENHANCED ENZYMATIC SACCHARIFICATION OF AGRI-FOOD SOLID WASTES BY MICROWAVE PRE-TREATMENT

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ABSTRACT: The second generation bioethanol production has been a focus area of research and development activities worldwide. Bio-wastes generated in the agriculture and food industry can be considered a low priced and reproducible feedstock for biofuel technologies. Enhancing of the efficiency of enzymatic saccharification of lignocellulosic materials is a key issue to make the bioethanol fermentation more cost effective and increase the overall capacity of the process. Microwave (MW) pre-treatment is a novel technology to increase the sugar yield and ethanol production due to the accelerated cellulose hydrolysis. In our work, the effects of MW irradiation with different intensities and combination of them with acid and alkaline pre-treatments were investigated on the efficiency of saccharification of sorghum bagasse and black currant press residues. Our results show, that the MW pre-treatments could significantly increase the amount of hydrolyzed sugar from cellulose and the rate of decomposition was also accelerated. The highest degree of saccharification was observed after combined alkaline-microwave pre-treatment for both the sorghum bagasse and berry press residue, but the positive effect of MW irradiation on cellulose hydrolysis was limited using high intensity irradiation with longer exposure time.
KEYWORDS: bioethanol, lignocellulosic biomass, enzymatic saccharification, microwave pre-treatment

❖ INTRODUCTION

The world is facing an energy crisis due to increasing concern related to fossil use, i.e. environmental impact, climate change, finite availability and security of supply. Two main concepts have been come into the limelight to solve the problem of increased energy demand. One of them is the using of nuclear energy; other is the utilization of renewable energy sources. With the high environmental risk of nuclear wastes and the threat of nuclear power plant accidents has resulted in global tendency to support the development of renewable energy supplying system.

Rapidly rising motor fuel prices and the enhanced needs for them has stressed to increase the biofuel production. Biofuels are liquid or gaseous fuels made from plants and residues such as agricultural crops, municipal wastes and agricultural or forestry by-products. Photosynthetic reactions during biomass growth can remove atmospheric carbon-dioxide as well as producing saccharides and oxygen, which can eliminate the atmospheric CO_2 increment originating from production and utilization of biomass energy.

Fuel ethanol can be produced through fermentation from plants containing sugar, starch or lignocellulose. Global effects of first generation bioethanol produced from materials containing sugar or starch are controversial because of the raised competition between the food and energy sectors for the raw materials [1]. Technologies producing second generation bioethanol use lignocellulosic biomass as feedstock because of its relative abundance, reproducibility and low price. Biofuel production from agri-food wastes can also contribute to make waste management more socially acceptable, sustainable and cost effective [2]. Considering of the 1993/31 Council Directive of the European Parliament the amount of landfilled biodegradable waste shall be reduced to 35% by 2020, as compared to levels of the basis year of 1995, what motivate professionals and the industrial sector to focus on the development of novel and up-to-date technologies according to the waste-to-energy concept [3].

A key issue for processing of the lignocellulosic raw material for bioethanol production is the disruption of complex structure of cell-wall polymers to liberate the monosaccharide [4]. The conversion of cellulose to ethanol includes mainly three steps: pre-treatment, saccharification and fermentation. Pre-treatments 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, thermal methods, acid and alkaline pre-treatment, enzymatic degradation has been developed to enhance the cellulose degradation, remove hemicellulose and lignin or alter the structure of them [5, 6]. There can be found reports dealing with the examination of the efficacy of simultaneous saccharification and fermentation (SSF) and optimize the process parameter for it. SSF has many advantages over the conventional ethanol fermentation process because of enhanced ethanol yield due to the minimized end-product inhibition; furthermore the overall cost of technology can be reduced by eliminating the need for separated reactor for saccharification and yeast fermentation, respectively [7].

Microwave irradiation has been successfully applied in many fields of organic chemistry, biotechnology and environmental engineering [8]. Thermal and a-thermal effects of the microwave (MW) irradiation play role in the “hot-spot” overheating phenomena, and the different dielectric parameter of cell components led to selective heating manifested in the different thermal stress, which contributes in the intensive degradation of cell wall components such as cellulose and pectin [9].

Generally, MW operation can be successfully adopted in materials which have polar compounds such as water, and the acidic or alkaline condition could enhance absorption of energy transferred by MW due to the added ions. MW pre-treatment solely has verified positive effects on cell wall destruction and releasing of organic matter into the soluble phase, but combining of it with addition of chemicals such as alkali, acid and oxidizer agents cause synergetic mechanism to accelerate the decomposition under aerobic and anaerobic condition., as well [10, 11]. MW irradiation under acidic or alkaline conditions can effectively break down the crystalline structure of cellulose. Furthermore it is suitable to solubilize cellulose and hemicelluloses fraction into monomer sugars and oligomers, thus the conversion rate can be improved [12].

Comparison with the conventional alkali pretreatment of wheat straw the microwave-assisted alkali pretreatment required shorter reaction time and lower enzyme loading and, furthermore, higher ethanol yield can be achieved [13]. In the study of Singh et al. [14], the degree of saccharification from rice straw was investigated by using microwave-alkaline pretreatment and it is concluded optimum irradiation time and alkali concentration for combined MW pretreatment of 22.5 minutes and 2.75 %, respectively and the effect of MW power was termed as non-significant. However in earlier study on microwave enhanced enzymatic saccharification without alkaline addition using rice straw as feedstock determined both MW intensity and the irradiation time significant factors with optimum values of 680 W, and 24 minutes, respectively [15].

Moreover, the surface area of MW irradiated cellulose can be more accessible to cellulase enzyme. Fibrous structure of cellulose contains amorphous and crystalline regions alternated. Results of diffuse infrared spectrum reflectance methods verified, that MW irradiation increases the molecular motion in the amorphous region of cellulose and polarize the macromolecules resulting in a reduction of the level of crystallinity with increasing of the decomposition rate [16].

Some study suggests to use MW and thermal pre-treatment with dosage of acid in high concentration and operating at high temperature range (over 100°C) and under high pressure to achieve higher cellulose conversion rate and enhanced ethanol yield. It can be noticed that the high temperature and high pressure aid to increase the degree of cellulose hydrolysis. But, on the other hand, in strong acidic condition at high temperature the glucose released from cellulose may convert further to hydroxymethyl furfural (HMF), what decrease the overall yield of the process operating with enzymatic hydrolysis followed by fermentation, due to the inhibitory effect of phenolic side-products during ethanol fermentation stage [17]. With the optimum pH of enzymatic hydrolysis of cellulose has been determined in a range of 3.5 – 5.5, another advantage of low concentration acid pre-treatment may be that pre-treated biomass can be added directly into the hydrolysis stage without neutralization, additionally the final wastes of operation can be utilized as fertilizer. Therefore, from energetic and efficacy points of view the realistic option for industrial-scale MW pre-treatment for enhanced saccharification is the low temperature operation combined with the diluted acid or alkaline addition. In our work, we investigate the effect of alkali and acid hydrolysis solely and combination of them with conventional heat treatment and microwave irradiation on the enzymatic saccharification and bioethanol production from agri-food solid wastes such as press residues from berry and sweet sorghum processing.

❖ MATERIALS AND METHODS – RAW MATERIALS AND PRE-TREATMENTS

Sweet sorghum bagasse *(Sorghum bicolor)* used in our experiments came from harvesting after juice extraction. A press residue was cut to obtain an average particle size of 3 mm. Black currant press residue originated from juice processing. Sweet sorghum bagasse and black currant press cake has an average total solid (TS) content of 68.1 w/w% and 38.1 w/w%, respectively. In order to avoid decomposition before saccharification the samples were stored in a sealed plastic bag at -18°C.

Microwave (MW) pre-treatments were carried out in a special designed cavity resonator equipped with magnetron operating at a frequency of 2450 MHz. With adjusting of heating voltage by a toroidal-core transformer the power of magnetron was changed continuously in the range of 50W to 900W. The intensity of MW pretreatments was given in Wg^{-1} units, related to the quantity of irradiated samples. For the conventional heat (CH) treatments, a laboratory heater block (Medline CM 307, UK) was used with automatic temperature control. The heating time was 60 minutes at a temperature of 90°C. To make comparable the experimental results the volume of heated samples was 100 mL with TS concentration of 25±0.1 w/w % in all tests. To adjust pH 1 M HCl solution and 1M NaOH solution was added, respectively.

❖ MATERIALS AND METHODS – ENZYMATIC SACCHARIFICATION AND ANALYTICAL METHOD

For enzymatic hydrolysis cellulase from *Trichoderma reesei* (Sigma) and cellobiase from *Aspergillus niger* (Sigma) was applied dosed in a concentration of 20 □Lg_{TS}^{-1}. Hydrolysis tests were conducted in Minifors laboratory fermentor (Infors HT, Switzerland), by using volume of 500 mL with adjusted TS concentration of 5.0±0.1 w/w %. The temperature and pH for enzymatic hydrolysis were controlled at 30±0.2°C and 5.0±0.1, respectively.

To examine the enzymatic hydrolysis the samples taken from fermentation broth were centrifuged and the supernatants were used to analyze the quantity of released reducing sugars. Reducing sugar concentration were estimated by using of 3,5-dinitrosalicylic acid (DNS) photometric method, with glucose as standard [18].

❖ RESULTS AND DISCUSSION

Firstly, the time-depending cellulose hydrolysis to monomer sugar from sweet sorghum bagasse was investigated. In Figure 1 the produced glucose content related to TS basis was indicated after acidic and alkaline pretreatment and combination of them with conventional heating (CH).

Results show, that both acidic (at a pH of 2) and alkaline (pH 12) pre-treatments increased the total amount of sugar produced in followed enzymatic hydrolysis and, additionally, the rate of hydrolysis was also accelerated. Saturation behavior of curves related to sugar producing versus time indicated that the maximum of saccharification can be achievable until the seventh day of enzymatic hydrolysis. The highest concentration of produced glucose can be observed after the alkaline pre-treatment in combination with heating at 90°C for 60 minutes.

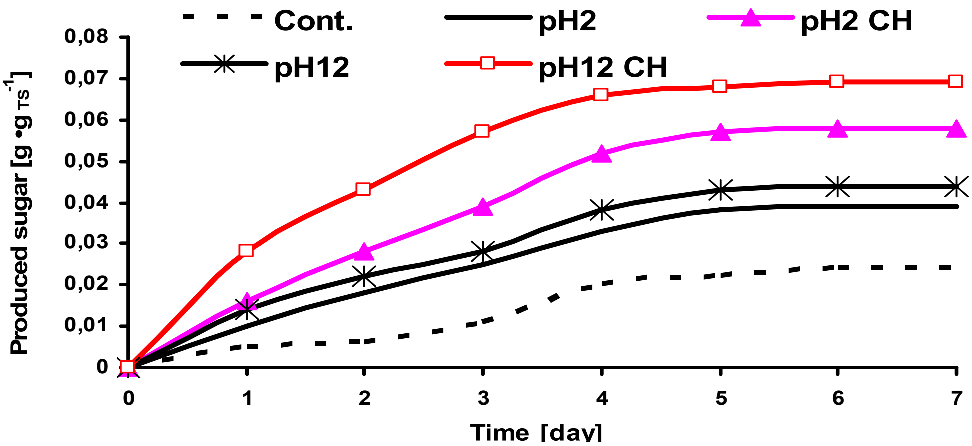

Figure 1. Produced sugar from sweet sorghum bagasse during enzymatic hydrolysis after acidic (pH2) alkaline (pH12) pre-treatment and combination of them with conventional heating (CH)

Since we focused on the determination of the maximum degree of cellulose hydrolysis, the condition of combined heat treatment (heating rate, temperature, duration time) was not further examined and optimized.

In comparison with the conventional heating the positive effect of MW pre-treatment could be manifested in higher sugar yield from sorghum bagasse. The synergetic effect of MW irradiation with alkaline pre-treatment for lignocellulosic raw materials was also concluded in the study of Sing et al. [13] and Cheng et al.[11]. In our experiments, the most effective procedure was the alkaline - MW pre-treatment at 2 Wg^{-1} power level. It can be noticed, that significant difference was not observed in the quantity of produced sugar after MW pre-treatments for 10 minutes at 0.5 and 2 Wg^{-1}, respectively. However, the rate of cellulose hydrolysis was higher after more intensive irradiation than of that after irradiation at 0.5 Wg^{-1} (Figure 2.).

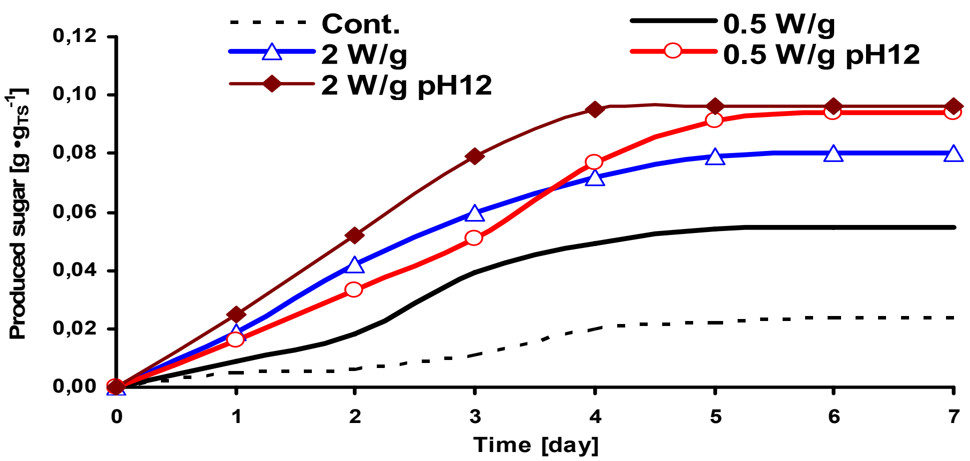

Figure 2. Produced sugar from enzymatic saccharification of sweet sorghum bagasse after MW pre-treatment solely and under alkaline condition
(irradiation time was 10 minutes for all MW pre-treatments)

The results of enzymatic cellulose hydrolysis for black currant press-residues were similar to those of obtained from the saccharification of sweet sorghum bagasse (data not shown in details).
The effect of MW pre-treatment on the hydrolysis rate of cellulose depends on the intensity of irradiation and the duration time, as well. The strong destructive effect of MW pre-treatment can increase the amount of liberated sugar from the cell wall, but in the presence of amino acids at a high temperature the Maillard reaction can also take place in biosolids what contribute to the decrease of reducing sugar content. In order to examine the effect of MW pre-treatments with different intensities (in a range of 0.5-2 Wg^{-1}) and irradiation time (in a range of 5-20 minutes) the total amount of produced sugar from sweet sorghum bagasse and black currant press-residues were also determined.

Total sugar yield from the hydrolysis of sorghum bagasse and black currant press-residue after MW pre-treatment with different intensities and irradiation time in acidic and alkaline suspension are shown in Figure 3 and Figure 4 , respectively. MW pre-treatment solely resulted in an increment of 72-260% for sugar yield from sweet sorghum bagasse, but in the case of berry press residue the MW irradiation without chemical addition did not affect significantly the efficiency of saccharification. Moreover, the acidified condition for cellulose hydrolysis of bagasse led to a slightly increment in the amount of produced sugar.

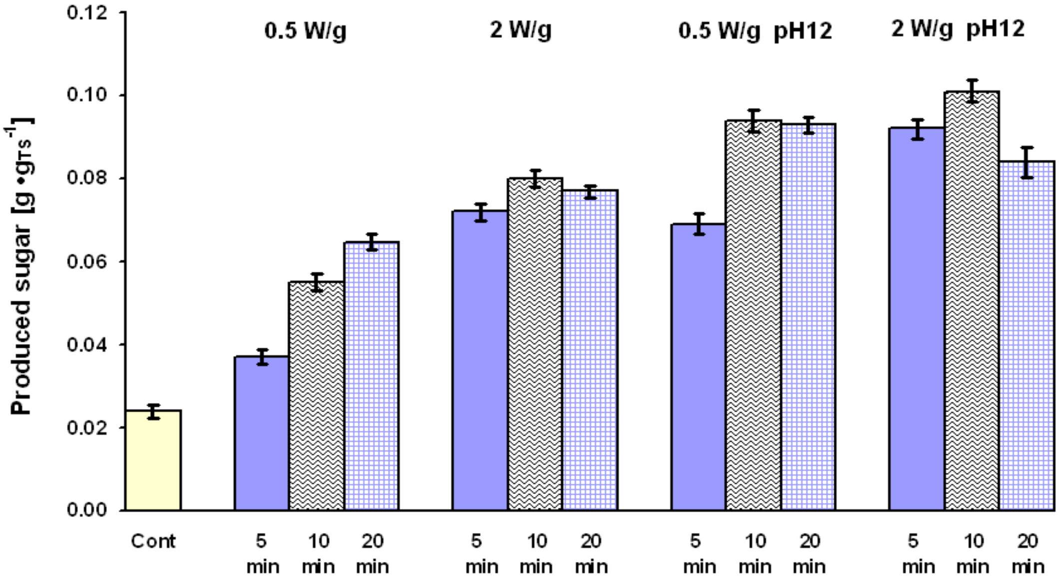

Figure 3. Quantity of total sugar produced in enzymatic hydrolysis of sweet sorghum bagasse after different pre-treatment methods

Significant worsening effect on the reducing sugar content was found at 2 Wg^{-1} MW power level by applying longer than 10 minutes irradiation by both the sorghum bagasse and black currant press residue under an alkaline condition. Under acidified condition the negative effect of high intensity MW irradiation for longer duration time was just observed by the black currant press-cake hydrolysis.

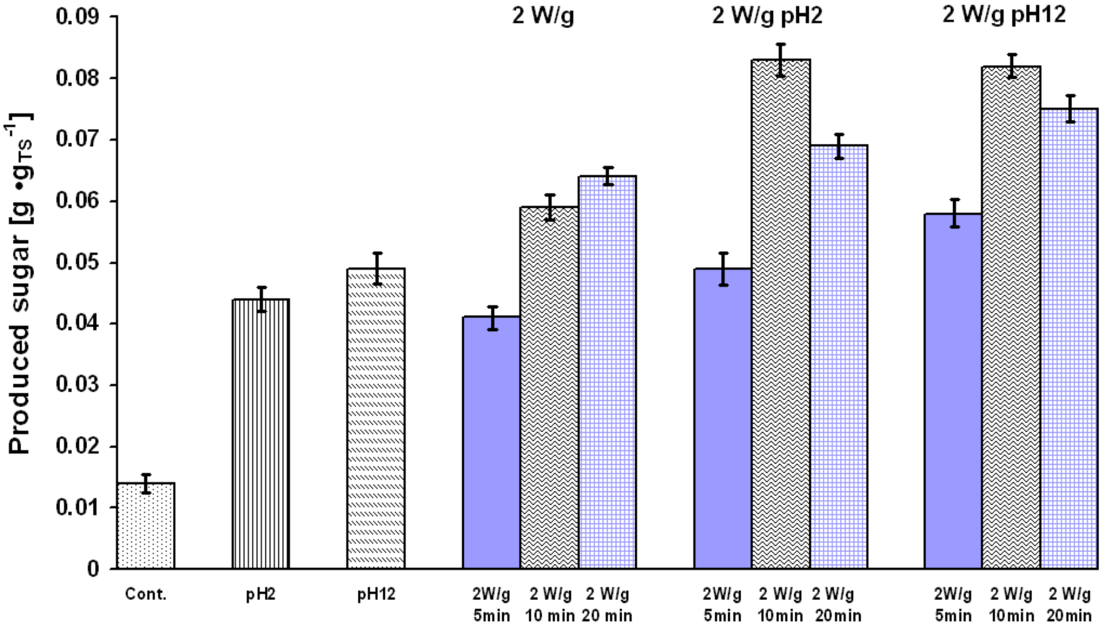

Figure 4. Quantity of total sugar produced in enzymatic hydrolysis of black currant press-cake after different pre-treatment methods

A higher degree of saccharification can be achieved by using sorghum bagasse feed, and in this case the difference between the amount of sugar produced from untretated and MW pre-treated was higher than that of observed from berry residues. The lower degree of hydrolysis from black currant press-cake was limited by the relatively high amount of the seed fractions (28 w/w% on TS basis). However the acidic condition can enhance the efficiency of the degradation of seed structure and therefore significant difference was not observed in the maximum achievable sugar product from alkaline and acidic pre-treated berry press residue (Figure 4.).

❖ CONCLUSIONS

In our work, the efficiency of cellulose saccharification from sweet sorghum bagasse and black currant press residue were investigated using alkaline and acidic pre-treatment in combination with conventional heat treatment and microwave irradiation. Our experimental data show that MW pre-treatment is suitable to increase the total amount of sugar produced in the enzymatic degradation process and additionally the rate of saccharification was also accelerated. By applying of well designed MW pre-treatment the increment in sugar produced during cellulose hydrolysis from berry press residues and sorghum bagasse was higher than 500% and 350%, respectively. With higher intensity MW irradiation the process time can be shortened, but mainly the acidic condition and high temperature generated at high power levels contribute to decrease of the reducing sugar concentration due to the effects of Maillard-reaction.

❖ ACKNOWLEDGEMENT

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