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Chemical Industry & Chemical Engineering Quarterly 14 (4) 231–234 (2008) CI&CEQ

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SHORT COMMUNICATION

UDC 537-962:663.142/.143:547.262

A MICROWAVE-ASSISTED LIQUEFACTION AS A PRETREATMENT FOR THE BIOETHANOL PRODUCTION BY THE SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF CORN MEAL

A microwave-assisted liquefaction as a pretreatment for the bioethanol production by the simultaneous saccharification and fermentation (SSF) of corn meal using Saccharomyces cerevisiae var. ellipsoideus yeast in a batch system was studied. An optimal power of microwaves of 80 W and the 5-min duration of the microwave treatment were selected by following the concentration of glucose released from the corn meal suspensions at hidromodul of 1:3 (corn meal to water ratio) in the liquefaction step. The results indicated that the microwave pretreatment could increase the maximum ethanol concentration produced in the SSF process for 13.4 %. Consequently, a significant increase of the ethanol productivity on substrate ($Y_{P/S}$), as well as the volumetric ethanol productivity (P) in this process, could be achieved.

Key words: bioethanol; microwaves; simultaneous saccharification and fermentation; Saccharomyces cerevisiae var. ellipsoideus; corn meal.

Bioethanol produced from renewable biomass, such as sugar, starch, or lignocellulosic materials, is one of the alternative energy resources that is both renewable and environmentally friendly. Today, bioethanol is world's main and one of the most promising biofuels [1]. The production of bioethanol from renewable agricultural residues has become a priority in European Union, with the decision to replace up to 20 % of classic fuel by ethanol until the year of 2020 [2,3]. Significant scientific and technological investments will be needed to achieve this objective. Currently, approximately 80 % of total world ethanol production is obtained from the bioprocess which is the fermentation of simple sugars by yeast [4]. In Serbia, one of the most suitable and available agricultural raw material for the industrial bioethanol production is corn. Corn starch cannot be metabolized directly by yeast, but must first be broken down into simple hexose sugars. This is usually performed by enzymatic liquefaction and saccharification, which produces a relatively clean glucose stream that is then fermented to ethanol by yeasts. A novel and economically more favorable process for the bioethanol production is a simultaneous saccharification and fermentation (SSF) which is currently attracting a lot of attention primarily due to a lower energy consumption, a decreased substrate inhibition of yeast and reduced process time [5,6].

Some previous studies have shown that the microwave heating influences the process of swelling and gelatinization of starch granules and thus could be very efficient in destroying the starch crystalline arrangement and obtaining a soft gel [7,8]. These phenolmena could also enhance the enzyme susceptibility needed for efficient hydrolysis, which may later on improve the outcome of the fermentation. In this study, we investigated the microwave-assisted liquefaction as a pretreatment for the bioethanol production by SSF of corn meal with *Saccharomyces cerevisiae* var. *ellipsoideus*; the kinetics of the SSF process, as well as bioethanol yield, and the productivity were assessed.

EXPERIMENTAL

Materials

Corn meal obtained by a dry milling process was a product of the corn processing factory (RJ Corn

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Paper received: October 7, 2008.
Paper revised: October 23, 2008.
Paper accepted: October 26, 2008.

Product, Sremska Mitrovica, Serbia). The corn meal consisted of particles with the diameter 0.2-1.7 mm (95 % or more particles pass through a 1.70 mm sieve). The content of the main components in the corn meal, determined by chemical analysis was the following (w/w): starch 76.75 %, proteins 6.35 %, lipids 4.50 %, fibers 1.36 %, ash 0.70 % and water 10.34 %. Termamyl SC, a heat-stable α-amylase from *Bacillus licheniformis* (enzyme activity was 133 KNU/g) was used for the corn meal liquefaction and SAN Extra L, *Aspergillus niger* glucoamylase (activity of 437 AGU/g) was used for the corn meal saccharification. The enzymes were a gift from Novozymes, Denmark. *Saccharomyces cerevisiae* var. *ellipsoideus* was used for the fermentation of hydrolyzed corn meal. The culture originated from the collection of the Department of Biochemical Engineering and Biotechnology, Faculty of Technology and Metallurgy, Belgrade (BIB-TMFB), and was maintained on a malt agar slant. The agar slant consisted of malt extract (3 g/L), yeast extract (3 g/L), peptone (5 g/L), agar (20 g/L) and distilled water (up to 1 L). Before used as an inoculum for the fermentation, the culture was aerobically propagated in 500 ml flasks in a shaking bath at 30 °C for 48 h and then separated by centrifugation. The liquid media consisted of yeast extract (3 g/L), peptone (3.5 g/L), KH_2PO_4 (2.0 g/L), $MgSO_4 \cdot 7H_2O$ (1.0 g/L), $(NH_4)_2SO_4$ (1.0 g/L), glucose (10 g/L) and distilled water.

Hydrolysis and fermentation

A 100 g of corn meal was mixed with water at the weight ratio (hidromodul) 1:3, and 60 ppm of Ca^{2+} (as $CaCl_2$) ions was added. The liquefaction was carried out in flasks in a thermostated water bath at 85 °C and pH of 6.0 for up to 1 h by adding 26 μL of enzyme Termamyl SC per 100 g of starch. The liquefaction and SSF process were performed in flasks in a thermostated water bath at 30 °C with shaking (100 rpm) with 2 % (v/v) of inoculum of *Saccharomyces cerevisiae* var. *ellipsoideus* as described by Mojović *et al.* [5].

Microwave treatment

Samples of the mixture of corn meal and water at the weight ratio (hidromodul) 1:3 placed in glass flasks were subjected to the microwave treatment in a microwave oven (Sanyo, EM-S9515W) just after the addition of liquefying enyzme. Parameters such as heat power (from 20 to 240 W) and the duration of heating between 1-10 min were investigated. After the microwave pretreatment, the glass flasks were kept in a thermostated water bath at 85 °C for up to 1 h and then subjected to SSF.

Analytical methods

During the corn meal hydrolysis and fermentation, the content of reducing sugars, calculated as glucose, was determined by 3,5-dinitrosalicylic acid [9]. A standard curve was drawn by measuring the absorbance of known concentrations of glucose solutions at 570 nm. The ethanol concentration was determined based on the density of alcohol distillate at 20 °C and expressed in weight % (w/w) [10]. The indirect counting method, *i.e.*, a pour plate technique was used to determine the number of viable cells. Serial dilutions of the samples were performed, and after the incubation time at 30 °C, the colonies grown in Petri dishes were used to count the number of viable cells, and expressed as colony forming units (CFU). At least three measurements were made for each condition and the data given were averages.

RESULTS AND DISCUSSION

The effect of the microwave treatment on the liquefaction of corn starch

The samples of corn meal and water at the weight ratio (hidromodul) 1:3 were subjected to the microwave treatment immediately after the addition of the enzyme Termamyl SC. Figure 1 presents the effect of microwave power on glucose concentration obtained after the liquefaction of samples. As shown in Figure 1, the increase of the power over 80 W did not cause the further increase of the liquefaction meas-

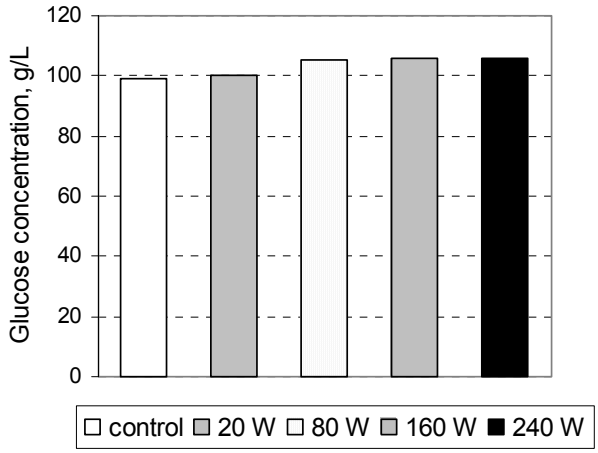

Figure 1. The effect of microwave power on the glucose concentration obtained after the liquefaction of samples of corn meal suspension (hidromodul 1:3). The microwave treatment was performed within 4 min just after the addition of the liquefying enzyme. Temperatures measured in the samples at the end of the microwave treatment were: 68 (20 W), 96 (80 W), 105 (160 W) and 122 °C (240 W). Experimental conditions for liquefaction: 26 μL of Termamyl SC per 100 g of starch at hidromodul 1:3; thermostated water bath at 85 °C up to 1 h; pH 6.0, 100 rpm.

ured by the concentration of released glucose. For this reason this microwave power was selected as the optimal one for further experiments. In this case the increase of glucose concentration for 6.4 % over untreated control sample was observed.

Figure 2 presents the influence of the duration of the microwave treatment (80 W) on the concentration of glucose achieved after the liquefaction of the samples of corn meal suspension. The treatment during 5 min was selected for further experiments since during that time maximal glucose concentrations were attained. Similarly, relatively short duration of the microwave treatment was also selected by other investigators as appropriate for swelling and gelatinization of starch granules and for destroying the starch crystalline arrangement [7,8,11].

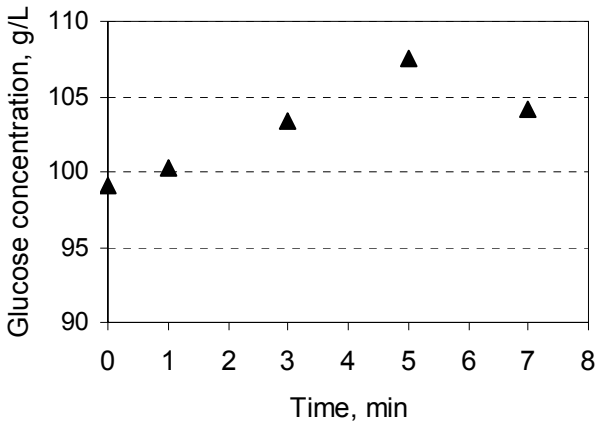

Figure 2. The effect of time of the microwave treatment on the glucose concentration obtained after the liquefaction of samples of corn meal suspensions (hidromodul 1:3). The microwave treatment was performed just after the addition of the liquefying enzyme with the power of 80 W. Other experimental conditions for liquefaction were as in Fig. 1.

SSF fermentation of corn meal after the microwave pretreatment

Figures 3A and 3B show ethanol, glucose and viable yeast biomass profiles during the SSF fermentation of corn meal by *Saccharomyces cerevisiae* var. *ellipsoideus* after the microwave pretreatment. Maximum ethanol concentration of 9.91 % (w/w) was achieved after 44 h of the fermentation. After that, a decline of the ethanol concentration could be noticed because of the exhaustion of the released glucose and the transition of the yeast metabolism towards utilization of ethanol as a carbon source. This phenomenon can also be seen in Figure 3B which shows a number of viable cells during the SSF fermentation of corn meal by *S. cerevisiae* var. *ellipsoideus*. For these reasons, the SSF process should be stopped after 44 h of the proceeding time. The advantages of the SSF process compared to the conventional separate hydrolysis and fermentation (SHF) process are a lower substrate inhibition because the fermenting organism consumes the released sugars in accordance with their production in the process of hydrolysis. In addition, the process is energy efficient (since the saccharification is performed at lower temperature, *e.g.* at the temperature of fermentation) and ends at least 4 h before the conventional one (4 h is required for saccharification when carried out separately). These advantages are also noticed and reported previously by us [5] or by other researchers [12-14].

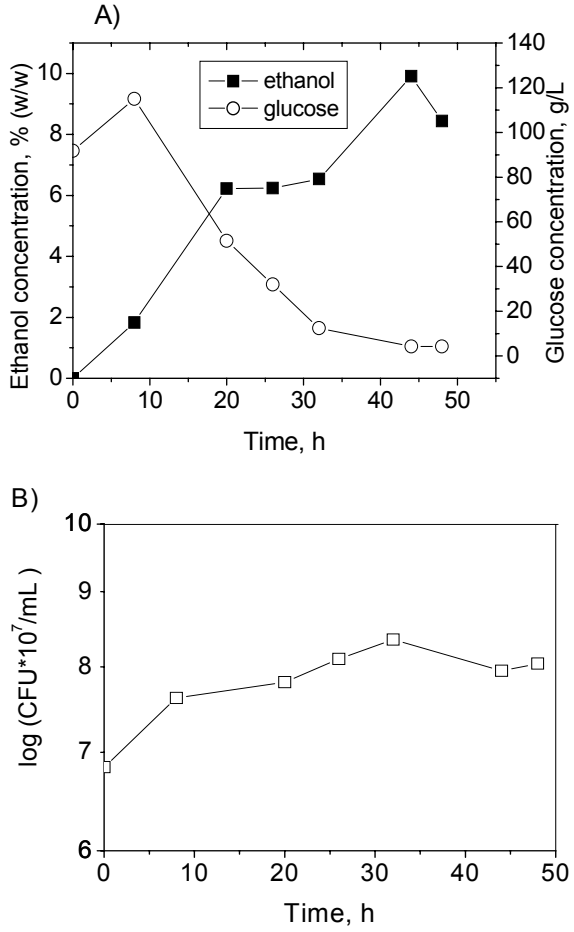

Figure 3. A) The ethanol and glucose concentration during the SSF process of corn meal by S. cerevisiae var. ellipsoideus after the microwave pretreatment. B) The number of viable cells during the SSF process of corn meal by S. cerevisiae var. ellipsoideus after the microwave pretreatment. The microwave treatment of 80 W was performed for 5 min just after the addition of the liquefying enzyme (sample temperature reached 96 °C at the end of the treatment). The process conditions: liquefaction with 26 μL of Termamyl SC per 100 g of starch at hidromodul 1:3 in a thermostated water bath at 85 °C for up to 1 h; pH 6.0, 100 rpm; SSF process with 156 μL of SAN Extra L per 100 g of starch at 30 °C, pH 5.0, 100 rpm.

The comparison of the significant process parameters achieved in the batch SSF of corn meal by *S. cerevisiae* var. *ellipsoideus* with and without a prior microwave treatment is presented in Table 1. The results indicate that the microwave pretreatment can increase the maximum ethanol concentration (for 13.4 %) and consequently significantly increase the ethanol productivity on the substrate ($Y_{P/S}$) as well as the volumetric ethanol productivity (P) in the SSF of corn meal by *S. cerevisiae* var. *ellipsoideus.* This improvement could be attributed primarily to the acceleration of the starch hydrolysis and the enhanced release of fermentable sugars [7,8,11,13,15].

Table 1. The comparison of the significant process parameters achieved in the batch SSF process of corn meal by S. cerevisiae var. ellipsoideus with and without a prior microwave treatment

SSF[a] process of corn meal	Maximum % (w/w) of ethanol	Theoretical yield[b] of ethanol, %	$Y_{P/S}$ g/g	P g/l h
With pretreatment	9.91	92.27	0.52	2.25
Without pretreatment (control)	8.74	81.38	0.46	1.98

[a]Process conditions are the same as in Figure 3; [b]the yields were calculated based on starch content: 76.75% (w/w)

CONCLUSIONS

A microwave-assisted liquefaction as a pretreatment for the bioethanol production by the simultaneous saccharification and fermentation (SSF) of corn meal using *Saccharomyces cerevisiae* var. *ellipsoideus* yeast in a batch system was studied in this paper. An optimal power of microwaves of 80 W and the duration of the treatment of 5 min (sample temperature reached 96 °C at the end of the treatment) were selected according to the concentration of glucose released from the corn meal suspensions (hidromodul 1:3) in the liquefaction step. By applying this microwave pretreatment, maximum ethanol concentration of 9.91 % was attained in the SSF process after 44 h. When compared to the control without pretreatment, the ethanol concentration was increased for 13.4 %. Consequently, the ethanol productivity on the substrate ($Y_{P/S}$) as well as the volumetric ethanol productivity (P) in the SSF of corn meal by *S. cerevisiae* var. *ellipsoideus* was significantly increased. This improvement is primarily attributed to the acceleration of the starch hydrolysis and the enhanced release of fermentable sugars

Acknowledgements

This work was funded by the Serbian Ministry of Science and Technological Development (TR 18002).

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