

Improvement of Bioethanol Production from Corn by Ultrasound and Microwave Pretreatments

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Bioethanol production by simultaneous saccharification and fermentation (SSF) of corn meal by *Saccharomyces cerevisiae* var. *ellipsoideus* yeast in a batch system with prior ultrasound or microwave treatment was studied. The SSF process kinetics were assessed and determined. Based on the obtained results, both pretreatments improved ethanol production during the SSF process. The ultrasound and microwave pretreatments increased the maximum ethanol concentration produced in the SSF process for 11.15 % and 13.40 % (compared to the control sample), respectively. The application of microwave pretreatment resulted in higher increase in ethanol concentration compared to the ultrasound pretreatment.

1. Introduction

Today, world faces the complex economical and ecological problems concerning progressive energy consumption. This has led to a major interest in expanding the use of bioenergy and development of alternative energy resources, such as bioethanol, that are both renewable and environmentally friendly (Balat et al., 2008; Kim and Dale, 2005). In Serbia, one of the most suitable and available agricultural raw material for industrial bioethanol production is corn, since the average annual corn yield is approximately 40% higher than the calculated domestic needs in last few years (Mojović et al., 2009). The current world bioethanol research is driven by the need to reduce the costs of production by improvement in feedstock pretreatment, shortening of fermentation time, lowering the enzyme dosages and improving the overall starch hydrolysis. Some previous studies (Khanal et al., 2007; Palav and Seetharaman, 2007) have shown that application of ultrasound or microwave pretreatment may significantly increase the conversion of starch materials to glucose as well as overall ethanol yield. Besides the pretreatment, the process mode used for saccharification and fermentation, such as SSF process, is also an important factor affecting the production costs and improving ethanol productivity (Mojović et al., 2006; Nikolić et al., 2009; Öhgren et al., 2007).

The aim of this study was to investigate the possibilities of improving ethanol productivity by applying an ultrasound and microwave pretreatment in the bioethanol

production by simultaneous saccharification and fermentation of corn meal with *Saccharomyces cerevisiae* var. *ellipsoideus* yeast in a batch system. Additionally, changes in physical properties of corn meal suspensions before and after the ultrasound or microwave pretreatment were examined at microscopic level by SEM studies.

2. Materials and Methods

2.1 Starch

Corn meal obtained by dry milling process was a product of corn processing factory RJ Corn Product, Sremska Mitrovica, Serbia. The corn meal consisted of particles with 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 was the following: starch 76.75 % (w/w), proteins 6.35 % (w/w), lipids 4.50 % (w/w), fibers 1.36%, ash 0.70% (w/w) and water 10.34 % (w/w), as determined by chemical analysis in our previous study (Nikolić et al., 2008).

2.2 Enzymes and microorganisms

Termamyl SC, a heat-stable α -amylase from *Bacillus licheniformis* was used for corn meal liquefaction. The enzyme activity was 133 KNU/g (KNU - kilo novo unit). SAN Extra L, *Aspergillus niger* glucoamylase, activity 437 AGU/g, (AGU- amyloglucosidase unit), was used for corn meal saccharification. The enzymes were gift from Novozymes, Denmark. *Saccharomyces cerevisiae* var. *ellipsoideus* was used for the fermentation of hydrolyzed corn meal. The culture originated from the collection of Department of Biochemical Engineering and Biotechnology, Faculty of Technology and Metallurgy, Belgrade, 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 use 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), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1.0 g/L), $(\text{NH}_4)_2\text{SO}_4$ (1.0 g/L), glucose (10 g/L) and distilled water.

2.3 Ultrasound and microwave pretreatment

Samples of the mixture of corn meal and water at the weight ratio (hidromodul) 1:3 placed in glass flasks were exposed to ultrasound or microwave pretreatment before the addition of liquefying enzyme Termamyl SC. The ultrasound pretreatment was carried out in a sonicator (Model: USK 28, power 600 W, EI Niš, Serbia) at sonication time of 5 min, temperature of 60 °C and frequency of 40 kHz. The microwave pretreatment was performed in a microwave oven (Model: R-677, Sharp Electronics, UK) at microwave power of 80 W and time of 5 min. The control samples were not subjected to the pretreatment.

2.4 Liquefaction and SSF experiments

The amount of 100 g of corn meal was mixed with water at the weight ratio (hidromodul) 1:3. Then, 60 ppm of Ca^{2+} (as CaCl_2) ions were added. The liquefaction was carried out at 85 °C and pH of 6.0 for 1 h by adding 0.026% (v of enzyme/w of starch) enzyme Termamyl SC. The liquefaction and SSF process were performed in flasks in a thermostated water bath with shaking (100 rpm), as described by Mojović et

al. (2006). The liquefied mash was cooled, pH was adjusted to 5.0 using 2 M HCl, and KH_2PO_4 (4.0 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.4 g/L) and $(\text{NH}_4)_2\text{SO}_4$ (2.0 g/L) were added. The SSF process was initiated by adding 0.156% (v of enzyme/w of starch) enzyme SAN Extra L and 2% (v/v) of inoculum of *S. cerevisiae* var. *ellipsoideus* to the liquefied mash, and carried out up to 48 h at 30 °C. Initial viable cell number was $\sim 10^6$ CFU/ml. It was considered that the pasteurization of the substrate achieved during the enzymatic liquefaction (85 °C for 1 h) was sufficient thermal treatment, and thus no additional sterilization prior to SSF process was performed.

2.5 Scanning Electron Microscopy (SEM)

The surface structure of the control (without ultrasound and microwave pretreatment) and ultrasound and microwave pretreated samples of corn meal suspensions were observed by scanning electron microscopy (SEM). A thin layer of the sample was mounted on the copper sample-holder, using a double sided carbon tape and coated with gold of 10 nm thicknesses to make the samples conductive. The SEM studies were carried out using a scanning electron microscope (JSM5800, JEOL, Tokyo, Japan) at acceleration voltage of 20 kV.

2.6 Analytical methods

During the liquefaction and SSF process, the content of reducing sugars, calculated as glucose, was determined by 3,5-dinitrosalicylic acid (Miller, 1959). 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 the alcohol distillate at 20 °C and expressed in % (w/w) (Official Methods of Analysis, 2000). At least three measurements were made for each condition and the data given represents the average values of the measurements.

3. Results and Discussion

3.1 Scanning Electron Microscopy Examinations

The changes in physical structure of control (without pretreatment) and ultrasound or microwave pretreated samples of corn meal suspensions, before and after liquefaction, were imaged by SEM, as presented in Figure 1. The ultrasound and microwave pretreatments of the presented samples were performed under optimal conditions (ultrasound: 60 °C, 40 kHz, 5 min; microwaves: 96 °C, 80 W, 5 min) determined in our previous studies (Nikolić et al., 2008; Nikolić et al., 2010).

SEM images in Figure 1 (a), (b) and (c) show that both ultrasound and microwaves affected the decomposition of the starch granules even before the liquefaction started. As shown in Figure 1 (d), (e) and (f), after liquefaction the sizes of starch granules were smaller in the pretreated samples than in the control sample because the ultrasound and microwaves stimulated starch granules degradation and glucose release, but microwaves in higher level. In contrast, conventional heating observed in the control sample caused less change in structure.

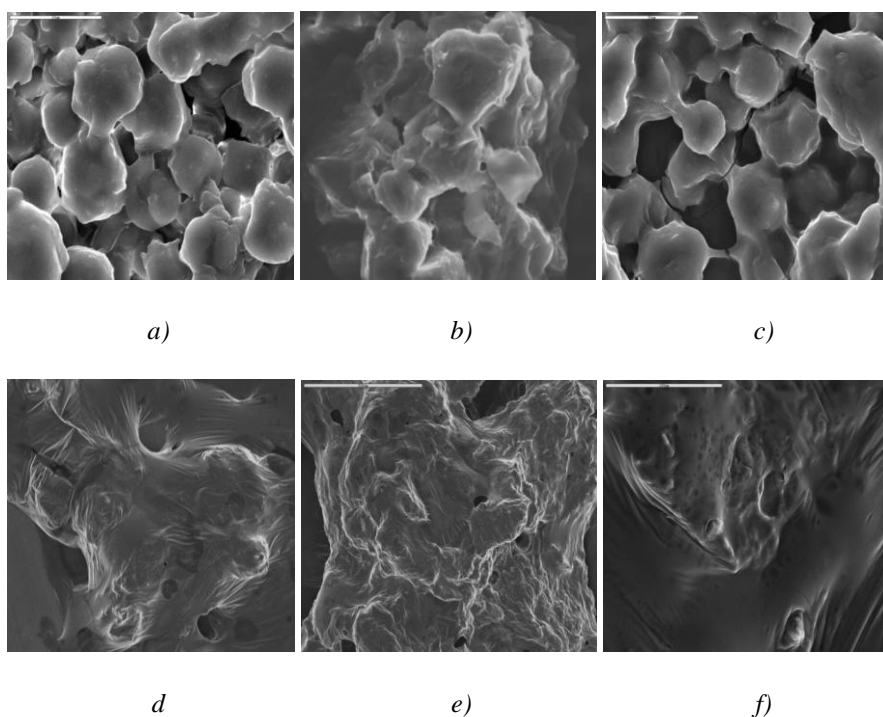


Figure 1: SEM images of samples of corn meal suspensions: (a) control sample (without pretreatment) before liquefaction, (b) ultrasound pretreated sample before liquefaction, (c) microwave pretreated sample before liquefaction, (d) control sample after liquefaction, (e) ultrasound pretreated sample after liquefaction, (f) microwave pretreated sample after liquefaction. The length of the scale bar is equivalent to 20 microns in Figures 1 a, b and c (magnification 2000 \times), and 500 microns in Figures 1 d, e and f (magnification 100 \times).

3.2 SSF of liquefied corn meal after the ultrasound and microwave pretreatments

Figure 2 presents the time course of ethanol production and glucose consumption in the SSF process of liquefied corn meal suspension by *S. cerevisiae* var. *ellipsoideus*, with and without the ultrasound or microwave pretreatment. The pretreatments were performed under optimal conditions determined in our previous studies (Nikolić et al., 2008; Nikolić et al., 2010).

As shown in Figure 2, the ultrasound and microwave pretreatments increased the maximum ethanol concentration by 11.15 and 13.40 % (compared to the control sample), respectively, after 32 h of SSF process. The application of microwave pretreatment resulted in higher increase in ethanol concentrations compared to the ultrasound pretreatment. This was probably due to the fact that the temperature of 96 °C reached during microwave treatment at a power of 80 W was higher compared to the optimal sonication temperature of 60 °C, and also the mechanism of microwave action on destroying the starch crystalline arrangement was probably different compared to the action of ultrasound. The maximum ethanol concentration of 9.87 % (w/w), ethanol

yield of 0.52 g/g, percentage of theoretical ethanol yield of 90.80 % and volumetric productivity of 3.08 g/(l·h) were achieved after 32 h of the SSF process on corn meal with prior microwave treatment. As shown in Figure 2, the glucose consumption was in accordance with the results of ethanol concentration since the glucose was consumed as a carbon source by the yeast. At the end of the SSF process, in the microwave pretreated sample the glucose concentration was 0.88 g/L indicating the end of fermentation.

The improvement in ethanol production obtained in pretreated samples could be attributed primarily to the effect of ultrasound and microwaves on the disintegration of corn starch granules, the acceleration of the starch hydrolysis, the enhanced release of fermentable sugars and thereby the increased ethanol productivity (Khanal et al., 2007; Huang et al., 2007). Future work is needed to scale-up system designs to large batch or continuous processes in order to fully realize the potential benefits of ultrasound or microwave pretreatment. However, it should be noted that a critical assessment of the costs and benefit analysis are needed because the initial capital investment and operation cost of the pretreatment are not cheap.

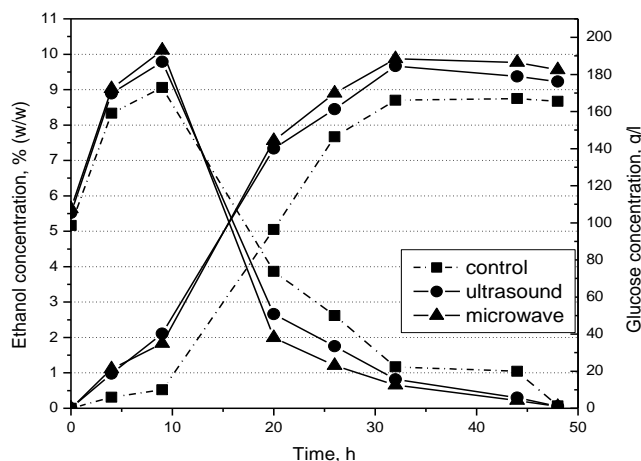


Figure 2: Time course of ethanol production and glucose consumption in the SSF process of corn meal hydrolyzates by *S. cerevisiae* var. *ellipsoideus* with and without the ultrasound and microwave pretreatments. Solid lines – pretreated sample, dashed lines – control sample.

4. Conclusions

The present investigation shows that ultrasound and microwave pretreatments increase the maximum ethanol concentration obtained in the SSF process for 11.15 and 13.40 % (compared to the control sample), respectively. The maximum ethanol concentration of 9.87 % (w/w) and the percentage of theoretical ethanol yield of 90.80 % were achieved after 32 h of the SSF of corn meal with prior microwave treatment. SEM images showed that ultrasound and microwaves stimulated disruption of corn starch structure either before or after liquefaction step, and thereby enhanced glucose concentration and

consequently ethanol productivity in the SSF process. It is needed to scale-up system designs to large batch or continuous processes in order to fully realize the potential benefits of ultrasound pretreatment, which is a part of our further research.

Acknowledgements

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

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