ISSN 1330-9862
(FTB-2032)

preliminary communication

Improvement of Ethanol Fermentation of Corn Semolina Hydrolyzates with Immobilized Yeast by Medium Supplementation

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Received: January 14, 2008
Accepted: May 7, 2008

Summary

The possibilities of improving ethanol fermentation of enzymatically obtained corn semolina hydrolyzates with alginate-immobilized yeast *Saccharomyces cerevisiae* var. *ellipsoideus* by medium supplementation with mineral salts as sources of magnesium, zinc, calcium and copper ions, and vitamins (pantothenate, thiamine, pyridoxine, biotin and inositol), separately or as combined mixtures, have been investigated. Among all tested minerals, alone or combined, the most efficient in improving ethanol productivity during fermentation of corn semolina hydrolyzates was a mixture of magnesium and zinc salts: $MgSO_4$ (2 g/L) and $ZnSO_4$ (0.3 g/L). Positive effects were also obtained with the addition of copper ions ($CuCl_2$, 1 mg/L) or calcium ions ($CaCl_2$, 40 mg/L). Among vitamins, the most effective was Ca-pantothenate (1 g/L), which caused an increase in the fermentation efficiency for approx. 8 %, compared to the control sample. Based on these results, an effective mixture of vitamins and minerals consisting of $MgSO_4$ (2 g/L), $ZnSO_4$ (0.3 g/L), $CuCl_2$ (1 mg/L), Ca-pantothenate (1 g/L) and inositol (1 g/L) was arranged for the supplementation of the medium based on corn semolina hydrolyzates. The supplementation with this mixture provided an increase of the fermentation efficiency for 20 % compared to the control sample, without supplementation.

Key words: bioethanol, fermentation, *Saccharomyces cerevisiae* var. *ellipsoideus*, alginate-immobilized yeast

Introduction

Bioethanol produced from renewable biomass, such as sugar, starch, or lignocellulosic materials, is one of the alternative energy resources, which is both renewable and environmentally friendly. Today, bioethanol is one of the main and most promising biofuels (*1,2*). Present world bioethanol production is around 50 million m^3 per year and it is constantly expanding with a possibility to reach 120 million m^3 per year until 2025 (*3,4*). Many countries have established the national agenda to employ bioethanol as an alternative fuel (*5,6*). The production of bioethanol from renewable agricultural residues has become a priority in the European Union, with the decision to replace up to 20 % of classic fuel by ethanol within the next 15 years (*7,8*). Significant scientific and technological investments will be needed to achieve this objective.

The priority in future ethanol production is put on lignocellulosic processing, which is considered as one of the most promising second-generation biofuel technologies (*9*). However, utilization of lignocellulosic material

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for fuel ethanol is still under improvement. Sugar-based (molasses, sugar cane, sugar beet) and starch-based (corn, wheat, potato, rice, *etc.*) feedstocks are currently predominant at the industrial level and they are still economically favourable compared to lignocelluloses. Currently, approx. 80 % of total world ethanol production is obtained from the fermentation of simple sugars by yeast (*10*). In Serbia, one of the most suitable and available agricultural raw material for industrial bioethanol production is corn. Corn starch cannot be metabolized directly by yeast, but it first has to 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.

The yeast *Saccharomyces cerevisiae* still remains the major industrial ethanol producer (*11*). Efficient ethanol production requires a rapid fermentation leading to high ethanol concentrations; therefore, a yeast strain must have a good specific growth rate and specific ethanol production rate at high osmotic pressure and ethanol concentration. This goal is not easy to achieve since many parameters during batch fermentation can cause the decrease of the specific rate of yeast growth, and inhibition can be caused either by product or substrate. Many strategies have been explored to overcome the substrate and product inhibition and to improve the ethanol tolerance of yeasts. Among them, the most explored are immobilization of yeasts in/on adequate matrices such as calcium alginate, κ-carragenan gel, polyacrylamide, γ-alumina (*12*,*13*), wooden chips (*14*), PVA gel (*15*), orange peel (*16*), *etc.* This approach is often combined with the choice of an appropriate process mode, such as fed-batch, continuous or semi-continuous fermentation (*17*) and/or with the manipulation of the yeast metabolism either by medium composition (*18–22*), or by means of genetic engineering (*23–25*). Novel and economically more favourable processes for bioethanol production on starchy and lignocellulosic feedstock with simultaneous saccharification and fermentation (SSF) are currently attracting a lot of attention (*26*,*27*).

In this paper, ethanol fermentation of corn semolina hydrolyzates by immobilized *Saccharomyces cerevisiae* var. *elipsoideus* has been studied. This yeast was found to be the most superior for the fermentation of corn semolina hydrolyzates among four yeast strains tested. Previously, it had been demonstrated that higher ethanol productivity could be achieved in the fermentation of corn semolina hydrolyzates by immobilized yeast, which was mostly due to higher ethanol tolerance obtained (*28*). This paper aims to improve the fermentation of corn semolina hydrolyzates with immobilized yeast by medium supplementation. The supplementation was performed by the addition of various minerals and vitamins, separately or in combination.

Materials and Methods

Starch

Corn semolina obtained by dry milling process was a product of corn processing factory (RJ Corn Product, Sremska Mitrovica, Serbia). The corn semolina consisted of 95 % or more particles that pass through a 1.70-mm sieve; 45 % or more particles that pass through a 0.71-mm sieve; 35 % or less of particles that pass through a 0.212-mm sieve. The mass fraction of the main components of the corn semolina, determined by chemical analysis, was the following (in %): starch 73.75, proteins 9.35, lipids 5.86, ash 0.70, and water 10.34.

Enzymes and microorganisms

Termamyl® SC, a heat-stable α-amylase from *Bacillus licheniformis*, was used for the liquefaction of corn semolina starch. The enzyme activity was 133 of KNU/g (KNU, Kilo Novo Units of α-amylases, *i.e.* the amount of enzyme that breaks down 5.26 g of starch per hour according to Novozyme's standard method for the determination of α-amylase). SAN Extra® L, *Aspergillus niger* glucoamylase, with the activity of 437 AGU/g (AGU, amyloglucosidase unit is the amount of enzyme that hydrolyzes 1 μM of maltose per minute under specified conditions) was used for saccharification of corn semolina starch. The enzymes were a gift from Novozyme, Denmark.

Saccharomyces cerevisiae var. *ellipsoideus* was used for the fermentation of hydrolyzed corn starch. The culture originated from the collection of BIB-TMF, 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 propagated aerobically in 500-mL flasks in a shaking bath at 30 °C for 48 h as described by Mojović *et al.* (*26*), and then separated by centrifugation.

Immobilization and cultivation procedure

The yeast cells were immobilized in Ca-alginate using an electrostatic drop generation method. The 2 % (by mass) Na-alginate solution was prepared by dissolving 4.8 g of sodium alginate powder (medium viscosity, Sigma-Aldrich, USA) into 240 mL of distilled water. Polymer/cell suspension was formed by mixing 240 mL of Na-alginate solution with 60 mL of thick yeast suspension at room temperature. Spherical microbeads were formed by extrusion of Na-alginate/yeast cell suspension through a blunt stainless steel needle using a syringe pump (Pump 11, Harvard Apparatus, USA) with a 20-mL plastic syringe and an electrostatic droplet generator (Nisco Encapsulator, Nisco Engineering AG, Switzerland). The cell suspension was forced out of the tip of the needle at constant flow rate (0.25 mL/min), and droplets were formed by the action of electrostatic and gravitational forces. Electrostatic potential was formed by connecting the positive electrode of a high voltage DC unit to the gelling bath, which was 2.65 % (by volume per mass) $CaCl_2$ solution. In this way, the yeast cells were entrapped in a gel matrix of Ca-alginate. The immobilized particles were rather uniform in size with the mean diameter of 0.6 mm. After gelling, the microbeads were placed in double distilled water to remove the unreacted material. Until use, the microbeads with cells were stored in a physiological solution at 4 °C.

Hydrolysis of corn starch

A mass of 100 g of corn semolina was mixed with water at the mass ratio of 1:3, and the mixture was then

treated with enzymes in two steps, liquefaction and saccharification. The liquefaction was carried out at 85 °C and at pH=6.0 for 1 h by the addition of 0.026 % (by volume per mass of starch) of enzyme Termamyl® SC just before the heating of the suspension up to 85 °C. After that, the liquefied mash was saccharified at 55 °C and at pH=5.0 for 4 h with 0.156 % (by volume per mass of starch) of enzyme SAN Extra® L. The hydrolysis was performed in flasks in a thermostated water bath with shaking (150 rpm), as described previously (*26*).

Ethanol fermentation of corn semolina hydrolyzates

Starch hydrolyzates obtained by the two-step hydrolysis of the corn semolina were subjected to ethanol fermentation by yeasts under anaerobic conditions (pH=5.0, temperature 30 °C, mixing rate 100 rpm). Fermentation was performed in flasks in thermostated water bath with shaking. 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 fermentation was performed. The mash obtained after hydrolysis with initial glucose concentration of around 150 g/L was fermented by immobilized yeast cells for a period of up to 48 h. Initial viable cell number of approx. $2.5 \cdot 10^7$ CFU/mL was provided by the addition of 5 % (by volume) of immobilized yeast in the fermentation mixture. During the fermentation, the consumption of the substrate, viable number of cells as well as the formation of ethanol were followed.

The effect of the addition of the following vitamins and minerals, alone or in combination, to corn starch hydrolyzates was tested: thiamine (5 mg/L), pyridoxine (5 mg/L), inositol (1 g/L), Ca-panthotenate (2 g/L), $CaCl_2$ (40–120 mg/L), $CuCl_2$ (1–3 mg/L), $MgSO_4$ (2 g/L), $ZnSO_4$ (0.3 g/L). All vitamins and minerals used were of analytical grade. Samples with and without (control) the addition of supplements were tested simultaneously under the same experimental conditions in order to make comparisons. Fermentation efficiency (percentage by mass) was calculated as follows:

$$\text{Fermentation efficiency} = (\text{Experimental mass of ethanol} / \text{Theoretical mass of ethanol based on starch content}) \cdot 100 \quad /1/$$

Analytical methods

During the fermentation of hydrolyzed semolina, the content of reducing sugars, calculated as glucose, was determined by 3,5-dinitrosalicylic acid (DNS) method (*29*). A volume of 3 mL of DNS was mixed with a volume of 3 mL of the sample solution in a lightly capped test tube and heated at 90 °C for 7 min. After that, the colour was stabilized by the addition of 1 mL of a 40 % potassium sodium tartrate solution and the tubes were cooled to room temperature in a cold water bath. Then, the absorbances were measured at 570 nm by Ultrospec 3300 spectrophotometer (Amersham Biosciences, Sweden). A standard curve was drawn by measuring the absorbance of known concentrations of glucose solutions. The ethanol concentration was determined based on the density of alcohol distillate at 20 °C and expressed in percentage by mass. Direct counting method, *i.e.* spread plate technique was used to determine the number of viable cells, in which a few serial dilutions were made before spreading. After the preset incubation time at 30 °C, 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 average values.

Results and Discussion

Effect of the addition of minerals

The effect of supplementation of the medium with magnesium, zinc, calcium and copper ions on ethanol fermentation of corn semolina hydrolyzates by immobilized *S. cerevisiae* var. *ellipsoideus* was studied. Comparative results of ethanol mass fraction after 20 and 48 h of fermentation are presented in Table 1, while Fig. 1 shows the efficiency of ethanol fermentation obtained in the samples after 48 h.

Table 1. The effect of medium supplementation with minerals on the mass fraction of ethanol during fermentation of corn semolina hydrolyzates by alginate-immobilized *S. cerevisiae* var. *ellipsoideus*

Minerals	*w*(ethanol)/%	
	20 h	48 h
Control – immobilized cells without medium supplementation	4.48±0.09	8.01±0.16
$\gamma(CuCl_2)$/(1 mg/L)	4.67±0.09	8.32±0.15
$\gamma(CuCl_2)$/(2 mg/L)	4.30±0.10	7.58±0.15
$\gamma(CuCl_2)$/(3 mg/L)	4.12±0.11	7.26±0.16
$\gamma(CaCl_2)$/(40 mg/L)	4.73±0.12	8.36±0.15
$\gamma(CaCl_2)$/(80 mg/L)	4.68±0.11	8.25±0.18
$\gamma(MgSO_4)$/(2 g/L)+$\gamma(ZnSO_4)$/(0.3 g/L)	4.79±0.10	8.41±0.17

Process conditions: pH=5.0, 30 °C, mixing rate 100 rpm, initial glucose concentration ~150 g/L, initial viable cell number ~$2.5 \cdot 10^7$ CFU/mL provided by 5 % (by volume) immobilized particles. Presented data are averages of at least three measurements

Results presented in Table 1 suggest the importance of mineral supplementation to the fermentation medium necessary to activate *S. cerevisiae* var. *ellipsoideus* metabolism. Generally, the addition of all investigated minerals (copper, calcium, magnesium and zinc) contributed to the achievement of higher ethanol concentrations, improved sugar consumption and higher cell densities in alginate matrix (data not presented) when compared to the fermentation with immobilized cells without the addition of minerals. Recently, we have demonstrated that immobilized *S. cerevisiae* var. *ellipsoideus* yeast was superior to the free yeast regarding higher initial substrate concentrations. This was attributed to the protection of cells from the toxic effect of ethanol by alginate particles and thus preservation of yeast viability (*28*). The benefit of utilizing immobilized system for fermentation of corn hydrolyzates was apparent at glucose concentration of 175 g/L

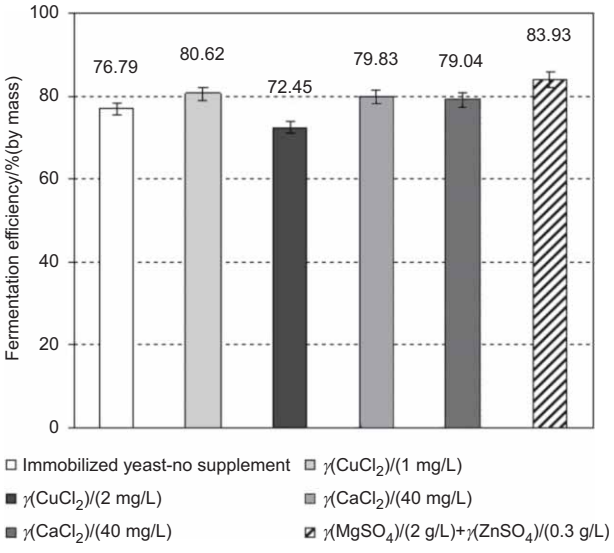

Fig. 1. Fermentation efficiency of corn semolina hydrolyzates by immobilized *S. cerevisiae* var. *ellipsoideus* with the addition of various minerals. Samples were analyzed after 48 h of fermentation. Process conditions as in Table 1

and higher (*28*). In addition, further convenience of yeast-immobilized system for continuous ethanol fermentation was documented by Wendhausen *et al.* (*30*) and Prasad and Mishra (*31*).

Effect of various minerals on ethanol fermentation by yeast has been studied extensively and still remains an issue of interest (*19*,*20*,*32*,*33*). It is known that mineral salts take part in yeast metabolism as the activators of enzymes or are part of the enzyme in their active centre. Among all tested minerals, alone or combined (Table 1), the most efficient in improving the ethanol productivity in fermentation of corn semolina hydrolyzates was a combination of magnesium and zinc ($MgSO_4$ 2 g/L and $ZnSO_4$ 0.3 g/L) (Fig. 1). It is also reported that magnesium ions are of extreme importance in the amelioration of harmful effects of ethanol toxicity and temperature shock in *S. cerevisiae* strain (*20*). Both heat and ethanol stress can cause disruption of cellular ionic homeostasis, leading to a reduction of metabolic activity and eventually cell death. However, magnesium ions decrease the proton and, in particular, anion permeability of the plasmalemma by interacting with membrane phospholipids, resulting in the stabilization of the membrane bilayer (*33*). Based on these findings, it is clear that the addition of magnesium is of crucial importance in fermentations with high substrate and product concentration. Similarly, the addition of zinc (*34*,*35*) is also of great importance in yeast metabolism, particularly in the fermentations of zinc-deficient substrates.

Our study has demonstrated (Table 1) that a very precise consideration should be taken regarding the supplementation of the media with copper ions. Copper ions enhanced ethanol yield and the yeast proliferation at the concentration of 1 mg/L. Furthermore, with additional increase of the concentration of copper ions (to 2 and 3 mg/L), the beneficial effect on the mechanical stability of the alginate-immobilized particles was observed, which is in agreement with the observations of Kurosawa *et al.* (*36*). On the other hand, a detrimental effect of copper ions on fermentation efficiency was detected. This detrimental effect is attributed to toxicity of higher concentrations of copper ions. Similar effect of copper ions on fermentation by yeast had previously been detected by Pejin and Razmovski (*32*). As they reported, the toxic effect was more pronounced on cell viability and proliferation than on anaerobic glucose utilization. Despite the need for the precise determination of the concentration of copper ions, supplementation with copper ions could be specifically beneficial for the fermentation of starch-based hydrolyzates in simultaneous saccharification and fermentation (SSF) process due to the fact that copper can activate the starch hydrolyzing enzymes, *e.g.* α-amylase (*37*), leading to higher efficiency of the overall SSF process. Besides copper ions, which are reported as the most efficient α-amylase activators, calcium and magnesium ions are also recognized as α-amylase activators (*37*), and thus could enhance the efficiency of the SSF on starch-based feedstocks.

According to the results presented in Table 1 and Fig. 1, the addition of calcium ions in the fermentation of corn semolina hydrolyzates by immobilized *S. cerevisiae* var. *ellipsoideus* yeast is recommended for several reasons. The main reason is demonstrated improvement of ethanol concentration and fermentation efficiency. Similar beneficial effects are achieved with the addition of 40 and 80 mg/L of calcium ions, thus the recommended dose is the lower one. In addition, calcium ions have positive effect on the stabilization of alginate particles, as well as on the activity of starch hydrolyzing enzymes, *e.g.* α-amylase.

Effect of the addition of vitamins

The effect of supplementation of the medium with inositol (1 g/L), mixture of thiamine (5 mg/L), pyridoxine (5 mg/L) and biotin (10 μg/L), and Ca-pantothenate (1 g/L) on ethanol fermentation of the hydrolyzates of corn semolina by immobilized *S. cerevisiae* var. *ellipsoideus* was studied. Comparative results of ethanol concentration after 20 and 48 h of fermentation are presented in Table 2, while Fig. 2 shows the efficiency of alcohol fermentation obtained in the samples after 48 h.

The highest ethanol fermentation efficiency on corn semolina hydrolyzates by immobilized *S. cerevisiae* var. *ellipsoideus* of 82.08 % was obtained by the addition of

Table 2. The effect of medium supplementation with vitamins on ethanol mass fraction during fermentation of corn semolina hydrolyzates by alginate-immobilized *S. cerevisiae* var. *ellipsoideus*

Vitamins	*w*(ethanol)/%	
	20 h	48 h
Control – immobilized cells without medium supplementation	4.48±0.09	8.01±0.16
γ(inositol)/(1 g/L)	4.70±0.10	8.46±0.16
γ(thiamine)/(5 mg/L)+γ(pyridoxine)/(5 mg/L)+γ(biotin)/(10 μg/L)	4.61±0.12	8.25±0.15
γ(Ca-pantothenate)/(1 g/L)	4.81±0.11	8.56±0.18

Process conditions as in Table 1

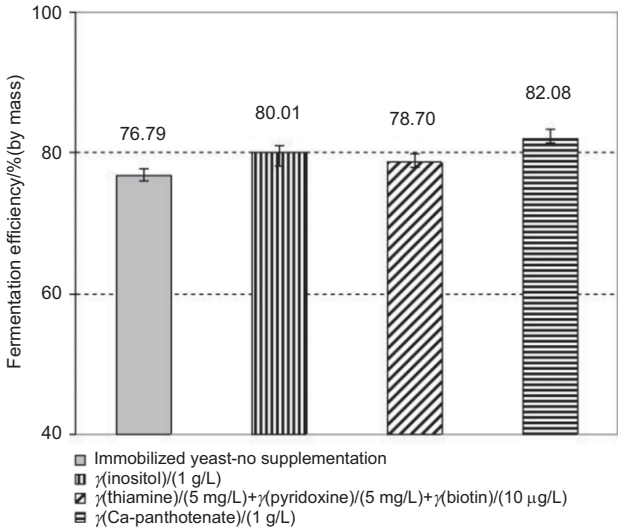

Fig. 2. Fermentation efficiency of corn semolina hydrolyzates by immobilized *S. cerevisiae* var. *ellipsoideus* with the addition of various vitamins. Samples were analyzed after 48 h of fermentation. Process conditions as in Table 1

Ca-panthotenate (1 g/L). The results demonstrated that the Ca-pantothenate caused an increase in the fermentation efficiency for approx. 8 %, compared to the control sample (from 76.79 to 82.08 %). The effect of Ca-pantothenate was stronger than the effect of the mixture of vitamins B (thiamine, pyridoxine and biotin), which increased fermentation efficiency for approx. 2 % (from 76.79 to 78.70 %). It has been reported that pantothenic acid increases the yeast tolerance of ethanol since it stimulates synthesis of lipids and thus reduces leakage of cell membranes of yeasts (*38*). Similarly, protective and positive effects of ethanol on yeast viability were ascribed to other vitamins of B group and particularly to biotin (*18,22*). The variations in the resulting quantitative effects of these vitamins on ethanol yield and fermentation efficiency could be a consequence of using different medium compositions, substrates and process conditions. Chemical analysis of vitamins present in corn semolina revealed the presence of particular amounts of vitamins such as thiamine, riboflavin, niacin, folate, and biotin. However, the contents are significantly reduced when compared to whole maize prior to milling process (*39*).

The results presented in Table 2 indicate a significance of inositol for ethanol fermentation of corn semolina hydrolyzate. By the addition of 1 g/L of inositol, fermentation efficiency was improved for approx. 4 %. Positive effect of inositol in ethanol production on synthetic glucose media by a high ethanol producing *Saccharomyces* sp. was reported by Chi *et al*. (*40*). It was shown that inositol positively affects cell viability and ethanol tolerance. This was attributed to the role of inositol in the synthesis of sterols, especially ergosterol (*41*) and other membrane components such as phospholipids (*40*).

Addition of minerals and vitamins

Based on the obtained results, in further fermentations the supplementation was performed with the following combination of minerals and vitamins: $MgSO_4$ 2 g/L, $ZnSO_4$ 0.3 g/L, $CuCl_2$ 1 mg/L, Ca-pantothenate 1 g/L and inositol 1 g/L. Process parameters such as concentration of ethanol, fermentation efficiency, yield of product on substrate ($Y_{P/S}$/(g ethanol/g starch)) and productivity of fermentation [P/(g/(L·h))] were assessed for alginate-immobilized system without medium supplementation and alginate-immobilized system supplemented with the mixture of vitamins and minerals. The results are presented in Table 3. The kinetics of fermentation in terms of ethanol production and sugar utilization of corn semolina hydrolyzates by immobilized *S. cerevisiae* var. *ellipsoideus* with and without the addition of the mixture of vitamins and minerals is presented in Fig. 3. Viable cell count during the fermentation can be seen in Fig. 4.

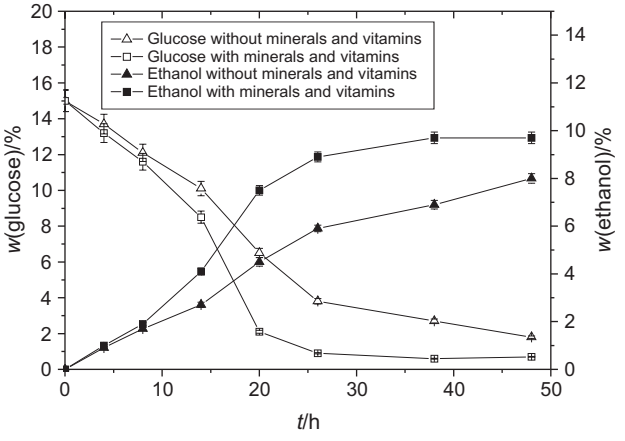

Fig. 3. Kinetics of ethanol production and glucose consumption during fermentation of corn semolina hydrolyzates by immobilized *S. cerevisiae* var. *ellipsoideus* with and without the addition of a mixture of vitamins and minerals. Process conditions as in Table 1. Mixture of vitamins and minerals consisted of: $MgSO_4$ 2 g/L, $ZnSO_4$ 0.3 g/L, $CuCl_2$ 1 mg/L, Ca-pantothenate 1 g/L and inositol 1 g/L

Table 3. The effect of medium supplementation with minerals and vitamins on ethanol fermentation of corn semolina hydrolyzates by alginate-immobilized *S. cerevisiae* var. *ellipsoideus*

Fermentation	w(ethanol) / %	Theoretical yield / %	$Y_{P/S}$ / g ethanol/g starch	P / g/(L·h)
Immobilized cells without medium supplementation	8.01±0.16	76.79±1.54	0.43±0.009	1.67±0.03
Immobilized cells with the addition of γ($MgSO_4$)/(2 g/L)+γ($ZnSO_4$)/(0.3 g/L)+ γ($CuCl_2$)/(1 mg/L)+γ(Ca-pantothenate)/(1 g/L)+ γ(inositol)/(1 g/L)	9.67±0.17	92.35±1.62	0.52±0.009	2.01±0.04

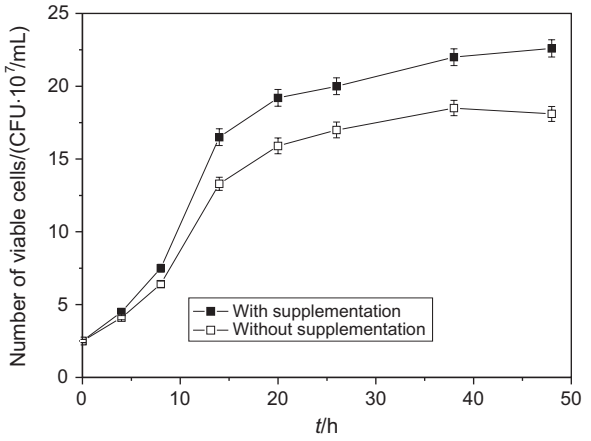

Fig. 4. Number of viable cells (CFU) during fermentation of corn semolina hydrolyzates by immobilized *S. cerevisiae* var. *ellipsoideus* with and without the addition of a mixture of vitamins and minerals. Process conditions as in Table 1. Mixture of vitamins and minerals consisted of: $MgSO_4$ 2 g/L, $ZnSO_4$ 0.3 g/L, $CuCl_2$ 1 mg/L, Ca-pantothenate 1 g/L and inositol 1 g/L

By medium supplementation with the chosen mixture of vitamins and minerals, ethanol concentration of 9.67 % was achieved, which represents 92.35 % of the theoretical ethanol yield on starch used as a substrate. This represents the increase in the fermentation efficiency for approx. 20 % (from 76.79 to 92.35 %) when compared with the control sample. The supplementation caused an increase of $Y_{P/S}$ of the immobilized system from 0.43 to 0.52 g ethanol/g starch, while process productivity of immobilized system increased from 1.67 to 2.01 g/(L·h).

As shown in Fig. 3, the supplementation of corn semolina hydrolyzates with the selected mixture of vitamins and minerals enhanced the dynamics of yeast growth and ethanol fermentation, as well as the glucose consumption. These effects were attributed primarily to increased viability of the alginate-immobilized yeast obtained in the supplemented medium (Fig. 4).

Conclusions

In conclusion, among all tested minerals, alone or combined, the most efficient in improving ethanol productivity in the fermentation of corn semolina hydrolyzates by alginate-immobilized *Saccharomyces cerevisiae* var. *ellipsoideus* yeast was a combination of magnesium and zinc ($MgSO_4$ 2 g/L and $ZnSO_4$ 0.3 g/L). Positive effects were also obtained with the addition of copper ions ($CuCl_2$ 1 mg/L) and calcium ions ($CaCl_2$ 40 mg/L). Among the tested vitamins, the most effective was Ca-pantothenate (1 g/L), which caused an increase in the fermentation efficiency for approx. 8 %, compared to the control sample. However, the mixture of vitamins and minerals which consisted of $MgSO_4$ 2 g/L, $ZnSO_4$ 0.3 g/L, $CuCl_2$ 1 mg/L, Ca-pantothenate 1 g/L and inositol 1 g/L, improved fermentation efficiency for 20 % and resulted in superior $Y_{P/S}$ and process productivity *P*. The effects were primarily attributed to improved viability of cells, and thus the rate and the productivity of ethanol fermentation.

Acknowledgements

This work was funded by the Serbian Ministry of Science and Technological Development (Projects:142075 and 18002).

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