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SHORT COMMUNICATION

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A CORN STEM AS BIOMATERIAL FOR *SACCHAROMYCES CEREVISIAE* CELLS IMMOBILIZATION FOR THE ETHANOL PRODUCTION

This study provides a preliminary contribution to the development of a bioprocess for the production of ethanol using Saccharomyces cerevisiae cells immobilized onto a corn stem. For this purpose, the yeast cells were submitted to the batch tests in situ adsorption onto 0.5 cm long corn stem. Cells immobilization was analyzed by optical microscopy. The number of the yeast cells, fermentation kinetics, the ethanol yield in the presence or the absence of the support in the fermentation medium was investigated. It was determined that the addition of the corn stem led to the abrupt increase of the yeast cells number in substrate, ethanol yield, pH value, a total dissolved salts content and substrate conductivity. The addition of 5 and 10g of the corn stem pith per liter of the medium decreased the amount of residual sugar. The results indicate that a corn stem might be a good carrier for the yeast cell immobilization, and also a cheap alternative recourse of mineral components with the possibility of application for improving ethanol productivities.

Key words: corn stem; yeast immobilization; fermentation; ethanol yield.

The ethanol production by immobilized yeast cells has been extensively investigated during the last few decades [1]. Four categories of immobilization techniques can be distinguished based on the physical mechanism of the cell location and the nature of the support mechanism: "attachment to a surface", "entrapment within a porous matrics", "containment behind a barier" and "self agregation" [2]. The yeast cell immobilization method by the surface adsorption seems to be more reasonable then other methods because of the fact that the yeast cell growth is not significantly affected and some yeast cells can be washed out of the fermentation system and continuously renewed. In addition, such supporting materials are readily cleaned and microbial contamination can be effectively prevented [3]. An industrial carrier for ethanol fermentations should be inexpensive, stable, reusable, nontoxic and should allow for high yeast cell concentrations with minimal internal mass transfer limitations [2]. The cells have been immobilized by the surface adsorption on a variety of natural and synthetic supports [1]. The main factor that influences the immobilization behavior of the yeast cells and their productivity is thought to be the surface characterristics of the carrier including a pore size, the water content, hydrophilicity and magnetism [4]. A corn stem, called a stalk, is low in cost, sustainable, environmentally friendly and abundantly available lignocelullosic raw material in many world regions [4-6]. Corn stalks have been used as forage for ruminants, a source of fibers for manufacturing the pulp for paper, a resource in the emerging cellulose-to-ethanol strategy for biofuels [5,7,8]. Corn stalks remaining in the field after the harvest contain 43 % polysaccharide consisting mainly of cellulose and hemi cellulose, 29 % lignin, 7 % proteins, 5 % ash, and 16 % others [8]. A corn stem has a heterogeneous structure. The outer ring is a concentration of stronger fibers bound by pectin and lignin. Despite a few fibers forming a part of vascular bundles, the corn stem pith has mainly a cellular parenchymatous tissue with the honeycomb microstructure [6,9]. The present paper describes the possibility of using the corn stem as a support material for the ethanol production. The aim of this study was to immobilize *Saccharomyces cerevisiae* cells on the corn stem pith tissue and evaluate the biocatalyst produced for efficiency to perform alcoholic fermentation.

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MATERIAL AND METHODS

Corn stalks of NS 640 maize hybrid were collected from ready-to-harvest corn fields from Budisava site, Serbia. In order to increase the specific surface area of the carrier, the stalks were manually cleaned to separate the fibrous tissue and nodes from the pith tissue [5]. The outer ring was easily peeled from the pith using knives [6]. The corn stem pith of the above ground internodes (7^{th}-10^{th}), cut into slices with a diameter of 1.5-2 cm (width) and 0.5 cm long, with the density of 0.05 g/cm^3, and 8.81 % measure content, was used as a support material [10-12]. The glucose solution used for alcoholic fermentation consisted of 74 g/l glucose in distilled water, and the pH was adjusted at 4.5 by the addition of H_2SO_4 prior to sterilization. The glucose medium in the absence and presence of 5 and 10 g/l of the support was sterillized by autoclaving at 120 °C for 30 min. Working microorganism was a commercial *Saccharomyces cerevisiae* strain (Alltech-Fermin, Serbia), commonly used in Serbian baking industry in form of pressed blocks (70 % w/w moisture) [13]. An amount of 40 g of wet pressed yeasts was suspended in 200 ml sterilized 0.9 % NaCl solution. To obtain always the same inoculums, the yeast cell concentration in this suspension was determined by counting with a Neubauer camera [12] and then appropriate aliquots were added to the fermentation medium. All the fermentations were performed under anaerobic conditions at 30 °C, in 500 ml Erlenmeyer flasks containing 200 ml of the same medium, inoculated with 1±0.1×108 yeast cells/ml. The flasks were maintained in a rotary shaker at 120 rpm for 72 h [14]. The quantification of the yeast cells in the medium was made by counting with a Neubauer camera. Carl Zeiss optical microscope connected to a camera Cannon S50 was used to capture the yeast cells immobilized onto the corn stem pith tissue. The fermentation kinetics was monitored by measuring the weight of produced CO_2, residual sugars and ethanol yields of the fermenting liquids at various time intervals (3, 5, 7, 24, 48 and 72 h from the beginning of fermentation). The concentration of ethanol and residual sugar was measured spectrophotometrically. Ethanol was determined by measuring the optical density at 600 nm after the standard distillation using a dichromate solution [15]. The sugar was estimated by DNS method [15].

In order to examine the influence of the carrier addition on a chemical composition of the fermentation medium, a set of extraction experiments was performed following the fermentation procedure, only without the addition of yeasts cells. For this purpose, the corn stem pith tissue was ground on a laboratory conical mill Miag-Braunschweig, type Doxy 71b/4 at 1375 r/min, until achieving the meal with the particles size below 1000 μm. pH value, the conductivity, a total dissolved salts content from the glucose medium in the absence and presence of 5 and 10 g/l of the support were monitored by a laboratory multiparameter analyzer Consort C863 (Consort, Belgium) and the sugar content was determined by DNS method at certain time intervals following the fermentation media sampling.

RESULTS AND DISCUSSION

The cell immobilization was presented by optical micrographs (Figure 1) showing that yeast cells are densely and homogenously adhered onto the surface of carrier, as a result of: a) natural entrapment into the honeycomb cellulosic material of parenchymatous tissue, b) physical adsorption by electrostatic forces or covalent binding between a yeast cell membrane and a carrier. As demonstrated by the uniform cell growth onto the surface of the corn stem parenchymatous cell walls (Figure 1), the cells immobilization was effecttive. This result suggests a possible recycling of the cells in repeated batch runs, also taking into account that cells grow even after 72 h of fermentation (Figure 2). Low fermentation times indicated that no period was needed for adaptation of biocatalyst in the fermentation environment.

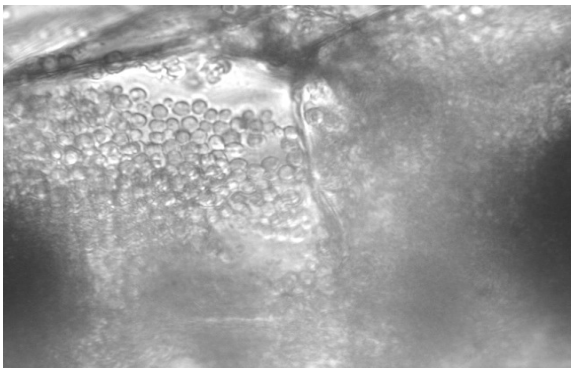

Figure 1. Optical microphotograph of Saccharomyces cerevisiae cells (400×) immobilized onto corn stem pith tissue done after 3 h of fermentation.

After 24 h of extraction, considering the carrier addition of 5 and 10 g per liter of the glucose solution, the values of following parameters increased: the sugar content of the glucose solution by 9.47 and 16.23 g/l, pH value by 2.6 and 2.7 units, conductivity by 210.6 and 291.6 μS/cm and total dissolved salts content by 58.3 and 154.3 g/l respectively, and with no further change in time.

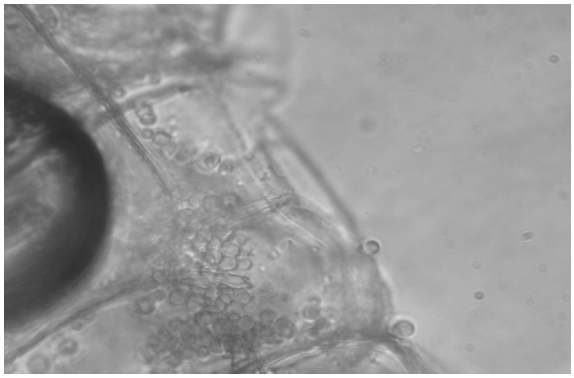

Figure 2. Optical microphotograph of Saccharomyces cerevisiae cells (400×) immobilized onto corn stem pith tissue even after 72 h.

The addition of 5 and 10 g of the corn stem pith per liter of the medium decreased the amount of residual sugar (Figure 3). The ethanol concentration after 72 h of fermentation was 33.3 g/l for free cells and 39.21 and 45.07 g/l for immobilized cells by the addition of 5 and 10 g/l carrier, respectively (Figure 4). The increase in the ethanol concentration, especially in the sample with 10 g/l of the corn stem pith per liter of the medium (Figur 4), after 48 h of fermentation is probably caused by the fermentation of the sugar extracted from the carrier. The dynamics of CO_2 production was in correlation with the ethanol production. The results presented in Figure 3 suggest that immobilized cells consume extracted sugars on the surface of the carrier causing no further time change in the amount of residual sugar in the medium caused by extraction. The observed pH behavior during the fermentation (Figure 5) was, indeed, expected considering the established alkaline nature of the examined carrier.

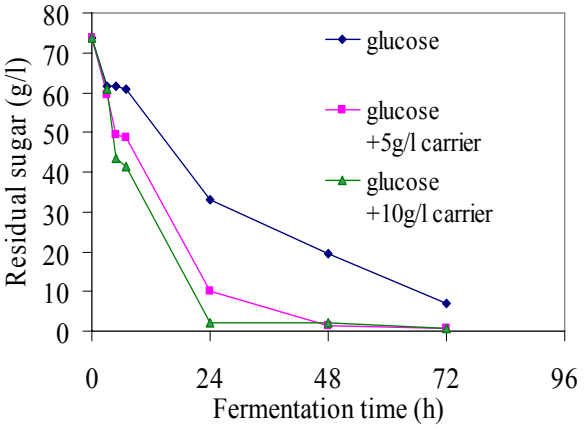

Figure 3. Kinetics of the glucose consumption by Saccharomyces cerevisiae cells immobilized on the corn stem pith and free cells.

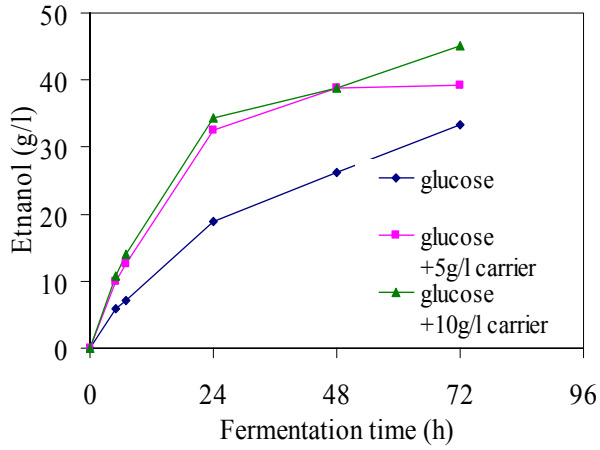

Figure 4. Ethanol concentration versus fermentation time.

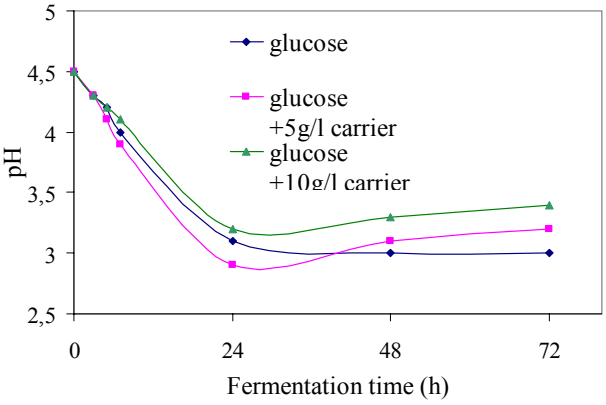

Figure 5. Variation of pH during the fermentation time.

It was determined that in the case of the absence of the corn stem pith, the number of free yeast cells remained constant during fermentation, while the presence of the support led to an abrupt increase of the number of free yeast cells in fermentation medium (Figure 6), probably due to the established increase of the yeast cells nutrients in the medium. The results

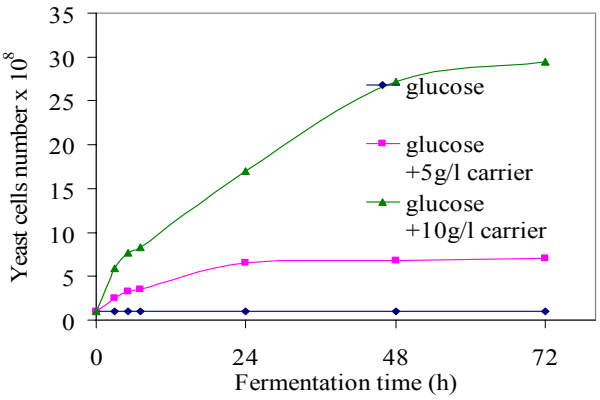

Figure 6. Free yeast cells count variation during fermentation.

demonstrated that the corn stem pith could be an interesting support for the cell immobilization, and also a cheap alternative recourse of mineral components with the possibility of application for improving ethanol productivities. Still, these results are far from ideal, because it is necessary to make more detailed studies in order to clarify the immobilization mechanism by *S. cerevisiae* onto the corn stem pith as a carrier.

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