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APPLICATION OF POROUS CERAMIC SINTER AS A SUPPORT FOR IMMOBILIZATION OF *SACCHAROMYCES CEREVISIAE* YEAST CELLS

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Aluminum ceramics with a high content of α–Al_2O_3 (*ca*. 97%) was analysed as a potential support for immobilized cells of *Saccharomyces cerevisiae* catalysing the process of ethanol fermentation. The study demonstrated that the mechanism of yeast immobilization consisted mainly in trapping the cells in the internal pores of the support and not on the adsorption surface. The presence of aluminum ions (a factor facilitating surface adsorption) in a suspension of yeast diminished adsorption capacity of the examined material. The amount of biomass bound by the support depended on the time of immobilization and yeast concentration in the solution (the porous material was immersed in). Immobilization of cells in porous aluminum ceramics did not evoke a reduction in their fermentation activity – dynamics of batch fermentation was similar to that of fermentation with free cells run under identical conditions.

INTRODUCTION

Immobilization of a biocatalysts is, principally, aimed at improving the productiveness of a reactor. It can be accomplished through accelerating the rate of the process by increasing the concentration of microorganisms in a reaction medium. One of the methods used to this end is immobilization of cells. Application of immobilized biocatalysts with the aim of productivity improvement is feasible only in continuous processes. Trapping the cells in reactor's space prevents their elution – at dilution rates higher than the specific rate of microorganisms growth. Conducting the process with an immobilized biocatalyst yields tangible benefits, *i.e.* reduces production costs [Luong & Tseng, 1984]. A variety of immobilization methods are known these days [Kourkoutas *et al*., 2004.]. The selection of one of them should be preceded by an analysis taking into account both technical (simplicity, easiness of use) and practical aspects (possibility of application on an industrial scale). Yet, the physiological aspects seem to be of the outmost significance. For this reason, a support should be characterised by inertness against a microorganism. Such a material seems to be porous ceramics [Kolot, 1980]. Its major constituents are metal oxides – usually biologically neutral and chemically non-toxic. The porous ceramics is characterised by high mechanical resistance, it is easy to clean and sterilize. As an experimental objective, we chose ceramics which, apart from the above-mentioned properties, was characterised by high porosity and specific spatial structure of pores – internally open and linked.

MATERIAL AND METHODS

IMMOBILIZATION

Support. The support used in the study was high-aluminum ceramics in the form of irregular grains 6÷10 mm in size. The major chemical components of the ceramic mass were: aluminum oxide α–Al_2O_3 (*ca.* 97%), magnesium oxide MgO (*ca.* 2.5%) and calcium oxide CaO (*ca.* 0.5%). The material was obtained with a technique of moulding profiles from sintered ceramic mass with flowing consistency. The binding of mass resulted from polymerization of aluminum oxychloride upon hydrolytic action of base oxides. After drying, the ceramic profiles were burnt at a temperature of 1600°C. The X-ray radiographic evaluation of the phase composition of the burnt ceramic material demonstrated the presence of mainly α–Al_2O_3 and small amount of $MgAl_2O_4$ sinter. The material was characterised with the following properties: total porosity – *ca*. 75%, pore sizes – 3÷1500 μm, and volumetric density – 1.25 g/mL. Photo 1 depicts the porous structure with visible links between pores. Photo 2 shows the microstructure of pores surface with visible α–Al_2O_3 grains *ca*. 3 μm in size. In turn, Photo 3 presents fracture of a bridge linking pores with intergranular pores with the size of several μm. The phase composition was determined by means of X-ray DSH phase analysis on a DRON-1 diffractometer, using filtered radiation CoK_α. Total porosity of the material was calculated based on the determined volumetric density and apparent density with the hydrostatic method. The structure and microstructure of

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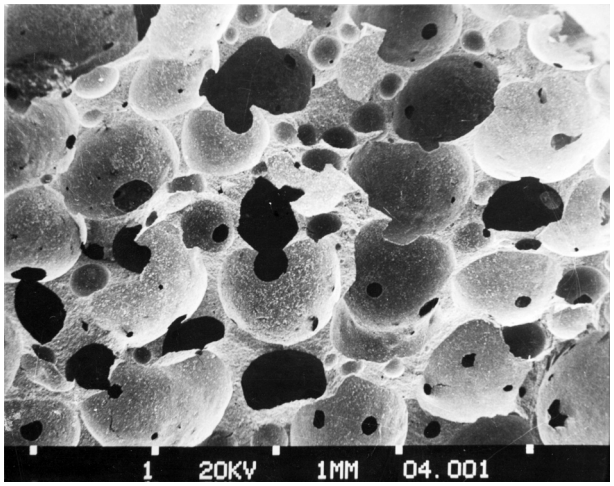

PHOTO 1. Porous structure of support (magnification x 3000).

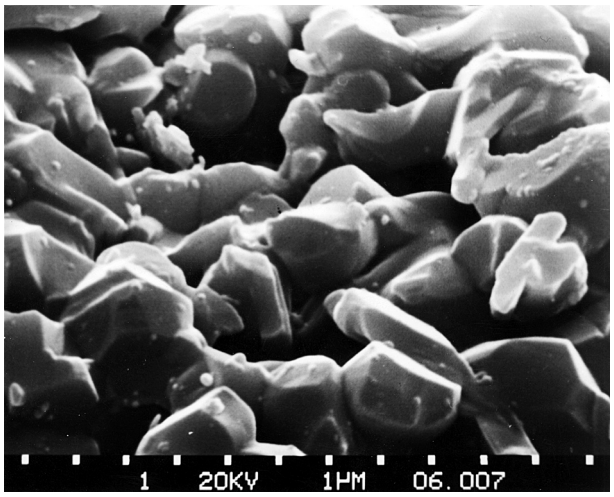

PHOTO 2. Microstructure of pore surfaces (magnification x 3000).

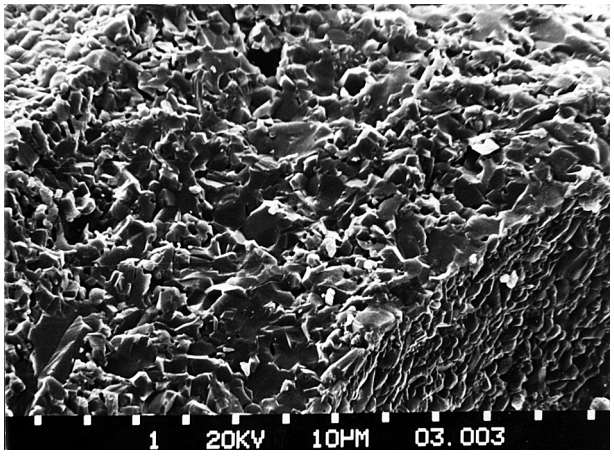

PHOTO 3. Fracture of a bridge linking the pores (magnification x 100).

sinter (size of pores and links between pores) was evaluated based on observations of sample fractures in a HEOL-35 scanning microscope. The support originating from the Institute of Building Engineering, Wrocław University of Technology [Bieniek *et al*., 1982].

Biological material. Distillery yeast *Saccharomyces cerevisiae* V30 stored on agar culture medium at a temperature of 4°C originated from a collection of the Chair of Fermentation, Wrocław University of Environmental and Life Sciences, Poland.

Yeast proliferation. The yeast were multiplied in static (in test-tubes) and dynamic cultures (in flasks on shakers) at a temperature of 30°C, for 48 h, on YPG medium with the following composition: 20 g/L of glucose, 10 g/L of peptone and 10 g/L of yeast extract, pH 5.0.

Immobilization technique. Support (30 g) was added to 100 mL of yeast suspension in physiological fluid (contained in 300-mL Erlenmeyer flask) with a specified concentration (5 g/L till fermentation) and pH 4.0; next the flask was transferred to a shaker (temp. of 30°C) and mixed for 24 h. Before the immobilization process, the sinter was subjected to burning (in a muffle furnace) at a temperature of 900°C for 24 h, then rinsed with distilled water and dried at a temperature of 120°C for 3 h. The amount of yeast adsorbed onto the support was computed from a difference between biomass concentration in the solution before and after the immobilization process.

FERMENTATION

Fermentation medium. For fermentation use was made of a sterile aqueous solution of glucose (pH 5.0) containing 2 g/L of yeast extract and the following amounts of mineral salts (converted into 100 g of glucose): $MgSO_4 \times 7H_2O$ – 0.5 g, KH_2PO_4 – 5 g, $(NH_4)_2SO_4$ – 2 g.

Batch fermentation. The batch fermentation was run in Erlenmeyer flasks (volume of 300 mL), containing 100 mL of the solution and 30 g of the support with immobilized yeast (according to the above-described method). The flasks were fixed in a water bath of a shaker, which enabled maintaining a stable temperature of the process, *i.e.* 32°C, and provided agitation of the culture medium.

ANALYTICAL ASSAYS

The concentration of yeast was determined with the spectrophotometric method using a Beckman apparatus type 560 (at λ=560 nm). Contents of glucose and ethanol were assayed with the method of high performance liquid chromatography using an HPLC Varian ProStar apparatus with RI detector under the following measurement conditions: HPX-C column, temp. of 85°C, eluent H_2O, pressure in the column – 53.7÷54.7 bar, and injection loop 10 μL.

RESULTS AND DISCUSSION

Experiments were conducted in two stages. At the first stage, analyses were carried out for the adsorption capacity of porous aluminum ceramics for immobilization of yeast

cells. The analyses were aimed at identifying a relationship that determines the effect of selected parameters of the immobilization process on the amount of biomass bound with the support. The second stage of the research focused on ethanol batch fermentations with immobilized and free cells and was aimed at evaluating their dynamics.

Immobilization

The support used in the experiments was porous ceramic material with a high content (*ca*. 97%) of aluminum oxide. Aluminum ceramics is characterised by a property enabling binding cells of *Saccharomyces cervisiae* with its surface. That property has been used for immobilization of yeast being a biocatalyst of the ethanol fermentation process [Kanellaki *et al*., 1989]. The mechanism of surface adsorption may result from the interaction of chemical, electrostatic and dispersion forces as well as from hydrogen binding strength. If those forces originate from various bodies, then the phenomenon of adhesion (adherence) occurs. Those forces include mainly those of Van der Waals'. They result from a difference of potentials on the surface of a support and a cell. In most cases, the potential of a cell wall is negative. The negative potential is also typical of glass and ceramic supports, the major constituents of which are metal oxides [Horitsu & Morishita, 1992]. A system in which two bodies feature a negative surface potential does not facilitate the process of adsorption. In such cases, use is made of treatments aimed at changing the character of cell or support potential. Michaux *et al.* [1982] reduced the negative potential of a cell wall of yeast by the addition of proteins and cationic polymers. To this end, Van Haecht *et al.* [1985] applied aluminum ions. Adhesion forces that determine cells adsorption on the surface of support are not high enough to prevent the phenomenon of desorption. Detachment of cells from the surface intensifies along with an increasing linear flow velocity of fermenting wort through the immobilized support. Another factor facilitating desorption is emission of carbon dioxide. Taking those facts into account, in the cell immobilization process use was made of material, the most significant properties of which included: high porosity (75%), appropriate diameter of pores (3÷1500 μm), and developed structure of pores (Figure 1). The size of pores and diameters of linking channels enabled assuming that this would facilitate the migration of yeast cells (3÷10 (m in diameter) into internal pores, and a winding system of channels linking the pores would evoked their trapping in the internal space of the support. In research on immobilization, determinations were carried out for a character of a dependency of adsorption capacity of porous ceramic material on three selected parameters of the process: concentration of aluminum ions (added to a yeast suspension as an agent reducing the potential of cells), time of immobilization and concentration of yeast. As a measure of the adsorption capacity of ceramic material there was adopted dry matter content of yeast (in grams) that was bound by 100 g of the support. Figure 1 depicts a dependency of the adsorption capacity of the support on the concentration of aluminum ions (addend to a yeast suspension as $Al(NO_3)_3$). Two cases were discussed. In the first, aluminum ions were added before fixing the support in a suspension with yeast, whereas in the second immobilization was carried out from the beginning without aluminum ions, which were then added after 24 h and the process was continued for the subsequent 12 h. Results obtained demonstrated that in the presence of aluminum ions, the amount of cells adsorbed on the support was twofold lower (Figure 1). It may be explained by the formation of cell agglomerates whose size made the internal diffusion impossible. The linking of single cells into larger structures was facilitated by the presence of aluminum ions that reduced the negative potential of a cell wall. Based on the relationships presented, it may be assumed that immobilized consisted mainly in trapping the cells in pores, and not on the surface adsorption. Such a mechanism has also been confirmed by results presented in Figure 2. A long period of immobilization (*ca.* 36 h), necessary to reach the maximum cell concentration on the support (*ca*. 1.75 g/100 g sinter), was due to diffusion resistances met by a cell traveling through winding and narrow channels linking the internal pores. The driving force of the diffusion process is the differences of concentrations in spaces between which mass transfer proceeds. In those cases, the yeast were observed to diffuse from the solution to the support's interior. Hence, the driving force was determined by yeast concentration in the suspension, which was confirmed by the results obtained. Similar findings were reported by Mustranta *et al.* [1987]. At yeast concentration of 5 g/L, the rate of cells immobilization reached 0.05 g/h (Figure 2). In turn at yeast concentration of *e.g.* 20 g/L, it increased to *ca.* 0.275 g/h (Figure 3). Increasing the concentration of yeast in the suspension to *ca*. 25 g/L allowed for the immobilization

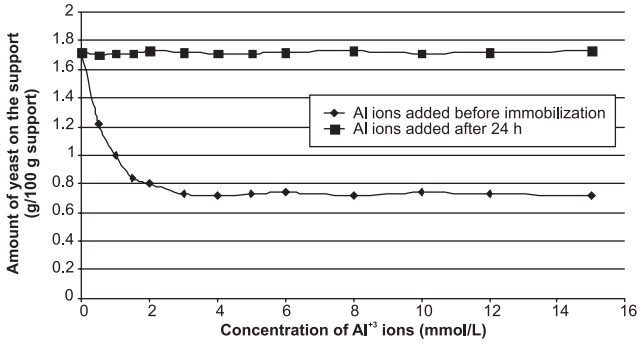

FIGURE 1. Amount of yeast adsorbed on the support after 36-h immobilization depending on aluminum ions concentration.

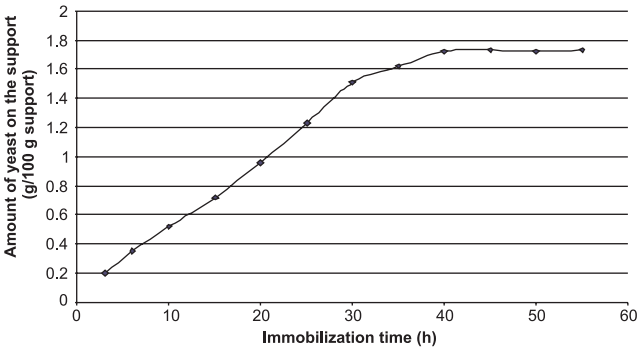

FIGURE 2. Dependency of the amount of yeast cells adsorbed on the support on immobilization time.

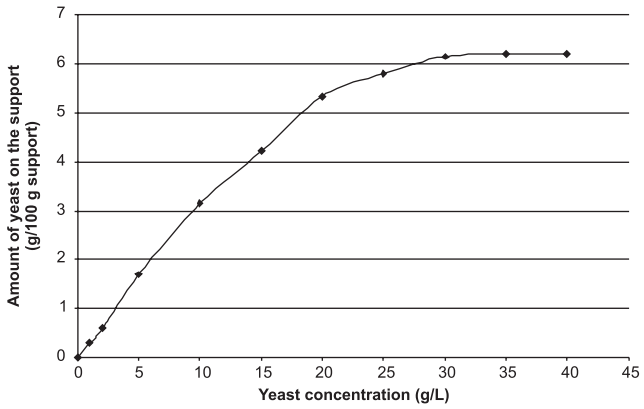

FIGURE 3. Dependency of the amount of yeast cells adsorbed on the initial concentration of biomass.

of *ca*. 6 g cells/100 g support (Figure 3). That value may be claimed as the "maximum volume" of the investigated material under experimental conditions applied. Such an amount of yeast on the carrier, considering material's porosity (75%) and bulk density (1.2 g/cm^3), corresponds to the theoretical (computed) concentration of biomass in the fermenting wort, *i.e*. 96 g/L.

Fermentation

Dynamics of the course of batch fermentation was one of the evaluation criteria of porous ceramic material usability as a support for immobilization of yeast cells. Analyses covered batch fermentation with cells adsorbed onto the support in the form of grains 6÷10 mm in size. After the immobilization process, the amount of cells adsorbed on the support accounted for 0.4 g/100 g support. Considering the mass of the support in a fermentation vessel (30 g) in the volume of added medium (0.1 L), the initial concentration of immobilized yeast reached 1.2 g/L. Under the same conditions and the same initial concentration of yeast, fermentation was run with unbound cells. The course of those processes was shown in Figure 4. In the case of fermentation with immobilized cells, the curve related to yeast depicts only the concentration of free cells – released from the support. Data presented indicate that the dynamics of both compared processes was alike. Differences in concentrations of biomasses result from the fact of not considering the immobilized cells. On termination of the fermentation process, the amount of yeast permanently bound with the support reached 0.65 g/100 g support. Having converted that value into concentration in wort, it appeared to account for *ca.* 2 g/L. This means that the total concentration of biomass in the solution, at the final stage of the process, oscillated around 10 g/L and was similar to yeast concentration recorded during the fermentation with free cells. The amount of biomass left on the support after the fermentation indicated that the adsorption capacity of sinter was utilized only to some extent. Thus, much longer period of fermentation is needed to fill the internal spaces of the porous material. It is possible in the continuous process.

CONCLUSIONS

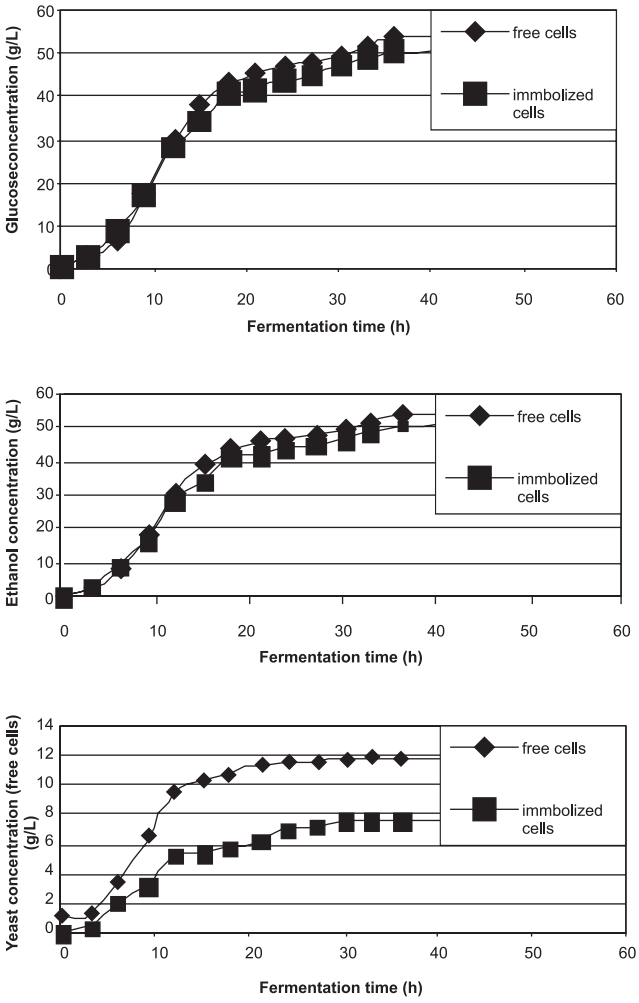

FIGURE 4. The course of batch fermentation at the initial concentration of glucose reaching 150 g/L.

Adsorption capacity of the support examined and mechanism determining the process of yeast immobilization were linked with the internal spatial structure – size of pores and systems of channels linking them. The high volumetric porosity of the material affords possibilities for obtaining high productivity of the reactor due to a high concentration of biomass. Complete assessment of the usability of that material as a support for immobilized cells requires investigations of the fermentation process in which the time the yeast stay in the reaction medium will be much longer as compared to the batch process, namely the continuous or feed-batch process.

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ZASTOSOWANIE POROWATEGO SPIEKU CERAMICZNEGO JAKO NOŚNIKA DO UNIERUCHAMIANIA KOMÓREK DROŻDŻY *SACCHAROMYCES CEREVISIAE*

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Badano porowatą ceramikę glinową o wysokiej zawartości $\alpha - Al_2O_3$ (ok. 97%) w aspekcie wykorzystania tego materiału jako nośnika unieruchomionych komórek *Saccharomyces cerevisiae* katalizujących proces fermentacji etanolowej. Wykazano, że mechanizm unieruchamiania drożdży polegał głównie na zamykaniu komórek w wewnętrznych porach nośnika, a nie na powierzchniowej adsorpcji. Obecność jonów glinu (czynnika sprzyjającego adsorpcji powierzchniowej) w zawiesinie drożdży obniżała zdolność adsorpcyjną badanego materiału. Ilość biomasy wiązana przez nośnik zależała od czasu unieruchamiania i stężenia drożdży w roztworze (w którym znajdował się materiał porowaty). Unieruchomienie komórek w porowatej ceramice glinowej nie spowodowało obniżenia ich aktywności fermentacyjnej – dynamika fermentacji okresowej była podobna do dynamiki fermentacji z komórkami wolnymi przebiegającej w identycznych warunkach.