NEW APPROACHES TO THE PRODUCTION OF LACTIC ACID: IMMOBILIZED BIOCATALYST BASED ON FUNGUS CELLS *Rhizopus oryzae* ENTRAPPED IN PVA CRYOGEL

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The possible wide application of lactic acid as monomer in the structure of new biodegradable polymers is discussed in the literature (Chen, 2001; Datta, 1995). Therefore, new biotechnological approaches allowing intensification of lactic acid production, as well as screening of new microbial producers, are of particular interest.

Now diverse bacterial strains are used for the production of L(+)–lactic acid in industry (Hofvendahl, 2000). All of them are needed in rich nutrient media with certain pH (no lower than 5.5) and high concentrations of carbohydrates. The growing cells are usually employed in the processes of lactic acid fermentation, whereas the main substrate is spended for both the cell biomass growth and product accumulation. As a result, the concentration of target product turns out lower than it can be under the conditions of absence of cell growth.

Immobilization of cells is the crusial approach, which can help to organize the production of lactic acid by non–growing cells for a long period of time. The use of fungus producers of lactic acid instead of bacteria is also very attractive approach, since fungi are resistant to the high concentrations of accumulated lactic acid (Park, 1998; Peimin, 1998). Fungi produce L(+)–lactic acid instead of racemic mixtures of D(–) and L(+)–forms, as it does in the case of bacterial cells. Thus, the investigation of biocatalysts developed on the basis of immobilized fungi cells in the processes of lactic acid production is of high actuality.

Fungi capable of lactic acid synthesis are the aerobic cells and they are needed in good aeration conditions during the growth and synthesis of product. This fact dictates strong requirements to the carrier used for cell immobilization. The high porosity together with mechanical strength can be among the main characteristics of the carrier matrix. Poly(vinyl alcohol) cryogel (PVA cryogel) (Lozinsky, 1998) is the carrier, which was examined in this study as the high porous carrier for the immobilized fungi cells.

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MATERIALS AND METHODS

The Rhizopus oryzae NRRL–395 cells were taken from the VKPM. Fungus spores were grown up on potato–dextrose medium containing agar (2%) and stored in refrigerator at 4°C.

The biocatalyst on the basis of immobilized fungus cells entrapped in PVA cryogel was prepared in accordance with patented procedure (Efremenko, 2002). The gel beads (1–1.5 mm in diameter) with immobilized spores were prepared using cryogranulating set–up (Lozinsky, 1992).

The following medium was used for the lactic acid fermentation: glucose – up to 120 g/l, yeast extract – 2 g/l, Tween 80 – 1 ml/l, pH 5 .06.0. To adjust the pH values of the medium to the optimal level $CaCO_3$ (5–10 g/l) was added at the beginning of immobilized biocatalyst cultivation.

The biocatalyst beads were used in batch and semi–batch processes, the beads were washed with 20 mM K/Na–phosphate buffer (pH 6.8) after each batch–cycle.

The concentrations of glucose and starch in the medium were analyzed spectrophotometrically by common methods, the concentration of lactic acid was estimated by HPLC and enzymatic method using L(+)–lactate dehydrogenase kit.

RESULTS AND DISCUSSION

New immobilized biocatalyst based on fungi R. oryzae entrapped in cryogel of poly(vinyl alcohol) (PVA) has been developed (Efremenko, 2002). The procedure of biocatalyst preparation consisted of two main stages:

(i) entrapment of spores in the cryogel carrier;

(ii) vegetation of fungus cells inside of the macro-porous PVA cryogel granules up to the steady state.

The optimization of conditions of the biocatalyst formation and use in the processes of lactic acid production from such substrates, as glucose and starch, has been performed. It was established that this biocatalyst was able producing L(+)–lactic acid with high yield and high concentrations (up to 180 g/l) from glucose, as well as from the acidic hydrolysates of starch.

The capability of free and immobilized fungi cells to synthesize the complex of extracellular amylolytic enzymes was also investigated. It was shown that native potato starch could be easily transformed into L(+)–lactic acid by the immobilized *R. oryzae* cells with the yield of process equal to about 50%.

Owing to favoring combination of unique characteristics of chosen gel carrier and the microorganism used, there was a number of incontestable advantages of new biocatalyst as compared to those based on bacterial cells traditionally employed in the lactic acid production. The new biocatalyst ensures the 99.5% yield of L(+)–form of lactic acid, whereas bacterial strains usually give no more than 95.1% of this isomer of lactic acid. Upon the comparison with bacterial producers the fungi do not need in complicated nutritional media for the intensive growth.

Immobilized spores *R. oryzae* can be stored in frozen state for a long time (at least for 5 months) without reducing the cell viability. The immobilized biocatalyst elaborated is much more active at the acidic pH values and can continue to function at higher concentrations of lactic acid than the bacterial cells do. Immobilized fungi could be used protractedly (at least for 400 h) with only 15%–loss of initial metabolic activity.

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