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Cryoimmobilized enzymes and cells in organic synthesis

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Abstract - Novel approaches to effectuation of complex organic reactions with the use of immobilized enzymes and cells are discussed. A new class of methods to obtain immobilized cells has been developed on the basis of entrapment of the cells in polymers upon their cryostructuring. The properties of the obtained heterogeneous catalysts and the basic advantages of cryoimmobilization are considered:
- the method is reagentless and the immobilization procedure is "gentle", which affords the entrapment of any cells, including those of animals and man;
- the possibility for effectuation of involved processes with regeneration of cofactors and enzymes;
- the possibility for creation of very stable systems functioning for a few years;
- the possibilities for conducting the reactions in organic solvents.

The properties of cryoimmobilized enzymes and cells are demostrated by some examples. These are a) the realization of a deep reduction of carbonic acid to ethanol, acetate and hydrocarbons; b) the bioelectrocatalytic reactions as an alternative to photosynthesis; c) the biocatalytic conversion of carbohydrates to alcohols, organic acids, amino acids and vitamins; and d) the biocatalytic conversion of polyunsaturated fatty acids to prostaglandins.

The development of methods for the work with living cells as catalysts of chemical reactions has led to the idea of creation of catalytic systems based on immobilized cells. The influence of the idea of using immobilized cells on the development of biotechnology is as grand and revolutionary as was at one time the influence of application of immobilized enzymes. Chemical and physical methods for cell immobilization are fairly diverse.

At present, various systems have been created that help entrap the catalytically active cells in matrices of various nature [1]. The living cells have been mostly immobilized on natural gel forming agents, such as carrageenan [2] and alginate [3]. Widely employed is the method of cell entrapment in polyacryamide gel [4]. Of common knowledge are the methods for "saturation" of polyuretane foams with living cells.

At Moscow University and the Institute of Organoelement Compounds (now the Institute of Food Substances), a novel methodology for production of biocatalysts based on immobilized living cells (Dr E.I. Rainina Dr V.I. Lozinsky and) has been elaborated. This methodology is based on the formation of gel structures in the process of cooling and subsequent freezing cell suspensions in polymer solutions [5]. The

method has been termed cryoimmobilization. By the present, the immobilization techniques have been optimized, the properties of the gels and catalysts, obtained on their basis, studied, the biotechnological fundamentals for production and application of catalysts and the pilot plant for cryo-immobilization designed.

The method is grounded on the following steps:

- the preparation of polymer solution (for instance, poly(vinyl alcohol)) and the suspending of cells in it;
- the formation of catalytic particles (various-dimension spheres, films and irregular granules granules);
- the programmable freezing of particles and formation of cryogels;
- the programmable thawing of particles and activation of catalytic capacity of cells.

The heterogeneous catalysts thus obtained are stable in wide ranges of pH, temperatures and the parameters of salt solutions. The method of cryoimmobilization of living cells and the catalysts obtained on its basis have the following advantages.

- The method of immobilization is the procedure in which the toxic reagents or effects are not used. This is a fairly mild immobilization technique. In some instances, it appears possible to retain a 100 % viability in the cells upon their entrapment in a cryogel matrix.

- A principal advantage of immobilization of living cells is the feasibility of very involved multienzyme coversions into one macrostep. The retention of methabolic reactions in the cell meets the problem of intracellular regeneration of all cofactors and enzymes if, for some reasons, the enzyme or enzymes will be inactivated.

- The method of cell cryoimmobilization helps obtain the persistently stabilized catalytic systems. Since the cells retain the reactivation and reparation potentials including the biosynthesis of enzymes, the catalytic systems can be used quite long. We employed the systems retaining the initial catalytic activity for an year.

- The resultant cryogels are mechanically stable and can be used in ordinary reactors for immobilized cells. Cryoimmobilization technique forms in gels the system of macropores, which makes the matrix material highly permeable for substrates and reaction products and impermeable for cells.

- Cryogel matrices, compared to other gels (agar and carrageenan) are stable at elevated temperatures (up to 65-70°C). This permits the reactions with thermophilic microorganisms and thermostable enzymes.

- The cells entrapped in the carrier are surrounded by water-containing gel medium. The gel matrix itself is insoluble in organic solvents. This makes possible to use the catalysts with cryoimmobilized cells for reactions in organic solvents.

- The systems with the cryoformed matrix are highly renewable. If the catalyst became inactivated for some reasons, PVA cryogel can be melted down at 80-90°C and the solution obtained can be reused for immobilization of new cells. This makes the system with immobilized cells wasteless and ecologically acceptable.

- The cryoimmobilization method makes possible to obtain the cells with co-immobilized enzymes, which affords effectuation of very involved processes with introduction, into catalytic systems, of new elements absent from the cells in use.

- The cryogels are permeable for proteins. This helps effectuate the production of exocellular enzymes and useful proteins.

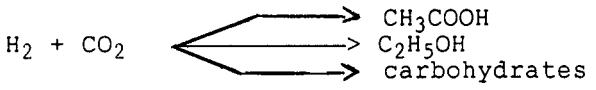
- The cells entrapped in the polymeric matrix at a high density of cell population have no actually conditions for further growth. This effect allows one to use the bacterial strains with unstable plasmids, obtained by genetic engineering. The absence of cell population growth upon immobilization helps avoid the loss of plasmids upon the cell growth [6].

These advantages make the cryoimmobilized cells a highly convenient tool for effectuation of complex synthetic processes.

Consider a few examples.

Deep reduction of carbonic acid

Cryoimmobilization of microorganisms afforded a biocatalytic effectuation of the following reactions:



Bioelectrosynthesis as an alternative to photosynthesis

The development of the systems of CO_2 reduction opened a possibility for constructing the systems of CO_2 electroreduction to energy-rich metabolites and fuels [7].

The simplest scheme of the electrosynthetic process looks as follows:

$$2H_2O \xrightarrow{\text{electrolysis}} 2H_2 + O_2$$

$$H_2 + CO_2 \xrightarrow[\text{immobilized acetogenic cells}]{} CH_3COOH$$

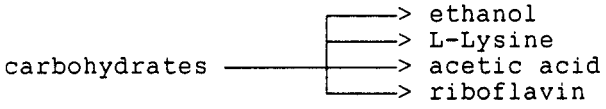
$$CH_3COOH + O_2 \xrightarrow[\text{immobilized Lysine-producing cells}]{NH_3} \text{L-Lysine}$$

Realization of the overall process

$$6\,CO_2 + 4H_2O + 2\,NH_3 \longrightarrow NH_2\text{-}(CH_2)_4\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}COOH + 7O_2$$

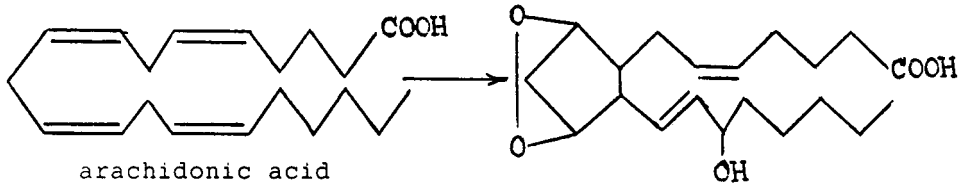
These are highly original processes, being in essence an alternative to photosynthetic reduction of CO_2. Possibilities for bioelectrosynthetic production of carbohydrates, monomers, alcohols, glycoles of organic acids, ketones, hydrocarbons and amino acids. Transformation of carbohydrates.

The biosynthetic processes with immobilized living cells help effectuate a vast range of processes of carbohydrate (glucose, saccharose and molasses) conversion.



Fine organic synthesis

Enzymatic modifications with immobilized cells afford a stereosynthetic synthesis to be conducted in one macrostep. For instance, using prostaglandin-H-synthetase of immobilized cells of vesicular glands, it becomes possible to modify polyunsaturated fatty acids to prostaglandins:



arachidonic acid

prostaglandin-H

The cryoimmobilized cells open new possibilities for realization of new synthetic processes.

REFERENCES

1. Brodelius P. and Vandamme E. J.. In: Biotechnology, Ed. by H.-J. Rehm, G. Reed. Weinheim 1987, v. 7a, 405-464
2. Chibata I, Tosa T and Sato T.. Meth. in Enzymol. 1987, v.135B, 189-198
3. Bucke C.. Meth. in Enzymol. 1087, v.135B, 175-189
4. Skryabin G.K., and Koshcheenko K.A.. Meth. in Enzymol.1987, v.135B, 198-215
5. Lozinsky V.I., Vainerman E.S. and Rogozhin S.V.. USSR, Pat. 1400071, 1986
6. Varfolomeyev S.D., Kaluzny S.V.. Biokimiya, 1991 in press
7. Rainina E.I., Pusheva M.A. and Borodulina N.S.. Microbiologia, 1991 in press