MAJOR PROGRESS IN DEVELOPMENT OF BIOCATALYTIC TECHNOLOGIES IN RUSSIA IN THE ADVANCE OF THE XXIst CENTURY

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Survey from the materials of the Project "Biocatalytic Technologies" of the subprogram "Newest Methods of Bioengineering" of the Federal targeted scientific-technological program "Research and methodology in the priority trends of development in civic science and technology.

The survey covers major progress in Enzyme Engineering in Russia for the late decade. Research in this field has been developed in the framework of the project "Biocatalytic Technologies" of the Governmental subprogram "Newest methods of bioengineering" To-date, new technologies have been elaborated using enzymes in food, pulp-and-paper and textile industries as well as in agriculture. Based on enzymes, new high-sensitivity methods, instruments and devices have been created for medical and ecological purposes as well as for control of food quality, industrial productions and environmental monitoring. Test batches of medicaments and materials based on enzymes and their regulators have been created and are under way for therapy of cardio-vascular, viral and oncological diseases as well as for surgery, stomatology and ophthalmology.

The contemporary position of biotechnology in the overall system of the world technologies is defined, among other factors, by development of research in Chemical Enzymology and Enzyme Engineering started about 30 years ago. The Enzyme Engineering is underlain by the high level of fundamental knowledge about enzymes as catalysts of biological processes, the availability of enzymes for possible technological application due to elaboration of efficient methods for their isolation from various sources, the development of methods for stabilization, immobilization and modification of biocatalysts imparted with new valuable properties. Large prospects for contemporary technologies are expected from genetic engineering of enzymes, which lays ground for effectuation of involved multistep catalytic processes.

Late decades in Russia have accumulated a large experience in research and application of enzymes in various fields of science, industry, agriculture, medicine and ecology. In all, the researches in these fields have been evoluted in the framework of the Governmental subprogram "Newest methods of bioengineering". To-date, an experimental-theoretical ground has been created to solve the tasks of Enzyme Engineering, including the methods for structural and mechanistic studies of individual enzyme activities, the new methods of chemical modification of enzymes with the changed activity, stability, membranotropy, the procedures for conducting the enzymatic processes in nontraditional media, the genetic engineering methods for production of some biotechnologically important enzymes with improved catalytic and physico-chemical properties.

The research performed in the framework of the project "Biocatalytic technologies" can be grouped into three major trends according to the field of their application: (1) Enzymes in industry; (2) Analysis and sensor-based technologies; (3) New medicaments based on enzymes and their regulators.

Enzymes in Industry

The most important results in this trend are those that led to elaboration of high-efficiency methods, preparations and approaches to the most important problems of biotechnology.

In Russia successful trials for cellulolytic enzyme complexes with new improved properties were conducted and their industrial production organized for agricultural, textile and pulp-and-paper industries in Berdsk biochemical plant. In 1999, the plant produced about a thousand arbitrary tons of enzymic preparations, carbohydrases. The same success crowned the works on application of cellulolytic enzymic complexes obtained in various conditions of cultivation of highly productive mutant producer strains *Penicillium verruculosum and Trichoderma reese* to bleach cellulose, to enzymatically depigment the colored fabric, to enzymatically retreat the secondary paper fibers as fodder additives [1–4].

Russian researchers derived high-activity immobilized forms of cyclodextrin transferases for microorganisms for industrial synthesis of beta-cyclodextrin. To do this, mineral cultivation cultures were optimized and the screening of the microbial *B. Species* strains conducted. The *B. Species* strain 739 was shown to be the most active producer of cyclodextrin transferase.

To solve ecological problems, a new wasteless biocatalytic technology was created to purify of the natural gas of hydrogen sulfide through biocatalytic oxidation of H_2S to elementary sulfur using a heterogeneous catalyst based on immobilized cells. A laboratory reactor was used to show a principal possibility for aerobic microbiological purification of waters containing soluble sulfides by use of Thiobacillus and Thiomicrospira genera microorganisms, as catalysts [5–7].

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For the same purposes, trials were performed for enzymic preparations based on organophosphorus hydrolase for destruction of organophosphorus toxic agents and pesticides. The results underlay a new procedure for protection from mass-destruction weapons. New methods were elaborated to create stable biocatalysts with phosphoesterase activity for decomposition of organophosphorus neurotoxins in real objects, wastes of agriculture and processing industries. Possible applications of these methods were shown for hydrolysis of toxic agent analogs [8–10].

For agricultural purposes, the enzymic preparations with cellulolytic and pectolytic activities were derived and industrially implemented. A repeated application of these preparations on stem apexes of short-day plants was shown to result in flowering of perilla plants not subjected to photoperiodic induction by a short day. The results will be used in the plant-growing institutions for regulation of flowering and fruiting [11–12].

Many problems of maintenance and therapy of agricultural animals may likewise be solved using the bioengineering approaches. In particular, in the framework of the subprogram, researches were performed aiming at elaboration of the technologies for production of a new composite veterinary preparation based on the enzyme complex "Lysoamidase" for therapy of serous, serocatarrhic and fibrinous mastitis of staphylococcus etiology in cattle. The research was based on the earlier created preparation "Lysomast" allowing for the necessity to create the drug against both gram-positive and gram-negative microflora [13]. Combinations of enzymes with various concentrations of preparations of non-antibiotic nature against pathogenic microorganisms were examined for bactericide and bacteriolytic actions. Most effective compositions of the enzymic preparation were obtained.

For application in food industry, the technology was developed for isolation of milk clotting enzyme from milk whey of transgenic sheep. The chemical and enzymatic properties were studied to demonstrate the identity of the transgenic enzyme to chymosin of calf. New chromophore substrates of chymosin were synthesized; their application simplifies the control of the enzyme activity and makes it more precise. The trial batches of milk clotting enzyme were obtained in paste and powder forms [14, 15].

In biotechnology, of great importance is the fundamental research aiming at development of new promising methods of enzyme modification. At present, works are going on to create the technologies for enzyme stabilization in water-organic mixtures. Methods were elaborated to produce "organophilic" films for incorporation therein of enzymes based on non-crosslinked rubbers (caoutchouc) by the example of laccase. Similar films can serve as an active element of analytical devices for reactions with hydrophobic substances [16–18]. Biocatalysts were created for regeneration of NADH and NADPH, based on recombinant formate dehydrogenase from the methylotrophic bacteria *Pseudomonas sp.* 101. The express system was elaborated, based on the plasmid pUC119 with the tandem of lac- and tac-promoters and the strains *E. coli* TG1 and *E. coli* BL21; the system increases the output of formate dehydrogenase from 7–8% to 40% of the overall amount of soluble *E. coli* proteins. The enzyme is expressed in soluble active form [19–21].

Analysis and Sensor Technologies

This research trend includes the creation of express analytical systems for environmental monitoring, the quality control of tap water, waste water, foods as well as the elaboration of methods for medical diagnosis. These systems include the equipment (electrochemical or optical biosensors or luminometers), the kits of reagents and the procedures for running the analysis.

Relevant materials and methods in immuno- and bioluminescent microassays were designed and applied. A limited production of Russia-invented immuno-enzymic test systems and devices have been organized for most important medical analyses. In particular, some new biosensors were created and new enzyme immunoassay kits commercialized; the bioluminescent tests were developed to analyze the biological contamination of various media water, foods and biological tissues. The methods are under way in the framework of a special project "Food Safety Program" oriented to the express-detection of biocontaminated foods and the detection therein of toxic agents. The Project is international; since the year of 2000, it is partially supported by IPP Program (USA) [22, 23].

An important progress in this field of biological engineering is the creation of new bioelectronic biosensor assays.

The developed methods for molecular designing the integral biosensors are based on liquid crystal dispersions of nucleic acids; they are aimed at analysis of some physiologically active agents including the biogenic oligopeptides, antibiotics, hydrolytic enzymes, toxins, natural anticoagulants, amino acids, proteins and their mixtures [24–28].

The following projects have been prepared: (1) Technological Conditions for the analyzer of glucose, the biosensor AG-301; (2) Instruction for users of the analyzer. The technology for a small-scale production of glucose oxide membranes for the analyzer was developed. Major technological parameters of the analyzer were optimized [29–32].

Industrial trials were conducted for the bioluminescent methods using luciferase enzyme for express-control of microbial contamination of milk; Russian preparations of pancreatin and neonol were used to create the reagent affording the researchers to standardize and simplify the assay [33–36].

To create the high-sensitivity devices for analysis of environmental pollutions, in particular, for detection of soluble phenolic compounds in waste waters from cellulosic industries, the two-enzyme membrane was created on the basis of immobilized tyrosinase and laccase; the membrane is coupled with the electrochemical detector. The two-enzyme system was used to create the laboratory device of the electrochemical analyzer for detecting a general pollution of waste waters with ecotoxicants [37–40].

To analyze the activity of neurotoxic esterase, a laboratory prototype of the amperometric biosensor was created; it will be used to solve the tasks of medical ecology (analysis of organophosphorus compounds) [41].

Like in the first trend, a great emphasis here was given to promising fundamental researches, to creation of model systems and to development of new instrumental approaches.

The new high-efficiency analytical methods can be applied in clinical conditions due to the obtained and characterized conjugates of pesticides and antibiotics with proteins and enzymes for immunization and running the assay. Test animals were vaccinated and polyclonal antisera tested. A flow cell was constructed for the portable luminometer. The interpolyelectrolyte reaction was characterized and suggested for application in microbase-table, immuno-filtration and immuno-sensor systems for pesticide detection; this approach was shown to notably reduce the time of analyses [42–49].

New Medicaments Based on Enzymes and Their Regulators

A very notable progress was reached in invention of new medicaments. Russian researchers developed unique methods and medicaments, the curative agents of which are enzymes and their regulators. The relationship between the pathologies and enzymatic disfunctions in the organism was examined. Precise and express methods are under way to quantify the related proteins. The efficient methods for chemical modification are advanced to exclude the toxicity of the curative agent, to increase its immunogenic potency and to impart it the target and prolonged effects, what helps to obviate many detrimental after-effects in therapy, to reduce the cost of therapy and in some instances to exclude the injections dangerous from the viewpoint of hospital infections.

Russian researchers elaborated the medicaments for rapid healing of wounds including the infected ones, burns and radiation lesions. The medicaments are made in the form of dressings, pastes, powders and are efficiently applicable in field conditions. To-date, the dressing and tegmental materials with proteinases and their inhibitors on various carriers are being produced for therapy of severe wounds (burns, radiation lesions, infected wounds, non-cicatrizing wounds of diabetic patients, bedsores, squeeze-caused lesions, lesions after transplantations, etc.). The medicaments are produced as test kits in the pilot plant at the Institute of textile fabrics and in pharmaceutical companies by special orders from Defense Ministry, Ministry of Extraordinary Situations of Russian Federation and various hospitals.

These medicaments can be exemplified by the bioresorbing sutural material "Dalcex-trypsin", the gauze dressings and napkins for therapy of aggravated wounds, the paste from crab pancreas "Morikrol" for therapy of operational cicatrices. This paste is produced for Defense Ministry in the amount 5-10 kg; the production is organized by the pharmaceutical company "TRINITA". Test kits of film and microgranular medicaments are being produced in the application forms for therapy of eyes, mouth cavity (paradontoses and paradontites) and inner ulcers. The pilot production of surgical sutural materials, based on proteinases and their inhibitors, is set going. All listed materials can be supplemented (when necessary) with antibiotics and other medicaments enhancing therapy and reducing the rejection. The required trials of the preparations and clinical verifications were carried out; there are international and Russian patents; documents are supplemented with Certificates of quality [50–60].

Research was performed to create the medicaments for therapy of thromboses and for prevention of acute infarctions. The drugs are based on natural activators of proteinases of the bloodstream and on genetically engineered proteins. Composite preparations of a new generation were obtained. They notably accelerate thrombolysis. Their application does not cause high concentrations of proteinases in the bloodstream, sharply reduces the possibility of after-effects: rethrombosis and occlusion of vessels which often occur during therapy of acute diseases. Russian researchers have implemented extremely important developments which help prevent the mortality during acute infarctions. Likewise, the preparations were created for prolonged cardiotherapy, on the basis of natural activators of the bloodstream system. The preparations are produced in the pilot plant at the All-Russian cardiological research center under RAMS [61–68].

The method for diagnosis and treatment of kidney and stomach diseases in children was developed; the cataract development was shown to be inhibited at diabetes by angiotensin-converting enzyme inhibitors [50–69].

Four technological schemes were elaborated to produce the biologically active additive "Insol" containing BBI, the Bowman-Birk soybean inhibitor. The processes differ technologically, ecological safety, output, quality and cost of the final product. The technological instruction for "Insol" production was authorized. "Insol" was preclinically trialed and studied for its influence on growth of reinoculated tumors and their metastases *in vivo* (mice). It was shown to inhibit the growth of such tumors and to influence the therapeutic effect of antitumor preparations. Note the developed technologies for production of preparations with inhibitors of proteolytic enzymes from cattle pancreas and soybeans; the preparations were successfully clinically trialed in 1992. They cease the lethal acute pancreatitis, inhibit the development of cancer tumors, are extremely potent against burns of large surfaces and at transplantation of tissues. At present, despite the available developments, Russia purchases the analogs of these preparations abroad (prices not lower than 1000 USD/kg). The demand for such drugs only in Moscow is about 10 kg/year. Provided the production in Russia has been restored, the country could save millions of dollars and lives of many people [70, 71].

This is not a complete list of prospects open to humankind due to application of enzymic biocatalysts and technologies elaborated on their basis. Biocatalytic technologies both simplify, improve and, in some cases, notably reduce the cost of the processes. They are often the only approaches possible to apply. Economical efficiency from application of Russian preparations and devices, based on biocatalysts, is so evident, in some cases, that the foreign researchers and commercial firms interests in these developments are understandable. This interest is most pronounced in international events with participation of Russian scientists. Of great importance

is the Federal financial support of Russian scientists, including those of the Lomonosov Moscow State University, engaged in most promising projects of the Federal targeted scientific-technological program "Research and developments on priority trends in civic science and technology" as well as in the project "Chemical Enzymology as the leading scientific school of MSU", headed by Prof. S.D. Varfolomeyev.

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