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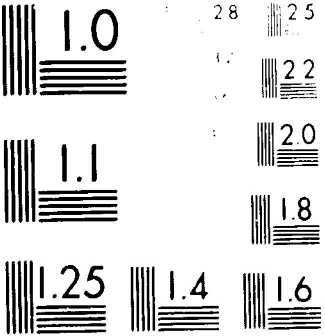
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BIOTECHNOLOGY OF ENZYMATIC CONVERSION OF CELLULOSE: FUNDAMENTALS AND APPLIED ASPECTS*

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In recent years, increasing attention has been received by natural resources ensuring the world's further development largely with respect to the growing food and fuel requirements.

Regarded as most promising are those natural resources which meet the following conditions: (1) they are largely universally distributed as opposed to being located in a limited area; (2) their conversion into foods or petrochemical synthesis products does not result in notable chemical or heat pollution of the environment; (3) their long-term exploitation, free from substantial disturbance of the ecological or geothermal balance of the Earth, is rendered possible by recycling. All these requirements are met (or can be met) by natural carbohydrate biopolymers. The most important of them are cellulose, hemicellulose and a number of other plant-origin polysaccharides. Their processing into respective monomers yields, with subsequent bioconversion, valuable materials to be used in food, microbiological, petrochemical, power and medical industries.

The world natural production of cellulose is estimated by different sources at 110 to 700 billion tons per year. The utilization of this raw material in agriculture, woodworking and other industries results in the accumulation of considerable amounts of wastes.

A vital scientific and technological objective of today is the development of approaches to converting carbohydrates from their inedible forms to food sugars, The degradation of cellulose to glucose can be performed by physical, chemical and enzymatic methods. In many respects, optimal is the biotechnical conversion of cellulose-containing raw materials. Enzymatic hydrolysis requires much softer conditions as opposed to acid hydrolysis, and the substances obtained contain far fewer by-products. The glycose syrup yielded by enzymatic cellulose hydrolysis is important as a component of a micro-organism growing medium (in particular, fodder protein), as a raw material for subsequent fermentation, e.g. for the production of alcohols, drugs, etc.) and as a semiproduct for subsequent chemical or enzymatic conversions (e.g. for producing the sweet glucose-fructose syrup). All the above accounts for the interest in the enzymes capable of converting cellulose into glucose shown in virtually all the developed countries.

One of the most promising fields of modern biotechnology is engineering enzymology. This new field unites the approaches of a wide range of natural sciences, primarily chemical enzymology, biochemistry, chemical technology and engineering economy. The major objective of engineering enzymology is the development of biotechnological processes using the catalytic action of enzymes normally isolated from biological systems.

Among the most significant trends in engineering enzymology, both fundamental and applied, is the bioconversion of recycled raw materials. Depending on the raw material type, final product and technology, it may include preliminary processing of the raw material, its enzymatic destruction to monomers, fermentation of the monomers into the final product, or direct microbiological conversion of the raw material into the intermediate or final product. The processes for which effective technologies are expected are as follows:

1. Enzymatic production of glucose from cellulose-containing raw materials (industrial and agricultural wastes).

2. Bioconversion of cellulosic and lignocellulosic materials into ethanol.

3. Hydrolytic (possibly, oxidative-hydrolytic) destruction of plant biomass to enhance its nutritive value for livestock.

4. Enzymatic or microbiological destruction of lignin to obtain alkylphenol, oxyphenol and other phenol derivatives as likely initial products of polymer chemistry.

It is believed that the processes 1 and 2 will have been implemented (to an adequate extent) industrially in 1986-1996, the other two, not until 1990-2000. The effective implementation of the above biotechnological processes will largely depend on the progress of molecular-level (fundamental) studies on the enzymic (microbiological) conversion of plant multi-component materials. In other words, the priority of the fundamental biotechnical research into recycled natural (plant) resources will be decisive.

It appears that the first biotechnology to be introduced industrially on a large scale will be the enzymatic production of glucose from cellulose-containing industrial and agricultural wastes. Along with fructose, the product of its enzymatic isomerization, glucose is a valuable food and medical product. In addition, glucose is a major component of nutritive media for the microbiological production of diverse compounds: vitamins, antibiotics, amino acids, etc.

The enzymatic hydrolysis of cellulose proceeds under the action of the so-called cellulase complex, containing several enzymes. These enzymes are well adsorbed on cellulose and remain associated with it in the course of gradual degradation of the substrate, so the process concerned is a reaction under the impact of immobilized (more precisely, auto-immobilized) enzymes. The reaction yields glucose or a mixture of oligosaccharides (largely, glucoses and cellobioses). The composition of hydrolysis products is largely a function of that of the cellulase enzyme complex and the type of the cellulose-containing raw material. In addition to the direct sugar production, the latter can be fermented by yeast, without isolation from the reaction system, to yield ethanol.

Major projects on cellulose enzymic hydrolysis are currently implemented in the USA, Japan, Sweden, Finland and India (on a smaller scale, the work is also being done in Poland, Czechoslovakia and Bulgaria). These projects are primarily aimed at producing ethanol but not food glucose, which is explained by several reasons. One of these is the energy situation. In addition, to produce food glucose from cellulose, the initial raw material should meet much more rigid requirements (an increased content of pure cellulose). Similarly rigid are the requirements for the enzyme preparations which should be definitely balanced for individual components of the cellulase complex, so that cellulose hydrolysis should yield glucose but not a mixture of cellulooligosaccharides which are not suitable food products.

As compared with other countries, the fundamental and applied studies on the enzymatic production of glucose from cellulose have been advanced.

In recent years, the USSR Academy of Sciences jointly with other organizations has carried out both fundamental and applied integrated research into enzymatic conversion of cellulose into glucose. The inter-stimulation of the above approaches has made it possible to elaborate the fundamentals of a number of new trends in physico-chemical enzymology and biotechnology of carbohydrates (e.g., with special reference to the development of the kinetic theory of the action of consequtively-parallel poly-enzymatic system, the revealing and generalization of the regularities of biopolymer conversion of enzymes and poly-enzymatic systems, elucidation of the role of physico-chemical and structural factors of insoluble biopolymers in the effectiveness of their enzymatic conversion, establishing the regularities of enzyme adsorption and their catalytic action on the surface of amorphous and crystallic polysaccharides. On the other hand, original instruments have been designed and new techniques of enzymatic conversion of cellulose into glucose developed as based on the understanding of the regularities of adsorption and kinetics of the action of cellulolytic enzymes. In the USSR, all the major aspects of the problem concerned: raw materials, enzymes, the process and instrumentation have been treated successfully, and currently a transition is being completed from a large-scale laboratory process to the development of a pilot plant for enzymatic conversion of cellulose into glucose.

The ensuring of large-scale industrial utilization of cellulose-containing-wastes through the action of enzymes would provide a new raw-material basis for sugar production whose importance can hardly be over-estimated. The major stages in the practical implementation of the enzymatic hydrolysis of cellulose-containing raw materials are as follows:

1. Choice of readily-available cellulose-containing raw materials whose conversion into glucose would be feasible both economically and ecologically.

2. Elaboration of the techniques of economically-effective and ecologically-pure preliminary processing of cellulose-containing raw materials to enhance the speed of enzymatic hydrolysis and the yield of the final product.

3. Microbiological production of a complex of cellulolytic enzymes with a high action effectiveness in relation to cellulose.

4. Engineering aspect of the process including designing a reactor for an effective enzymatic production of glucose from cellulose, regeneration of the enzymes and their recycling to provide a waste-free production and optimization of the process.

In the Soviet Union, all the above four problems of biotechnology are being successfully solved. Projects on the production of the cellulase-complex enzymes and their effect on cellulose-containing substances are under way in a number of the institutes of the USSR Academy of Sciences and republican Academies and also in other bodies.

The most suitable type of cellulose-containing raw material for enzymatic conversion into glucose is cotton -industry waste: short-stapled cotton (which is nearly pure cellulose) and cotton-plant stems. Short-stapled cotton is not utilized in textile industry forming several hundreds of thousands of tons of waste annually over the USSR. By means of biotechnological methods (enzymatic hydrolysis), one ton of short-stapled cotton can yield 0.5 tons of glucose with an economic effect of over 300 roubles per 1 ton of glucose per year. The greatest cotton waste are cotton-plant stems whose harvest may reach several millions of tons per year. One ton of cotton-plant stems is estimated to yield 0.8 tons of cellulose. This amount of cellulose can yield an amount of glucose, which, even by tentative estimates, is equal, with subsequent conversion into fructose, to 0.6-0.8 tons of sugar. The economic effect of the implementation of such production will amount to 300 to 500 roubles per 1 ton of cellulose annually.

The successful solution of the problem of biotechnological conversion of cotton wastes into sugars will, in its turn, provide insight into polysaccharification of timber wastes to provide fodder supplements for livestock.