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SYNTESIS OF ENZYMES IN MICROORGANISMS AND THEIR APPLICATION IN BIOTECHNOLOGIES

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Abstract. This article highlights the principles of enzyme biosynthesis processes carried out by microorganisms (bacteria and fungi) as well as the prospects for the application of these biocatalysts in modern biotechnology fields. During the research, the industrial-scale extraction of microbial enzymes, methods for increasing their activity, and their stability indicators were analyzed. In addition, the practical significance of enzymes in the food, pharmaceutical, textile, and environmental biotechnology sectors (waste utilization) was demonstrated through specific examples. The obtained results substantiate the economic efficiency and environmental safety of microbial synthesis products.

Keywords: microorganisms, enzyme synthesis, biotechnology, biocatalyst, biosynthesis, industrial fermentation, food industry.

Introduction

The development of biotechnology and microbiology, as well as the production of high value-added products from local raw materials, is currently considered one of the priority directions of state policy in the Republic of Uzbekistan. In particular, the Law of the Republic of Uzbekistan “On Science and Scientific Activity” and the Resolution of the President of the Republic of Uzbekistan No. PQ-4899 dated November 25, 2020, “On Measures for the Development of Biotechnology and the Improvement of the System for Ensuring the Country’s Biological Safety,” serve as a legal basis for accelerating modern research in this field. Within the framework of these strategic objectives, establishing enzyme synthesis through microorganisms and introducing them into industry are of great economic and environmental importance. Due to their high specificity, cost-effectiveness, and environmental friendliness, microbial enzymes fundamentally differ from chemical catalysts and are becoming a driving force in the food, pharmaceutical, and textile industries. This study scientifically analyzes the principles of bacterial and fungal biosynthesis and the issues of their effective application in biotechnological processes in order to implement the tasks set by the state regarding the introduction of innovative technologies into practice.

Main part

The expression and biosynthesis of enzymatic proteins in microbial cells represent a complex set of molecular and biochemical cascades strictly regulated at the stages of transcription and translation. Producer and destructor strains used in industrial microbiology regulate their metabolism through repression and induction mechanisms depending on the components of the external nutrient medium, thereby ensuring the synthesis of constitutive and adaptive (inducible) enzymes. For example, under conditions of catabolite repression (the glucose effect), when easily assimilated carbohydrates are present in the nutrient medium, the cell completely blocks the transcription of hydrolase genes responsible for degrading complex polymers by reducing the level of cyclic adenosine monophosphate (cAMP). The secretion of exoenzymes outside the cell membrane is carried out by special signal peptides (signal sequences) and Sec or Tat transport systems, and this process is directly dependent on ATP hydrolysis from an energetic point of view.

The relationship between microbial population growth kinetics and the synthesis of target biocatalysts is analyzed using the Monod, Michaelis–Menten, and Luedeking–Piret mathematical models, which quantitatively characterize whether product formation is growth-associated or non-growth-associated in nature. In industrial bioreactors, maintaining an optimal balance of osmotic pressure in the nutrient medium, dissolved oxygen partial pressure (pO2), pH level, and temperature gradients is essential for maximizing enzymatic productivity, since any disruption in microcirculation may lead the cell to switch to the synthesis of "stress proteins." Modern genetic engineering and CRISPR-Cas9 technologies are used to enhance promoter regions of producer strains, introduce plasmid vectors ensuring high protein expression, and generate targeted point mutations in the genetic code of enzymes in order to increase their thermostability.

The efficiency of biotechnological processes is determined by the correct selection and classification of microbial strains capable of degrading specific substrate types with high specificity in the environment, according to the International Enzyme Classification (EC) system. Amylolytic enzymes, which hydrolyze starch polymers into oligosaccharides by cleaving α-1,4-glycosidic bonds, are synthesized in high titers mainly by thermophilic and mesophilic microorganisms such as Bacillus subtilis, Bacillus amyloliquefaciens, and Aspergillus oryzae.

The cellulase enzyme complex, consisting of endoglucanase, exoglucanase, and β-glucosidase, is capable of degrading lignocellulosic biomass, one of the most abundant renewable resources in nature. These enzymes are produced as metabolic products of the fungus Trichoderma reesei and are widely used in industry for the complete saccharification of plant tissues. Proteolytic enzymes (alkaline, neutral, and acidic proteases), which hydrolyze peptide bonds in proteins, are isolated from the culture liquids of Bacillus licheniformis and Aspergillus niger. These enzymes are distinguished by their ability to maintain stability even in the presence of high pH values and surfactants.

The lipase group of enzymes, which break down hydrophobic lipids into glycerol and free fatty acids, is produced through inducible pathways by Pseudomonas fluorescens bacteria and Candida rugosa yeasts. These enzymes catalyze not only hydrolysis reactions but also esterification processes in non-aqueous environments. Pectinase and xylanase enzymes, which degrade pectin substances in plant raw materials and facilitate juice extraction, are produced using fungi belonging to the genera Aspergillus and Penicillium through solid-state or submerged fermentation methods.

These industrial microbiology objects are considered leading producers in the global biotechnology market due to their genetic stability and ability to grow rapidly even on inexpensive substrates. Depending on their field of application, the resulting enzyme preparations are purified to high activity levels through membrane ultrafiltration or chromatographic methods. The study of the physiological characteristics of microbial strains serves as an important foundation for reducing the production cost of industrial biocatalysts and modernizing local manufacturing processes. In order to preserve the enzymatic activity of these microbial hydrolases, various metal ions such as Ca^{2+} and Mg^{2+} are used as cofactors, significantly enhancing their thermal stability.

As a result of screening studies conducted by scientists, new extremophilic strains capable of functioning under extreme conditions, including high salinity and elevated temperatures, are being isolated, and their enzyme genes are actively analyzed. A deeper understanding of the nature of biocatalytic processes and their kinetic properties ultimately guarantees the production of high-quality import-substituting enzymatic products for the food and pharmaceutical industries.

Table 1
Biotechnological Characteristics of Major Industrially Important Microbial Enzymes

Enzyme Group (EC Code)	Typical Producer Strain	Specific Substrate	Optimal Industrial Working Conditions
α-Amylase (EC 3.2.1.1)	Bacillus licheniformis	Starch (α-1,4-glycosidic bond)	Temperature: 70–90 °C, pH: 6.0–7.0
Alkaline Protease (EC 3.4.21.62)	Bacillus clausii	Native proteins (peptide bond)	Temperature: 50–60 °C, pH: 9.0–11.0
Endoglucanase (EC 3.2.1.4)	Trichoderma reesei	Amorphous cellulose	Temperature: 45–55 °C, pH: 4.8–5.5
Triacylglycerol Lipase (EC 3.1.1.3)	Burkholderia cepacia	Triglycerides (fats)	Temperature: 37–45 °C, pH: 7.5–8.5
Endo-polygalacturonase (EC 3.2.1.15)	Aspergillus niger	Pectic acid	Temperature: 40–50 °C, pH: 3.5–4.5

Modern food and textile industries cannot be imagined without biocatalysts, since they not only increase reaction rates by 10^8 to 10^{12} times compared to chemical reagents, but also significantly reduce energy consumption. In the bakery industry, microbial amylases and xylanases partially hydrolyze starch and pentosans in dough, improving gas retention capacity, increasing bread volume, and slowing down the staling process.

In the dairy industry, recombinant chymosin (rennin) enzyme produced through genetic engineering from Kluyveromyces lactis yeast is used instead of traditional calf rennet. This enzyme ensures the specific coagulation of proteins during cheese production and improves the organoleptic properties of the final product. In juice and wine production, the use of microbial pectinases decreases the viscosity of fruit and berry raw materials, increases extraction yield by 15–20%, and completely destroys colloidal particles responsible for turbidity, thereby clarifying the final juice product.

Cocktails of proteases, lipases, and amylases incorporated into synthetic detergents are capable of degrading blood, juice, fat, and food stains at the molecular level even at relatively low temperatures of 30–40°C, which significantly reduces household energy consumption. In the textile industry, cellulases are used for the microhydrolysis of fine fibers on the surface of cotton and denim fabrics, providing softness to the material (biopolishing), while environmentally friendly biobleaching technologies without chlorine compounds have been fully implemented.

Fruit Raw Material (High Pectin Content) + Pectinase Enzyme
→ (Hydrolysis process: degradation of colloids)
→ Viscosity ↓
→ Juice yield ↑ (15–20% higher) and excellent clarity

The extremely high stereospecificity and biocompatibility of microbial enzymes have opened new prospects for their application in pharmaceutical industries, both in drug synthesis and as therapeutic agents in clinical medicine. In medical biotechnology, the enzyme L-asparaginase isolated from Escherichia coli strains plays a crucial role in the treatment of acute lymphoblastic leukemia because it breaks down the amino acid L-asparagine in the bloodstream, which tumor cells are unable to synthesize independently. As a result, cancer cells are deprived of nutrients and undergo apoptosis (cell death).

In cardiovascular pathologies, particularly acute myocardial infarction and thromboembolism, the enzyme streptokinase derived from Streptococcus bacteria is considered one of the most widely used emergency thrombolytic drugs for dissolving fibrin clots in blood vessels.

Microbial laccases and peroxidases are widely used in the degradation of xenobiotics, phenols, and synthetic dyes contained in industrial wastewater. These biocatalysts decompose toxic compounds into non-toxic oligomers or ultimately into carbon dioxide and water through radical-based mechanisms. In addition, the enzymatic degradation of cellulose- and lignin-rich agricultural residues by microbial enzyme complexes serves as the basis for the production of second-generation bioethanol and biobutanol, which are important components of green energy technologies.

Currently, technologies for the immobilization of microbial enzymes onto natural polymers such as chitosan and alginate, as well as onto magnetic nanometal frameworks, are rapidly developing in order to stabilize enzymes and enable their repeated use. These approaches reduce the cost of biotechnological processes and facilitate the transition toward continuous automated cassette bioreactor systems.

Conclusion

The study of enzyme biosynthesis in microorganisms and their biotechnological applications remains one of the leading directions of modern science. Due to their high specificity, environmental safety, and economic efficiency, microbial biocatalysts are gradually replacing traditional heavy chemical technologies in industrial production. It has been practically proven that bacteria and fungi, through the regulation of metabolic processes, represent powerful resources capable of bringing revolutionary changes to the food, textile, and pharmaceutical industries.

In particular, the enzymatic potential of these microorganisms is virtually limitless in the fields of waste utilization and the development of environmentally friendly bioenergy sources. Increasing strain productivity through innovative methods such as genetic engineering and CRISPR-Cas9 technologies guarantees a future transition toward completely eco-friendly and waste-free “green economy” principles. The microbial enzyme industry is considered one of the main driving forces of the biotechnological revolution aimed at rebuilding the foundations of human industry in harmony with nature.

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