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Production of enzymes by biotechnological methods: A review

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Abstract: *enzymes are essential biological catalysts widely utilized in diverse industries such as pharmaceuticals, food processing, biofuels, and environmental technology due to their high specificity, efficiency, and eco-friendliness. With increasing global demand, conventional methods of enzyme extraction have become inadequate, leading to the development of advanced biotechnological approaches. The aim of this review is to analyze contemporary strategies for enzyme production using biotechnological methods, evaluate their benefits and limitations, and explore emerging trends aimed at improving enzyme yield, stability, and industrial applicability. The materials and methods used in this review involved a comprehensive analysis of scientific publications over the past three years obtained from international databases, using keywords such as enzyme production, fermentation, recombinant DNA, and protein engineering. The data were synthesized through critical review of original research, review articles, and industrial reports. Biotechnological enzyme production predominantly relies on microbial systems due to their rapid growth, adaptability, and cost-effective fermentation capabilities. Bacteria, fungi, and yeasts are employed in submerged or solid-state fermentation, offering scalability and precise process control. Genetically engineered microorganisms, particularly Escherichia coli, Saccharomyces cerevisiae, and Pichia pastoris, are frequently used as expression hosts, enabling high-yield production and post-translational modifications. The review highlights the significance of recombinant DNA technology, emphasizing cloning strategies, vector design, expression optimization, and fusion proteins for improved purification and secretion. Protein engineering techniques such as rational design and directed evolution allow the fine-tuning of enzyme properties, enhancing thermal stability, pH tolerance, and substrate specificity. These advances have had transformative effects on the pharmaceutical sector, enabling efficient drug synthesis and delivery systems, and supporting sustainable production practices across industries. Although plant- and animal-derived enzymes retain specific industrial roles, microbial enzymes remain dominant due to their robustness and efficiency. Fermentation techniques are central to enzyme production, with submerged fermentation favored for its automation potential, and solid-state fermentation offering higher concentrations and lower costs. The integration of synthetic biology, metagenomics, and AI-driven process control is expected to redefine future enzyme production. In conclusion, biotechnological methods have significantly enhanced the feasibility, scalability, and sustainability of industrial enzyme production. Continued innovation in genetic and protein engineering, coupled with process optimization, promises to expand enzyme applications across emerging industrial sectors.*

Keywords: Biotechnology, DNA, Enzymes Recombinant, Fermentation, Protein Engineering

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Introduction

Enzymes are biological catalysts that accelerate chemical reactions by lowering the activation energy without being consumed in the process. They play critical roles in physiological processes and industrial applications due to their specificity, catalytic efficiency, and eco-friendly nature. Global demand for enzymes continues to rise, driven by their applications in sectors such as food technology, pharmaceuticals, detergent production, biofuels, and environmental remediation. Traditional extraction of enzymes from natural sources is often costly and inefficient, prompting the development of advanced biotechnological methods for enzyme production.

Biotechnology-based strategies, particularly those employing microbial systems and genetic engineering, have become the cornerstone of modern enzyme manufacturing. These methods enable controlled, scalable, and sustainable production of enzymes with desired properties. This article reviews the current biotechnological approaches for enzyme production, highlighting traditional and modern techniques, advancements in strain improvement, and innovations in process optimization [1].

Aim

The purpose of this review is to analyze contemporary biotechnological methods for enzyme production, evaluate their advantages and limitations, and explore emerging trends that enhance enzyme yield, stability, and functional diversity for industrial applications.

Materials and methods

This review is based on an analysis of scientific literature published over the last three years, retrieved from databases including **PubMed**, **Scopus**, **Web of Science**, and **Google Scholar**. The methods involved critical review of original research articles, review papers, and industrial reports, focusing on microbial sources, genetic engineering approaches, and bioprocess optimization techniques.

Review and discussion

The use of enzymes in industrial sectors has increased substantially due to their high specificity, catalytic efficiency, and environmentally friendly nature. Biotechnological methods, including fermentation and genetic engineering, have made large-scale production of enzymes feasible and cost-effective. These enzymes are applied in numerous industries to improve process efficiency, product quality, and sustainability. One of these industries is **Pharmaceutical**.

Biotechnologically produced enzymes have transformed pharmaceutical manufacturing by enabling stereospecific reactions, reducing the need for hazardous chemicals, and increasing overall process efficiency.

Enzymes, as essential biological catalysts, can be obtained from a variety of natural sources, each offering specific advantages, limitations, and potential applications in industrial biotechnology. The principal sources for large-scale enzyme production are microorganisms, plants, and animals. [3]. The selection of an appropriate source is guided by factors such as the physicochemical properties of the desired enzyme, production cost, scalability, and the simplicity of downstream purification.

Among the available sources, **microorganisms** are the most extensively exploited for industrial enzyme production due to their distinct benefits over plant and animal systems. Bacteria, fungi, and yeasts are especially preferred because of their rapid proliferation, adaptability to diverse environmental conditions, and suitability for high-yield fermentation processes. Microbial enzymes are typically produced using submerged fermentation (SmF) or solid-state fermentation (SSF), both of which enable precise control of growth parameters and enhanced enzyme output [2, 4].

One major advantage of microbial systems is their cost-effectiveness, as they can be cultivated on low-cost substrates such as agricultural residues and industrial waste. Furthermore, genetic engineering allows for the manipulation of microbial genomes to improve enzyme yield, enhance operational stability under extreme conditions, and modify catalytic characteristics. This adaptability is particularly beneficial for industrial processes conducted under high temperatures, fluctuating pH, or in the presence of organic solvents.

Bacterial strains, such as those from the *Bacillus* genus, are renowned for producing thermostable amylases and proteases, extensively utilized in the detergent and textile industries. Other genera, including *Pseudomonas* and *Streptomyces*, serve as prolific sources of lipases and cellulases [5]. Fungal microorganisms, particularly filamentous fungi like *Aspergillus* and *Trichoderma*, are highly valued for their ability to secrete substantial amounts of extracellular enzymes such as cellulases, xylanases, and pectinases, which are widely used in food, paper, and biofuel industries.

Yeasts, including *Saccharomyces cerevisiae* and *Pichia pastoris*, are commonly employed in recombinant DNA technology as expression systems for heterologous enzyme production. These eukaryotic hosts provide efficient post-translational modifications while being amenable to large-scale fermentation. Collectively, microbial systems dominate industrial enzyme biotechnology owing to their sustainability, scalability, and broad enzymatic diversity.

Plants remain a traditional but important source of industrial enzymes, where they provide specific catalytic properties that are difficult to replace. According to Cowan and Tombs (2022), examples include amylases derived from barley that are essential for brewing processes, papain obtained from papaya latex widely used as a natural meat tenderizer, and bromelain from pineapple stems, which demonstrates notable anti-inflammatory activity and finds applications in both food technology and pharmaceutical formulations. Beyond their role in food technology, plant-derived enzymes are increasingly significant in the pharmaceutical industry. They are applied not only as active pharmaceutical ingredients but also as auxiliary agents in drug formulation and targeted delivery systems. Their therapeutic potential–ranging from anti-inflammatory effects to digestive and proteolytic functions–underpins the development of innovative medicines and biopharmaceuticals. Moreover, advances in biotechnological production of plant enzymes are improving yield, stability, and scalability, strengthening their relevance for pharmaceutical biotechnology and industrial healthcare solutions [6-9].

Despite these applications, large-scale production of plant-derived enzymes is hampered by several constraints, including seasonal variability, inconsistent enzyme yield and activity, and the necessity for labor-intensive extraction and purification procedures. While microbial fermentation is generally more efficient, plant enzymes retain relevance in specialized applications where their specific catalytic features are indispensable [8].

Animal tissues and organs also serve as traditional enzyme sources, predominantly in the medical and pharmaceutical sectors. Enzymes such as trypsin, chymotrypsin, and lipase–extracted from the pancreas – are commonly used in digestive therapies and in peptide drug synthesis. Another well-known enzyme of animal origin is rennin (chymosin), obtained from calf stomachs and employed in cheese manufacturing [7].

Nonetheless, the industrial use of animal-derived enzymes has declined considerably due to ethical concerns, risk of disease transmission, supply inconsistencies, and elevated production costs. Furthermore, these enzymes often exhibit lower stability and specificity relative to their microbial counterparts, making them less suitable for industrial-scale use. Nevertheless, they remain important in niche fields such as pharmaceuticals and clinical diagnostics.

Fermentation processes are central to modern enzyme production, leveraging microorganisms as efficient biological factories. The two most prominent methods are **submerged fermentation (SmF) and solid-state fermentation (SSF)**, each differing in substrate type, moisture content, and operational parameters, all of which influence productivity and enzyme yield.

SmF involves cultivating microorganisms in a liquid nutrient medium under strictly controlled environmental conditions. This method is widely employed for the production of key industrial enzymes including amylases, proteases, lipases, and cellulases, which find extensive application in food processing, pharmaceuticals, and biofuel [10, 11].

In SmF, microorganisms–typically bacteria or fungi–are cultivated in sterile bioreactors supplied continuously with carbon, nitrogen, minerals, and essential growth factors. Adequate agitation and aeration are critical not only for ensuring oxygen transfer, nutrient distribution, and homogeneity,

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but also for maintaining the stringent process control required in pharmaceutical manufacturing. The use of SmF allows real-time monitoring and adjustment of pH, temperature, and oxygen levels, making the method highly scalable and compatible with Good Manufacturing Practice (GMP) standards and automation frequently demanded in pharmaceutical enzyme production for replacement therapy (lactase for lactose intolerance, pancreatin for enzyme deficiency), antibiotics synthesized by microorganisms (Penicillium → penicillin), recombinant proteins (insulin, interferons, monoclonal antibodies). (Patel et al., 2023). Furthermore, well-established downstream purification methods, such as centrifugation and filtration, are efficiently applied to recover enzymes with the high purity necessary for pharmaceutical applications. However, SmF also presents several challenges, including high water and energy consumption for aeration and agitation, and greater susceptibility to microbial contamination. In addition, enzymes are often produced in dilute form within large liquid volumes, requiring additional concentration and purification steps–processes that are particularly critical in ensuring the safety, efficacy, and regulatory compliance of pharmaceutical-grade enzyme preparations. [9, 12-13].

SSF serves as an alternative method wherein microorganisms are cultured on moist solid substrates in the absence of free-flowing water. Typical substrates include agro-industrial residues such as wheat bran, rice husk, corn cob, soybean meal, and sugarcane bagasse, which act both as nutrient sources and physical supports.

This technique is particularly effective for cultivating filamentous fungi like *Aspergillus*, *Penicillium*, and *Rhizopus*, which thrive in low-moisture conditions and can efficiently penetrate solid matrices. SSF is especially advantageous for the production of enzymes such as cellulases, xylanases, amylases, and proteases used in the feed, textile, and paper industries [4, 9].

SSF offers several benefits, including low water usage, higher enzyme concentration, and reduced production costs due to the utilization of inexpensive substrates. Nonetheless, the technique faces challenges in process monitoring and control, as parameters such as temperature, moisture, and oxygen levels may vary unevenly within the solid matrix. Additionally, limited scalability and labor-intensive extraction procedures pose constraints on widespread industrial adoption [14-16].

The advent of recombinant DNA technology has transformed enzyme production by enabling gene manipulation and expression in heterologous hosts. This strategy significantly improves enzyme yield, functional stability, and scalability [17-20]. Common expression hosts include *Escherichia coli*, *Saccharomyces cerevisiae*, and *Pichia pastoris*, each offering unique advantages in terms of growth kinetics and post-translational processing.

Principle of Recombinant Enzyme Production. The process begins with isolating or synthesizing the target gene, which is inserted into a suitable vector–typically a plasmid–designed for replication and expression in the host organism. Advanced cloning techniques such as Gibson assembly and CRISPR/Cas systems ensure precise gene insertion. Vectors contain regulatory elements like promoters, ribosome-binding sites, and selectable markers that facilitate effective transcription, translation, and identification of transformed cells [21].

Gene Cloning and Overexpression. A key benefit of recombinant technology is the ability to overexpress the enzyme-coding gene using inducible promoters, such as the lac promoter in *E. coli* or AOX1 in *P. pastoris.* Codon optimization further enhances expression efficiency by aligning gene codons with host preferences, particularly when expressing genes from thermophilic organisms in mesophilic hosts [22].

Role of Fusion Proteins and Signal Peptides. Fusion tags (e.g., His-tag, GST, MBP) aid in protein solubility, folding, and affinity purification. Signal peptides, such as the α-mating factor from *S. cerevisiae*, direct the recombinant enzyme to the secretory pathway, facilitating extracellular release and simplifying purification [23].

Expression Systems and Their Applications. *E. coli* is widely used for its rapid growth and high expression capability but lacks glycosylation machinery, making it less suitable for complex eukaryotic enzymes. *S. cerevisiae* and *P. pastoris*, in contrast, provide efficient post-translational modifications and are preferred for secreted or glycosylated enzymes [24].

Protein engineering enables the modification of enzyme structures to enhance their performance under industrial conditions. Given that natural enzymes are often optimized for physiological environments, their functionality may be limited in high-temperature, high-pH, or solvent-rich processes. Two principal strategies–rational design and directed evolution–are employed to overcome these limitations.

This approach relies on detailed structural and mechanistic knowledge, allowing specific amino acid substitutions via site-directed mutagenesis. Structural insights are typically derived from X-ray crystallography, NMR spectroscopy, or computational modeling. Modifications may improve solubility, thermal stability, or alter substrate specificity. For example, lipases have been engineered for increased methanol tolerance in biodiesel synthesis, and cellulases modified for high-temperature biomass hydrolysis.

However, rational design requires comprehensive knowledge of enzyme structure-function relationships, which is not always available, and may yield unpredictable results due to complex intramolecular interactions.

Directed evolution mimics natural selection by generating large libraries of mutated genes and screening for improved variants. Random mutagenesis techniques such as error-prone PCR or DNA shuffling introduce genetic diversity. Promising mutants are iteratively refined through multiple selection cycles.

This method has yielded enzymes with enhanced thermal tolerance, solvent resistance, and catalytic efficiency. Subtilisin proteases and thermostable polymerases are prominent industrial examples. Notably, Frances Arnold received the 2018 Nobel Prize in Chemistry for pioneering this technique, demonstrating its transformative potential in tailoring enzymes for industrial and medical applications.

Conclusions

Biotechnological methods have fundamentally transformed enzyme production, offering scalable, efficient, and sustainable alternatives to traditional extraction techniques. The use of microbial systems, particularly genetically engineered strains such as E. coli, S. cerevisiae, and P. pastoris, has significantly increased enzyme yield and allowed for fine-tuning of catalytic properties. Fermentation techniques, including submerged and solid-state fermentation, have enabled precise control over production parameters and reduced operational costs. Recombinant DNA technology and advanced cloning strategies have facilitated high-level expression of enzymes with improved stability and specificity. Protein engineering, through rational design and directed evolution, has expanded the functional diversity of enzymes for challenging industrial conditions. While plant and animal enzymes retain niche applications, microbial sources dominate due to their adaptability and productivity. The integration of synthetic biology, metagenomics, and AI-driven process optimization is expected to drive further innovation in this field. Despite some challenges related to regulatory compliance and production scalability, biotechnological enzyme production continues to evolve rapidly.

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Conflict of interests

No conflict of interests.

Consent to publication

All authors have got consent to publication of this article.

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Виробництво ферментів біотехнологічними методами: огляд

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Кафедра управління та економіки фармації з технологією ліків, Тернопільський національний медичний університет імені І. Я. Горбачевського Міністерства охорони здоров'я України, м. Тернопіль, Україна.

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Анотація: *ферменти є незамінними біологічними каталізаторами, які широко застосовуються в різних галузях промисловості, зокрема у фармації, харчовій промисловості, виробництві біопалива та екотехнологіях, завдяки високій специфічності, ефективності та екологічності. Зі зростанням глобального попиту традиційні методи отримання ферментів стали недостатніми, що зумовило розвиток сучасних біотехнологічних підходів. Метою даного огляду є аналіз сучасних стратегій біотехнологічного виробництва ферментів, оцінка їхніх переваг і недоліків, а також вивчення новітніх тенденцій, спрямованих на підвищення виходу, стабільності та промислової застосовності ферментів. Матеріали й методи огляду базувалися на всебічному аналізі наукових публікацій останніх трьох років, отриманих з міжнародних баз даних за ключовими словами: виробництво ферментів, ферментація, рекомбінантна ДНК, білкова інженерія. Дані були узагальнені шляхом критичного аналізу оригінальних досліджень, оглядових статей та галузевих звітів. Біотехнологічне виробництво ферментів переважно ґрунтується на використанні мікроорганізмів, завдяки їх швидкому росту, адаптивності та рентабельності ферментаційних процесів. Бактерії, гриби та дріжджі використовуються в зануреній або твердофазній ферментації, що забезпечує масштабованість та точний контроль процесу. Генетично модифіковані мікроорганізми, зокрема Escherichia coli, Saccharomyces cerevisiae та Pichia pastoris, часто використовуються як експресійні системи, що дає змогу отримувати високі виходи ферментів із посттрансляційними модифікаціями. В огляді підкреслюється значення технології рекомбінантної ДНК, з акцентом на стратегії клонування, конструкцію векторів, оптимізацію експресії та використання злитих білків для покращення очищення та секреції. Методи білкової інженерії, зокрема раціональний дизайн і спрямована еволюція, дають змогу тонко налаштовувати властивості ферментів, підвищуючи їх термостабільність, толерантність до pH та специфічність до субстрату. Ці досягнення суттєво вплинули на фармацевтичну галузь, забезпечуючи ефективний синтез лікарських засобів і систем доставки, а також підтримуючи сталий характер виробництва в різних галузях. Незважаючи на наявність промислових ніш для ферментів рослинного та тваринного походження, мікробні ферменти залишаються домінантними завдяки своїй надійності та ефективності. Методи ферментації є центральними для виробництва ферментів, причому занурена ферментація є кращою завдяки своєму потенціалу автоматизації, а твердофазна ферментація пропонує вищі концентрації та нижчі витрати. Інтеграція синтетичної біології, метагеноміки та управління процесами на основі штучного інтелекту очікувано визначатиме майбутнє ферментативного виробництва. Отже, біотехнологічні методи значно підвищили доцільність, масштабованість і сталість промислового виробництва ферментів. Подальші інновації в галузі генетичної та білкової інженерії разом з оптимізацією процесів сприятимуть розширенню застосування ферментів у нових промислових секторах.*

Ключові слова: біотехнологія, ДНК, рекомбінантні ферменти, ферментація, білкова інженерія.