

ENZYME PRODUCTION WITH FUNGAL CO-CULTURES

Iosvany López-Sandi¹, Roberto Parra Saldivar¹, H.N.M. Iqbal¹, Monserrat Franco Flores¹, Diana Castillo Martínez¹, Guadalupe Gutiérrez Soto¹*

Biomolecular Innovation Group, Facultad de Agronomía, Universidad Autónoma de Nuevo León.

Francisco Villa S/N, Col. ExHacienda El Canadá 66415, General Escobedo, N.L., México.

*Correspondencia: ggutierrez0402@gmail.com

n the field of modern biotechnology, enzymatic production through co-cultures is presented as an innovative and promising strategy, capable of overcoming the limitations of traditional monoclonal cultures. Fermentation practices have been able to take advantage of the natural synergy between various microorganisms, but their targeted application to obtain specific enzymes is a recent development. While this technique offers great potential, it also presents significant challenges that need to be addressed for its successful implementation. These include the control of microbial interactions, the optimization of culture conditions and the scalability of processes, which face both technical and economic difficulties. Despite these challenges, the prospects for co-crops are very optimistic. Continuous advances in synthetic biology, consortia engineering and modelling technologies will overcome these obstacles and position co-cultures as a fundamental tool in industrial biotechnology. In this way, they are expected to contribute to more efficient, sustainable and profitable enzyme production. The objective of this article is to detail the most relevant studies on enzyme production using co-cultures. To this end, an exhaustive review of the scientific literature will be carried out, identifying the production potential of this technique and exploring its applications in various industrial areas.

Introduction

Enzymes, catalytic biomolecules produced by living organisms, play a fundamental role in various biological processes. Their ability to accelerate specific chemical reactions makes them indispensable tools in various industries, such as food, pharmaceuticals, textiles, and biofuels. Enzyme production has traditionally been based on the cultivation of individual microorganisms, using simple substrates and controlled culture conditions. However, this approach has limitations in terms of productivity, the complexity of the enzymes obtained and environmental sustainability [1].

Enzyme production is a complex biotechnological process that involves the generation of enzymes, which are highly specific and sensitive biocatalyst proteins essential for various industrial applications, including food processing, pharmaceuticals, textiles, and more [2]. The process begins with the selection of enzyme sources, which can be microorganisms, plants or animals, with microbial sources being preferred due to their techno-economic advantages [3-4]. Enzymes can be produced through fermentation processes, which are classified into submerged and solid-state fermentation techniques. The production process can be optimized using genetic engineering and other modern techniques to improve yield and efficiency [2]. Agro-industrial waste, such as lignocellulosic biomass, is often used as substrates to reduce production costs and address environmental concerns related to waste disposal [4]. Agricultural enzyme production technology, for example, involves fermenting waste raw materials such as vegetable stems and leaves to create fertilizers that improve crop yields and quality [5]. After fermentation, downstream processing steps such as cell disruption, filtration, and chromatography are employed to purify and concentrate the enzymes in the bulk matrix, contributing significantly to the total cost of production [2]. The applications of enzymes in food processing are particularly notable for their high catalytic efficiency and specificity, which are advantageous over traditional extraction methods of microorganisms, plants, and mammalian tissues [6]. Despite the advances, challenges remain in producing enzymes economically and sustainably on a large scale, requiring continuous research and development in this field [4,6].

Eln this context, microbial co-culture has emerged as an innovative strategy in enzyme production, presenting significant advantages over traditional monoclonal cultures. This technique is based on taking advantage of synergistic interactions between different microbial species to enhance the efficiency and diversity of the enzymes produced. Recent studies have shown that co-culture of microorganisms can significantly increase the production of cellulolytic



and hemicellulolytic enzymes [6]. Early co-culture analyses focused on understanding the interactions between microorganisms, both natural and artificially induced [7]. However, at present, there is widespread recognition that co-cultures can also be effectively employed to optimize critical stages in a biosynthetic pathway, stimulate enzyme synthesis, and increase protein production [8]. Despite the significant challenges it presents, such as controlling microbial interactions and optimizing culture conditions, co-cultures are expected to play an increasingly crucial role in industrial biotechnology, offering more efficient and sustainable enzyme production.

What is a co-culture?

In co-cultures, the degradation and metabolization of substrates is achieved thanks to the joint metabolic activity of different microorganisms present in the same culture [9]. The use of co-cultures has been considered as an alternative to enhance the production of metabolites and enzymes, compared to monocultures. It is estimated that co-cultures of fungi of different species can increase the obtaining of enzymes with higher yield and efficiency [10].

Co-cultures of fungi for lignin degradation exist in nature, and they play an important role in the efficient decomposition of this complex polymer (Figure 1). Lignocellulosic biomass, which includes lignin, is the most abundant biomass on earth and is naturally degraded by entire communities of microorganisms, including fungi and bacteria, which act synergistically to recycle carbon [11]. In natural environments, such as forests, woody substrates are often broken down by diverse microbial communities, including several species of fungi that contribute to lignin degradation [12]. The interactions between these microorganisms can be classified as synergistic, antagonistic, or neutral, depending on their compatibility and specific environmental conditions [13].

How to produce fungal enzymes using co-culture?

The joint cultivation of two species of fungi in an environment with limited nutrients and specific environmental conditions favors their interaction. Depending on the nature of this interaction, fungi can act in antagonism or synergism. In the case of antagonism, fungi can produce metabolites that inhibit the growth of the other species present in the co-culture. In contrast, synergism is characterized by a positive interaction in which both fungi mutually benefit [14].



Figure 1. Co-culture in nature.

What are the benefits of co-cultures in enzyme production?

The production of enzymes in co-cultures offers several advantages over mono-culture (Figure 2), mainly due to improved enzyme activity, higher biomass production, and broader environmental adaptability. Co-cultures mimic natural biodegradation processes, often resulting in higher enzyme activities. There is an evidence that supports the effectiveness of co-cultures, highlighting their simplicity and efficiency. These methods do not require complex genetic manipulations or the use of expensive inducing chemical reagents [12]. In addition, the use of several species of fungi in a single culture allows the obtaining of enzyme extracts rich in different types of enzymes, such as cellulases, xylanases, and lignin-modifying enzymes [13]. Despite the potential of fungal co-cultures, there are some challenges that need to be addressed for their large-scale development.



- Synergy between microorganisms for greater enzyme production.
 - Obtaining enzymes with improved properties.
 - Complex substrates can be used.
 - Reduction of environmental impact.

Figure 2. Advantages and challenges of enzyme production in co-culture.

Challenges and future perspectives

Despite the promising applications of fungal co-cultures, their transition from laboratory to industry is not without obstacles. To fully realize the potential of this technology, it is critical to address challenges such as optimizing growing conditions, selecting suitable strains, and developing scalable processes (Figure 3).



Optimization of growing conditions

 It is necessary to develop strategies to optimize culture conditions and promote synergistic interactions between fungal species.



Fungal species selection

 It is necessary to carefully identify and select the fungal species that co-culture synergistically to achieve an efficient production of metabolites of interest.



Process scaling

 It is necessary to develop efficient methods to scale up fungal co-culture processes at an industrial level.

Figure 3. Challenges and prospects of co-cultivation scale up.

Conclusions

The utilization of fungal co-cultures can significantly increase the production of lignocellulosic enzymes, taking advantage of the synergy between different species to improve the efficiency of biotechnological processes. Not only does this strategy improve biomass degradation to produce biofuels and value-added chemicals, but it also has potential applications in bioremediation and other industries.





- Control of microbial interactions.
- Optimization of growing conditions.
 - Process scalability.



References

- Agarwal, PK (2006). Enzimas: una visión integrada de la estructura, la dinámica y la función. Fábricas de células microbianas, 5, 1-12. https://doi. org/10.1186/1475-2859-5-2
- Sharma, G., & Vimal, A. (2023). Industrial Processing of Commercially Significant Enzymes. Recent Innovations in Chemical Engineering (Formerly Recent Patents on Chemical Engineering), 16(1), 3-15. https://doi.org/10.2174/2405520416666230 301112734
- Yoo, Y. J., Feng, Y., Kim, Y. H., Yagonia, C. F. J., Yoo, Y. J., Feng, Y., ... & Yagonia, C. F. J. (2017). Production of Enzymes. Fundamentals of Enzyme Engineering, 23-33. https://doi.org/10.1007/978-94-024-1026-6_3

- Kaur, R., Panesar, P. S., & Singla, G. (2022). Production of Enzymes from Agro-Industrial Byproducts. In Valorization of Agro-Industrial Byproducts (pp. 89-116). CRC Press.
- 5. Liu, Daqing. (2017). Production technology for agricultural enzyme.
- Li, Q., Zhang, G., & Du, G. (2022). Production of food enzymes. In Current Developments in Biotechnology and Bioengineering (pp. 139-155). Elsevier. https://doi.org/10.1016/B978-0-12-823506-5.00015-1
- Chen, A., Wang, D., Ji, R., Li, J., Gu, S., Tang, R., & Ji, C. (2021). Structural and Catalytic Characterization of TsBGL, a β-Glucosidase From Thermofilum sp. ex4484_79. Frontiers in microbiology, 12, 723678. https://doi.org/10.3389/fmicb.2021.723678

- Rosero-Chasoy, G., Rodríguez-Jasso, R. M., Aguilar, C. N., Buitrón, G., Chairez, I., & Ruiz, H. A. (2021). Microbial co-culturing strategies for the production high value compounds, a reliable framework towards sustainable biorefinery implementation an overview. Bioresource technology, 321, 124458. https://doi.org/10.1016/j.biortech.2020.124458
- Ganesan, V., Li, Z., Wang, X., & Zhang, H. (2017). Heterologous biosynthesis of natural product naringenin by co-culture engineering. Synthetic and systems biotechnology, 2(3), 236–242. https://doi. org/10.1016/j.synbio.2017.08.0037
- Bader, J., Mast-Gerlach, E., Popović, M. K., Bajpai, R., & Stahl, U. (2010). Relevance of microbial coculture fermentations in biotechnology. Journal of applied microbiology, 109(2), 371-387.
- 11. Detain, J., Rémond, C., Rodrigues, C. M., Harakat, D., & Besaury, L. (2022). Co-elicitation of lignocelluloytic enzymatic activities and metabolites production in an Aspergillus-Streptomyces co-culture during lignocellulose fractionation. Current research in microbial sciences, 3, 100108. https:// doi.org/10.1016/j.crmicr.2022.100108
- Stefanović, S., Dragišić-Maksimović, J., Maksimović, V., Bartolić, D., Đikanović, D., Simonović-Radosavljević, J., ... & Marjanović, Ž. (2023). Functional differentiation of two autochthonous cohabiting strains of Pleurotus ostreatus and Cyclocybe aegerita from Serbia in lignin compound degradation. Botanica Serbica, 47(1), 135-143. https://doi.org/10.2298/ BOTSERB2301135S
- Soares, J. K. C., Vitali, V. M. V., & Vallim, M. A. (2022). Lignin degradation by co-cultured fungi: current status and future perspectives. Lilloa, 39-62. https:// doi.org/10.30550/j.lil/2022.59.S/2022.08.10
- 14. Albert, S., Chauhan, D., Pandya, B., & Padhiar, A. (2011). Screening of Trichoderma spp. as potential fungal partner in co-culturing with white rot fungi for efficient bio-pulping. Glob J Biotechnol Biochem, 6(3), 95-101.

