



Advances in biology through agronomy, aquaculture, coastal and environmental sciences

— Volume II —

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**Advances in biology through
agronomy, aquaculture, coastal and
environmental sciences**
Volume II



Pantanal Editora

2026

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Catalog sheet

Cataloging in publication
Prepared by a Librarian Janaina Ramos – CRB-8/9166

A244

Advances in biology through agronomy, aquaculture, coastal and environmental sciences - Volume II /
Editors Leandris Argente-Martínez, Ofelda Peñuelas-Rubio, Joe Luis Arias-Moscoso. – Nova
Xavantina-MT: Pantanal, 2026.
362p. ; il.

Book in PDF format

ISBN 978-65-85756-79-2

DOI <https://doi.org/10.46420/9786585756792>

1. Biology. 2. Agronomy. 3. Aquaculture. 4. Environmental sciences. I. Argente-Martínez, Leandris
(Editor). II. Peñuelas-Rubio, Ofelda (Editor). III. Arias-Moscoso, Joe Luis (Editor). IV. Title.

CDD 570

Index to systematic catalog

I. Biology



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Advances in Biology through Agronomy, Aquaculture, Coastal and Environmental Sciences, Volume II, is an e-book that continues the work of the first version (<https://editorapantanal.com.br/ebooks/2023/advances-in-biology-through-agronomy-aquaculture-coastal-and-environmental-sciences/ebook.pdf>), published in 2023 by Leandris Argente-Martínez and Ofelda Peñuelas-Rubio.

Our e-book compiles 25 manuscripts that present scientific results and reviews based on biological aspects. All of them contribute to explaining the responses of living organisms to agricultural, aquaculture, and coastal ecosystems, as well as those facing fragility or threat of climate change. This e-book was edited by Leandris Argente-Martínez, Ofelda Peñuelas-Rubio, and Joe Luis Arias-Moscato, members of the Consolidated Academic Group ITVAYA-CA-3.

We appreciate the contribution of all authors for their careful attention to the editorial committee's recommendations. The review process was double-blind and conducted by internationally renowned researchers.

Special thanks to the members of the BioSecuraNet International Network for Biotechnology and Innovative Strategies for Global Food Security at the National Technological Institute of Mexico.

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Fungal-based arsenic bioremediation: linking enzymatic detoxification mechanisms with bioreactor engineering

Received: 08/04/2026

Accepted: 29/04/2026

doi 10.46420/9786585756792cap22

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ABSTRACT

Arsenic contamination represents a major environmental challenge due to its toxicity, persistence, and impact on water resources used for human consumption and agriculture. Fungal-based bioremediation has emerged as a promising alternative to conventional physicochemical treatments, owing to the metabolic versatility and adaptive capacity of fungi to tolerate and transform arsenic species. This work presents an integrative approach that links enzymatic detoxification mechanisms in fungi with the design of a controlled bioreactor system aimed at improving experimental reproducibility and analytical precision. A systematic literature analysis was conducted to identify key biochemical pathways involved in arsenic detoxification, including arsenate uptake, enzymatic reduction to arsenite, thiol-mediated complexation, efflux through Ars systems, methylation, and antioxidant responses. Based on these biological requirements, a modular bioreactor was designed using computer-aided engineering tools, incorporating GL45-based cultivation units, integrated sensors for temperature, pH, and dissolved oxygen, and mechanical agitation. The system also includes an electronic architecture for real-time monitoring and feedback control, as well as an IoT-based platform for data acquisition and remote supervision. The proposed design enables precise control of environmental variables and continuous data collection, facilitating the study of fungal responses under defined conditions. By reducing experimental variability, this platform supports the establishment of robust correlations between operational parameters and enzymatic activity. Overall, this work provides a scalable and accessible framework that strengthens the integration of biotechnology and engineering, contributing to the advancement of fungal-based strategies for arsenic bioremediation.

Key words: arsenic bioremediation, fungal detoxification, bioreactor engineering.

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INTRODUCTION

Arsenic contamination is currently a serious and concerning environmental problem worldwide, as it often affects groundwater intended for human consumption (Monteiro De Oliveira et al., 2021) and/or agricultural activities (Patel et al., 2023). This heavy metal can exist in two chemical forms: arsenate [As(V)] or arsenite [As(III)] (Bjørklund et al., 2020). Both forms are toxic to living organisms, causing oxidative stress, cellular damage, and metabolic alterations (Ganie et al., 2024). Conventional physicochemical treatments for remediating this contaminant, such as chemical precipitation, ion exchange, or adsorption, typically involve high operating costs, high energy consumption, and the generation of secondary contaminating residues (Priya et al., 2023), which limits their long-term sustainability. Therefore, research and development of alternatives based on biological processes have become a priority in impactful environmental engineering.

In the previously mentioned context, an alternative that arises is the use of organisms such as fungi, which have high potential for the bioremediation of heavy metals such as arsenic, chromium, or lead (Sher; Rehman, 2019; Ayele; Godeto, 2021; Kumar; Singh, 2024). Their morphology generates a high surface-to-volume ratio (Alsharari et al., 2018), and their metabolic diversity allows significant interaction with contaminants (Deshmukh et al., 2016), making them useful in biosorption, bioaccumulation, and biotransformation processes (Bibbins-Martínez et al., 2023). Previous studies have shown that fungal species tolerate high concentrations of arsenic and are even capable of modifying its chemical state through mechanisms involving enzymatic processes dependent on arsenate reductases, glutathione, or metallothioneins (Singh et al., 2018; Gupta et al., 2022; Mohsin et al., 2023). However, there are still gaps in the understanding of the mechanisms involved due to the physiological complexity of fungi and the high metabolic variability influenced by culture conditions.

The study of detoxification mechanisms presents several challenges, notably those associated with cultivation in flasks or open batch-type systems (Bahera et al., 20219, where variations in temperature, oxygen availability, and pH occur (Xiao et al., 2022; Karayannis et al., 2023; Rocha et al., 2025). These fluctuations can significantly alter the metabolic and proteomic responses of the fungus, making reproducibility difficult and hindering the identification of correlations between culture conditions and enzymatic expression (Bakar et al., 2020; Rämä; Quandt, 2021). This may lead to discrepancies between studies and limitations in interpreting the fungus–arsenic interaction.

For this reason, the use of controlled cultivation platforms may be a valuable strategy for advancing the understanding of fungal mechanisms involved in arsenic bioremediation. Bioreactors enable regulation of critical process variables, reducing experimental noise and facilitating the collection of homogeneous biological samples for subsequent analysis (Lin et al., 2025). Automated control (Palladino et al., 2024) and remote monitoring (Hausmann et al., 2017), features of mechatronic

engineering, allow continuous recording of operating parameters, thereby establishing reproducible conditions useful for proteomic and molecular studies.

This chapter first reviews the key factors in arsenic bioremediation research and then proposes a conceptual design for an automated bioreactor with remote monitoring. In this way, it establishes a link between the biological requirements of fungi and the engineering operating variables necessary to generate standard experimental conditions in environmental bioremediation research.

MATERIALS AND METHODS

Literature Analysis of Enzymatic Detoxification Mechanisms

A systematic literature review was conducted to identify and analyze the main enzymatic detoxification mechanisms involved in fungal arsenic bioremediation. The search strategy was based on the use of structured Boolean queries designed to retrieve relevant scientific articles focusing specifically on fungal systems, while minimizing the inclusion of studies related to plants or other organisms.

Scientific databases including Google Scholar, Scopus, and Web of Science were consulted to ensure comprehensive coverage of peer-reviewed literature. The search process employed combinations of keywords related to arsenic speciation, transport mechanisms, enzymatic detoxification pathways, and fungal biology. Boolean operators (AND, OR, NOT) were used to refine the search, allowing the inclusion of synonymous terms and the exclusion of unrelated research areas.

For example, search strings included combinations such as: ("arsenate uptake" OR "As(V) transport") AND ("arsenite transport" OR aquaglyceroporin) AND (fungi OR fungal OR yeast) NOT (plant OR Arabidopsis OR rice). Additional queries were designed to target specific mechanisms, including arsenate reduction, glutathione-mediated detoxification, Ars systems, and oxidative stress responses. These queries were iteratively refined to improve specificity and relevance of the results.

Inclusion criteria considered peer-reviewed research articles that explicitly addressed arsenic detoxification mechanisms in fungi at the molecular, biochemical, or physiological level. Studies involving gene expression, protein characterization, enzymatic activity, and transport systems were prioritized. Review articles were also considered when they provided integrative insights or summarized experimental evidence relevant to the mechanisms of interest.

Exclusion criteria included studies focused primarily on plants, bacteria (unless used for comparative purposes), or environmental assessments lacking mechanistic detail. Articles without clear experimental or molecular evidence related to arsenic detoxification pathways were also excluded. This methodological approach ensured a structured and reproducible selection of literature, providing a robust foundation for the analysis of enzymatic detoxification mechanisms discussed.

Bioreactor Engineering Design and Development

The design of the bioreactor system for fungal-based arsenic bioremediation was carried out through a computer-aided engineering (CAE) approach, integrating mechanical, electronic, and system-level components into a unified platform. The entire design process was developed using SolidWorks, which enabled precise modeling, visualization, and iterative optimization of the proposed system.

The conceptual design phase involved the three-dimensional modeling of the bioreactor structure, including the support frame, control housing, and integration of GL45 glass vessels as cultivation units. Mechanical components such as the agitation system (motor and impeller), adjustable shaft, and sensor ports were designed to ensure proper alignment, stability, and compatibility with standard laboratory materials. Special attention was given to the modification of the GL45 cap, incorporating multiple inlets for sensor integration (pH, temperature, dissolved oxygen), aeration, and mechanical stirring, ensuring a functional and compact configuration.

For the electronic architecture, schematic-level planning was performed in parallel with the mechanical design to ensure proper spatial distribution and compatibility of components within the control unit. This included the arrangement of the power supply, microcontroller, signal conditioning modules, and actuator drivers. Although the electronic circuits were not simulated within the CAD environment, their physical integration and housing were designed in SolidWorks to ensure adequate heat dissipation, accessibility, and protection of components.

Additionally, the IoT-based monitoring system was conceptualized through a systems integration approach, where data flow between sensors, control units, and user interfaces was defined. The Human-Machine Interface (HMI) and remote monitoring capabilities were incorporated into the design as functional modules, allowing visualization of process variables and interaction with the system.

The design process followed an iterative methodology, where multiple versions of the system were evaluated in terms of spatial arrangement, ergonomics, modularity, and scalability. Virtual assemblies and renderings generated in SolidWorks were used to validate component compatibility, optimize layout, and communicate the system architecture. This approach ensured that the final design integrates mechanical robustness, electronic functionality, and digital connectivity, providing a comprehensive platform for controlled fungal bioremediation studies.

RESULTS AND DISCUSSION

Enzymatic Detoxification Mechanisms

Arsenic detoxification in fungal systems is a complex and highly regulated process involving coordinated biochemical and molecular responses. These mechanisms are strongly influenced by arsenic speciation, primarily As(V) and As(III), as well as by the intracellular redox state.

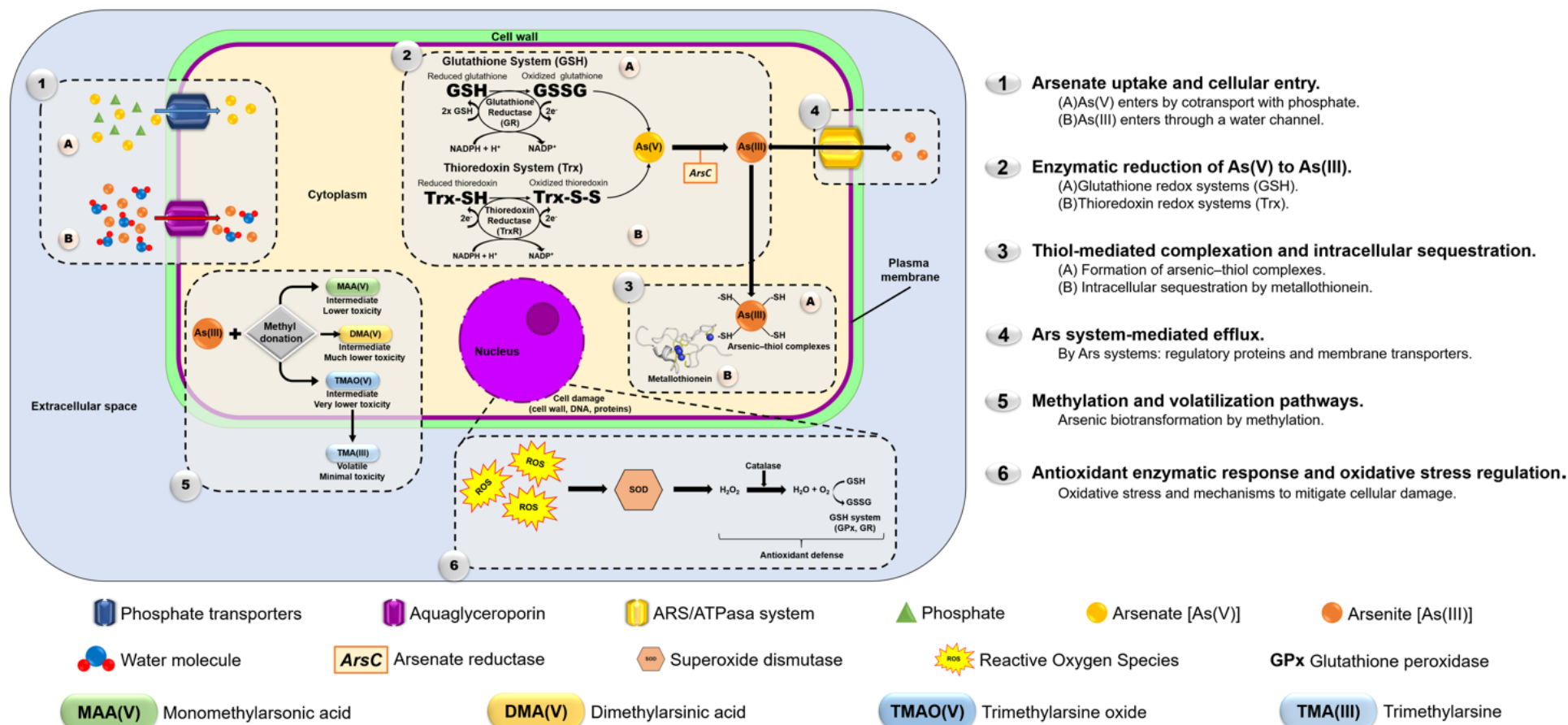


Figure 1. Integrated overview of fungal arsenic detoxification mechanisms, including cellular uptake pathways, enzymatic reduction of arsenate to arsenite, thiol-mediated complexation and intracellular sequestration, efflux via Ars transport systems, methylation and volatilization processes, and activation of antioxidant defenses under oxidative stress conditions.

In fungi, detoxification is achieved through a series of enzymatic and metabolic strategies that allow tolerance, transformation and removal of arsenic from the cellular environment (Figure 1). The main enzymatic detoxification mechanisms can be described as follows:

Arsenate uptake and cellular entry. As(V) enters fungal cells primarily through phosphate transporters due to its structural similarity to phosphate (PO_4^{3-}). This molecular similarity allows As(V) to be recognized by high- and low-affinity phosphate permeases in the plasma membrane. Consequently, arsenate uptake is closely linked to phosphate availability; under phosphate-limiting conditions, transporter expression is upregulated, inadvertently increasing arsenate influx and cellular toxicity. In contrast, As(III) predominates under reducing conditions and is uncharged at physiological pH. This enables its transport through aquaglyceroporins, a class of membrane channels that facilitate the passive diffusion of small neutral solutes such as glycerol. Unlike arsenate, arsenite uptake is less dependent on nutrient-regulated systems and more influenced by concentration gradients and membrane permeability. These distinct transport pathways have important implications for arsenic bioavailability and intracellular fate. Environmental factors such as pH, redox potential, and competing ions can modulate transporter activity and arsenic speciation. Therefore, cellular entry represents a critical control point that determines intracellular arsenic levels and directly influences the activation of downstream detoxification mechanisms (Liang et al., 2021; De Francisco et al., 2021; Sanaa-Qasem, 2025).

Enzymatic reduction of As(V) to As(III). One of the most critical steps in arsenic detoxification is the enzymatic reduction of As(V) to As(III), catalyzed by arsenate reductases. This reaction is essential because As(V), once inside the cell, can interfere with phosphate-dependent metabolic processes, whereas its reduction to As(III) allows further processing through detoxification pathways such as efflux and thiol complexation. Arsenate reductases are typically linked to cellular redox systems, primarily involving glutathione (GSH) or thioredoxin, which function as electron donors. These systems provide the reducing equivalents necessary for the conversion of As(V) to As(III), thereby coupling arsenic detoxification to the intracellular redox balance. As a result, the efficiency of this process is strongly influenced by the oxidative state of the cell and the availability of reducing molecules. Although As(III) is generally more toxic and reactive due to its high affinity for thiol groups, its formation is a strategic step in detoxification. This is because As(III) can be more readily sequestered through binding to thiol-containing compounds or actively exported out of the cell via specific transport systems. Therefore, arsenate reduction represents a central biochemical hub that connects arsenic transformation with redox homeostasis and downstream detoxification mechanisms (Mohsin et al., 2023; William; Magpantay, 2023; Georgiou-Siafis; Tsiftoglou, 2023; Khan et al., 2024).

Thiol-mediated complexation and intracellular sequestration. Once reduced, As(III) exhibits a high affinity for thiol groups (-SH) present in intracellular molecules such as GSH and cysteine-rich peptides. This interaction leads to the formation of arsenic–thiol complexes, which significantly

reduce the chemical reactivity and toxicity of arsenic by limiting its interaction with essential cellular components, particularly proteins and enzymes. Glutathione plays a central role in this process, acting both as a redox buffer and as a chelating agent. The formation of As(III)–GSH complexes facilitates intracellular detoxification by stabilizing arsenic in a less reactive form and preparing it for subsequent transport or compartmentalization. In addition, these complexes may serve as intermediates for further detoxification steps, including efflux or vacuolar sequestration. Metallothionein-like proteins, characterized by their high cysteine content, also contribute to arsenic binding. These proteins provide additional binding sites for As(III), enhancing the cell's capacity to sequester arsenic and mitigate its toxic effects. Overall, thiol-mediated complexation represents a crucial detoxification strategy that minimizes cellular damage by controlling arsenic reactivity and facilitating its safe handling within the intracellular environment (Robinson et al., 2021; Salama, 2025).

Ars system-mediated efflux. Fungal cells possess Ars-like detoxification systems that play a central role in maintaining low intracellular arsenic concentrations. These systems typically consist of regulatory proteins and membrane-associated transporters that coordinate arsenic sensing, transformation, and export. Once As(V) has been reduced to As(III), this more reactive form becomes the primary substrate for efflux mechanisms. Arsenite is actively transported out of the cell through specific efflux pumps, which may belong to families of membrane transporters such as ATP-dependent pumps or secondary transport systems. These transporters recognize As(III), often in its free or thiol-complexed form, and facilitate its extrusion across the plasma membrane, thereby reducing its intracellular accumulation and associated toxicity. The expression of Ars-related genes is commonly regulated in response to arsenic exposure, allowing cells to rapidly activate detoxification pathways under stress conditions. This regulatory component ensures an efficient and adaptive response to fluctuating arsenic levels in the environment. Importantly, the coupling of enzymatic reduction and active efflux constitutes one of the most effective detoxification strategies. While reduction converts As(V) into a transportable form, efflux systems complete the detoxification process by removing arsenic from the intracellular environment, thus preserving cellular integrity and function (Li et al., 2021; William; Magpantay, 2023; Sanaa-Qasem, 2025).

Methylation and volatilization pathways. In some fungal species, arsenic can undergo enzymatic methylation, leading to the formation of organic arsenic compounds such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). These transformations are typically mediated by methyltransferase enzymes that use S-adenosylmethionine (SAM) as a methyl donor. Through this process, inorganic arsenic species are converted into forms that are generally less reactive and, in some cases, less toxic. Methylation can also facilitate the volatilization of arsenic, generating gaseous compounds such as trimethylarsine. This volatilization process represents a potential detoxification route, as it enables the removal of arsenic from the cellular environment into the

atmosphere. However, the ecological implications of arsenic volatilization remain complex, as these compounds can be redistributed in the environment. Although these pathways are less characterized compared to reduction and efflux mechanisms, they contribute to the overall arsenic biotransformation capacity of fungi. The extent and efficiency of methylation and volatilization are influenced by environmental conditions, including substrate availability, redox state, and metabolic activity. Thus, these processes represent complementary detoxification strategies that may play a significant role in arsenic cycling and attenuation in contaminated environments (Naseem et al., 2021; Hamza et al., 2022; Mohsin et al., 2023).

Antioxidant enzymatic response and oxidative stress regulation. Arsenic exposure induces the generation of reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide and hydroxyl radicals, which disrupt cellular redox balance and lead to oxidative stress. These reactive molecules can damage essential biomolecules such as lipids, proteins and nucleic acids, ultimately impairing cellular function and viability. In response, fungal cells activate a coordinated antioxidant defense system to counteract oxidative damage. Key enzymatic components of this system include superoxide dismutase (SOD), which catalyzes the conversion of superoxide radicals into hydrogen peroxide; catalase (CAT), which decomposes hydrogen peroxide into water and oxygen; and glutathione-related enzymes such as glutathione peroxidase and glutathione reductase, which maintain intracellular redox homeostasis. GSH plays a dual role as both an antioxidant and a critical molecule in arsenic detoxification, linking oxidative stress response with thiol-mediated sequestration mechanisms. The regulation of these antioxidant pathways is essential for maintaining cellular integrity under arsenic stress conditions. Overall, the activation of antioxidant enzymatic systems represents a fundamental adaptive response that mitigates arsenic-induced damage and supports the functionality of other detoxification mechanisms within the cell (Hu et al., 2020; Yaakoub et al., 2022; Park; Son, 2024).

These mechanisms operate in an integrated manner, forming a coordinated detoxification network that enables fungi to survive under arsenic stress conditions. However, the efficiency and regulation of these pathways are highly dependent on environmental and cultivation conditions, including pH, oxygen availability, nutrient composition and arsenic speciation.

These limitations highlight the need for controlled experimental systems that allow precise manipulation of environmental variables and reproducibility of results.

In this context, bioreactor engineering emerges as a key strategy to bridge the gap between biological processes and experimental control. The following section presents the principles and design considerations of bioreactor systems tailored for the study of fungal-based arsenic bioremediation, emphasizing their role in improving the analysis of enzymatic detoxification mechanisms.

Bioreactor Engineering for Fungal-Based Arsenic Bioremediation

The conceptual design of the proposed bioreactor represents a key step toward bridging the gap between fungal physiology and controlled experimental conditions required for arsenic bioremediation studies. The system was conceived as a modular, low-cost, and scalable platform that integrates standard laboratory materials with essential process control capabilities. At its core, the design employs GL45 glass bottles (1–2 L) as cultivation vessels, enabling flexibility in experimental setups and facilitating replication. The modified caps incorporate multiple access ports that allow the integration of critical components, including temperature (PT100), pH, and dissolved oxygen sensors, as well as aeration inputs and a mechanical agitation system. The impeller ensures homogeneous mixing, which is essential for maintaining consistent nutrient distribution, oxygen transfer, and arsenic exposure within the culture medium. Additionally, the inclusion of a heating pad beneath the vessel allows precise temperature regulation, a critical parameter influencing fungal metabolism and enzymatic detoxification pathways. The vertical support structure with adjustable height enables adaptability to different vessel configurations and experimental requirements. Overall, this conceptual configuration ensures direct interaction between biological processes and controlled physical variables, thereby reducing environmental variability and improving reproducibility in fungal arsenic bioremediation experiments (Figure 2).

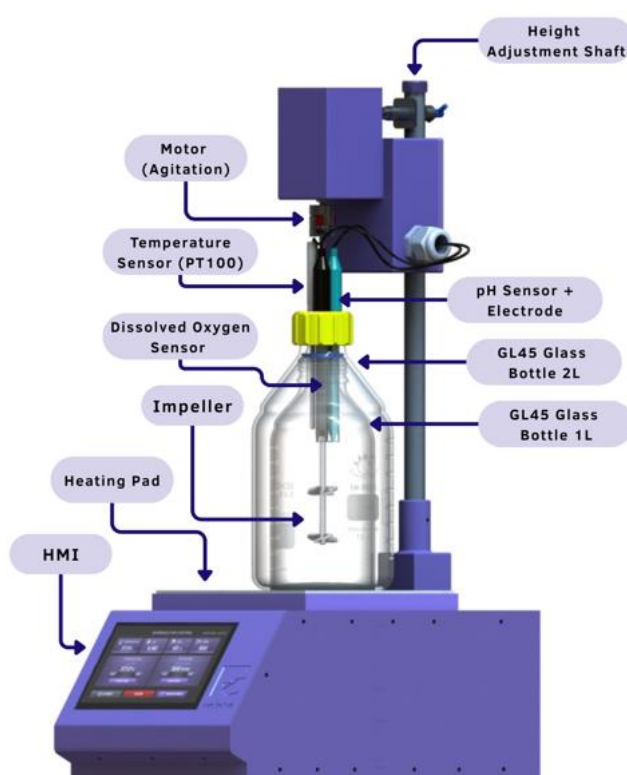


Figure 2. Conceptual design of the modular bioreactor system for fungal cultivation, illustrating the integration of sensing elements, mechanical agitation, and thermal regulation within a GL45-based vessel, along with its structural support and user interface for operation.

Building upon the physical configuration, the electronic architecture of the system establishes the functional backbone required for real-time monitoring and control of the bioprocess. The architecture is organized into four main subsystems: electrical power, control, sensors, and actuators. The power subsystem includes a regulated power supply with protective elements such as fuses, ensuring safe operation of the system. The control subsystem is centered on a microcontroller responsible for processing sensor signals and executing control algorithms, complemented by a Raspberry Pi module that enables higher-level monitoring, data visualization, and user interaction through the HMI interface. Sensor integration includes pH, temperature (PT100), dissolved oxygen, and motor encoder feedback, allowing continuous acquisition of key process variables. These signals are conditioned through an ADC/temperature amplifier before being interpreted by the control unit. On the actuation side, the system incorporates an H-bridge motor driver for agitation control and a MOSFET-based driver for the heating element, enabling precise regulation of mixing and temperature. This closed-loop configuration allows dynamic adjustment of operating conditions based on sensor feedback, ensuring stable and reproducible environments necessary for studying fungal enzymatic responses to arsenic stress (Figure 3).

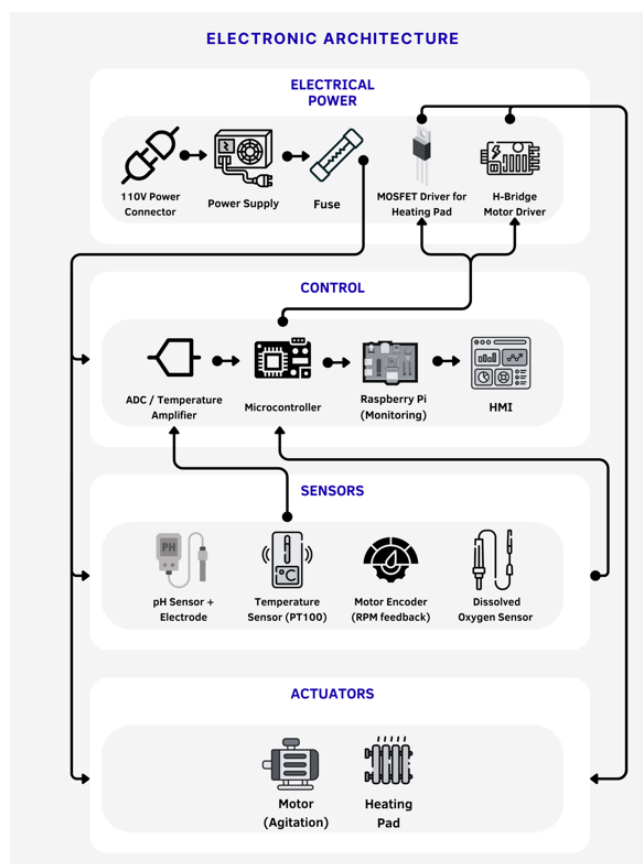


Figure 3. Electronic architecture of the bioreactor system, showing the organization of power supply, control units, sensor interfaces, and actuator drivers that enable signal acquisition, processing, and coordinated system operation.

Finally, the integration of an IoT-based monitoring and data acquisition system significantly enhances the analytical capabilities of the proposed bioreactor. The system enables continuous collection, storage, and visualization of process data, which are transmitted from the sensors through the control unit to a digital interface accessible via the HMI or remote devices. This architecture allows real-time tracking of key parameters such as temperature, pH, and dissolved oxygen, facilitating immediate detection of deviations and enabling timely corrective actions. Moreover, the implementation of remote monitoring supports experimental supervision without the need for constant physical presence, improving operational efficiency. The data acquisition system also enables the generation of structured datasets that can be used for advanced analyses, including pattern recognition, correlation of environmental conditions with enzymatic activity, and potential integration with predictive models. This digital layer not only reduces experimental variability but also strengthens the reproducibility and scalability of fungal bioremediation studies. By linking sensor data, control actions, and user interaction within a unified platform, the IoT design transforms the bioreactor into a smart system capable of supporting high-resolution studies of arsenic detoxification mechanisms (Figure 4).

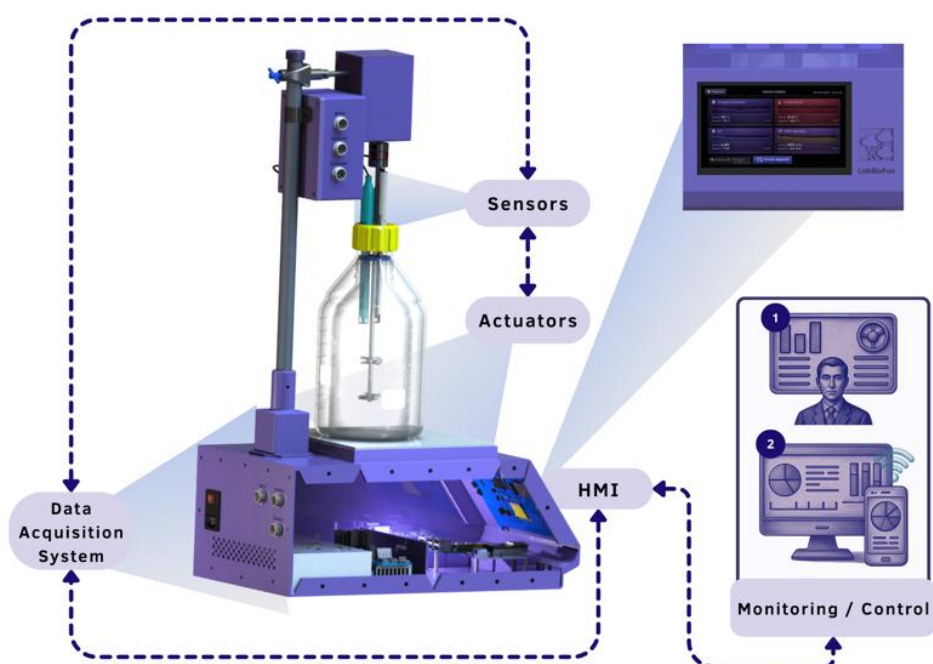


Figure 4. IoT-based system design illustrating the integration of data acquisition, real-time monitoring, and remote interaction between sensors, actuators, and user interfaces for process supervision and control.

CONCLUSIONS

This work establishes an integrative framework linking fungal arsenic detoxification mechanisms with controlled bioreactor engineering. The proposed design enables precise regulation of critical

variables and systematic data acquisition, creating conditions suitable for reproducible and high-resolution studies. By combining modular hardware, real-time monitoring, and IoT-based control, the system provides a robust platform to explore the relationship between environmental parameters and enzymatic responses. This approach not only strengthens experimental reliability but also supports the development of scalable and efficient strategies for arsenic bioremediation in complex environmental contexts.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support provided by Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI) for the postdoctoral fellowship (CVU: 560733) and the national scholarship (CVU: 123456), which made this work possible.

We also express our sincere appreciation to the Red Internacional de Biotecnología y Estrategias Innovadoras para la Seguridad Alimentaria Global (BioSecuraNet) for fostering collaboration and supporting research initiatives in the field of environmental biotechnology and sustainable development.

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9786585756792



BioSecuraNet

**Red Internacional de Biotecnología y
Estrategias Innovadoras
para la Seguridad Alimentaria Global**

Pantanal Editora

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