AgriSustain-An International Journal

Agriculture

Review

Synthetic Biology; An Advancement in Heavy Metal Detection and Detoxification

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Abstract

Heavy metal contamination, arising from natural processes and human activities, poses significant environmental and health challenges. Conventional remediation methods are often costly and inefficient, driving interest in synthetic biology as a promising alternative. Synthetic biology utilizes engineered organisms and systems to detect, bioremediate, and treat heavy metal pollution in environmentally friendly and cost-effective ways. Detection advances include biosensors for metals like mercury, arsenic, lead, cadmium, and copper, employing innovative genetic circuits, cell-free systems, and reporter genes for enhanced sensitivity and specificity. Bioremediation strategies leverage biosorption and bioaccumulation, utilizing engineered microbes to bind and sequester heavy metals, improving removal efficiency. However, challenges such as cytotoxicity, environmental adaptability, and ethical concerns over GMO use limit commercialization. Advances in CRISPR-based tools, biofilm engineering, and immobilization techniques, combined with addressing biosafety issues, are paving the way for scalable applications. These efforts highlight the transformative potential of synthetic biology for sustainable heavy metal remediation and environmental protection.

Keywords: Heavy metals, synthetic biology, biosorption and bioaccumulation

OPEN ACCESS

CITATION

Kumawat, R., Jha, S., Joshi, D. and Rawat, A. Synthetic Biology; An Advancement in Heavy Metal Detection and De-toxification. *AgriSustain-an International Journal,* 2025, 03(1), 25-30.

ARTICLE INFORMATION

Received: November 2024

Revised: December 2024

Accepted: December 2024

DOI: **10.5281/zenodo.15094953**

Introduction

Heavy metals, such as lead, mercury, and arsenic, are dense elements with high atomic weights that contaminate air, soil, and water through both natural processes and human activities like industrial operations. While essential in various industries and products, improper disposal leads to severe environmental and health consequences, including organ failure and death, with potential harm to future generations. Current treatments for heavy metal exposure are costly, inefficient, and often harmful. Synthetic biology offers promising solutions by developing environmentally friendly, cost-effective methods for detection, bioremediation, and treatment, addressing both the challenges and advancements in managing heavy metal pollution and poisoning.

Synthetic biology leverages natural biological systems to develop solutions for heavy metal remediation by redesigning existing biological mechanisms. Organisms naturally evolved to tolerate heavy metals often use operons—genetic systems encoding proteins for metal resistance, such as the mer operon for mercury or ars operon for arsenic. These systems activate in the presence of metals to produce proteins and enzymes that enable metal efflux or conversion [1]. Additionally, metallothioneins (MTs) and phytochelatins (PCs), found across various microorganisms, bind, and sequester heavy metal ions to reduce toxicity (Fig. 1). Certain microorganisms also transform inorganic lead into less toxic volatile forms or precipitate metals through complexation with specific molecules. Microbes further resist heavy metal exposure by limiting ion mobility and using binding molecules like lipopolysaccharides and polysaccharides, showcasing diverse natural strategies for surviving metal-rich environments.

Synthetic biology approaches to combat heavy metal toxicity

Detection of various heavy metals

Mercury detection in polluted environments is critical due to its severe health and ecological impacts, and synthetic biology has enabled the development of efficient, sensitive, and cost-effective microbial biosensors. Researchers have engineered various genetic circuits, such as Pmer/merR-lucGR, which use luciferin-based bioluminescence for detection within specific concentration ranges [2]. To enhance detection in soil, circuits like pmerRBPmerlux paired with rhamnolipid biosurfactants of Escherichia coli MC106 improve mercury release into water for measurement. Intracellular mercury-specific biosensors assess bioavailability using systems like MerA or MerR-efe, which facilitate measurable outputs such as gas production. Additionally, cell-free systems employing plasmids with reporter genes like EmGFP or LucFF provide robust detection capabilities, showing adaptability to environmental changes and comparable sensitivity to whole-cell systems, with a detection limit as low as 1 ppb [3]. These advances highlight diverse approaches to address mercury pollution effectively.

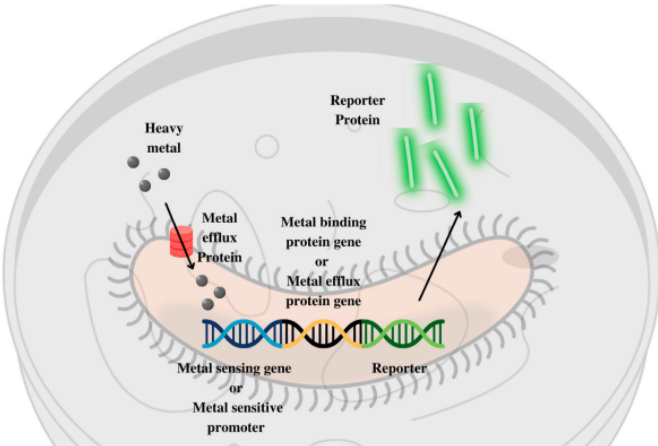

Fig. 1 General mechanism of detection of heavy metals [4]

Research on arsenic detection has focused on enhancing sensitivity and practicality, leveraging synthetic biology to create innovative biosensors. Initial efforts improved sensitivity by modifying untranslated regions and incorporating auxiliary ArsR binding sites, while modular signal amplification further advanced detection capabilities. Mutant ArsR proteins, identified through high-throughput error-PCR screening, demonstrated improved limits of detection. However, challenges with bacterial biosensor stability led to the development of a cell-free system using evolved ArsR mutants, achieving detection limits as low as 3.65 μg/L, compliant with WHO standards [5]. Other biosensors, like Pars/arsR-phiYFP, offered time- and dose-dependent fluorescence responses to arsenite and arsenate, with detection ranges extending up to 25 μM. Systems like luxCDABE/arsR/luxAB utilized bioluminescence reduction for cost-effective pollutant monitoring, while arsR/crtI biosensors exhibited visible red pigmentation changes upon arsenite exposure [6]. The Pars/arsR-lacZ system introduced pH-based detection, with pH shifts indicating arsenate presence, achieving a detection limit of 5 ppb, surpassing WHO recommendations [7]. These advancements highlight diverse approaches for arsenic detection, balancing sensitivity, ease of use, and cost-effectiveness.

Studies on lead biosensors has explored diverse approaches to enhance sensitivity, specificity, and detection limits. One study utilized the promoter-pbrR-GFP system cloned onto a low-copy plasmid, achieving high sensitivity across multiple bacterial hosts [8]. Another approach, the pGL3-luc/pbr biosensor, demonstrated specificity for lead in the presence of other metals, detecting concentrations between 1 and 100 μM without interference [9]. Luminescent biosensors using Bacillus subtilis and Staphylococcus aureus detected trace amounts of lead compounds (~0.01 μM) within two hours, though specificity remained a challenge [10]. Similarly, Alcaligenes eutrophus biosensors detected ~331 μg/mL of lead with high specificity, influenced by experimental conditions [9]. To address limitations like nutrient availability and plasmid copy number, researchers developed six genetic circuits with positive feedback loops, reconfiguring regulatory elements to enhance biosensor sensitivity and effectiveness by up to tenfold. These advancements highlight the potential for optimizing lead biosensors to achieve improved performance and practical application.

Research on cadmium and copper biosensors has led to innovative approaches using synthetic biology. For cadmium, Guo [11] designed biosensors with single, dual, and triple-signal outputs based on artificial cad operons, achieving sensitivity in the range of 0.1–3.125 μM Cd (II). Other studies developed dual-sensing systems using CadR and CadC regulators paired with fluorescent reporters like mCherry and eGFP, yielding quantitative detection proportional to cadmium levels [12]. Whole-cell sensors with toggled circuits and amplification modules improved detection limits (down to 0.01 μM) and specificity for cadmium while minimizing interference from other metals. For copper detection, microbial fuel cells (MFCs) integrated with genetically modified Escherichia coli expressing porins and riboflavin-related genes demonstrated a linear voltage response to Cu^{2+} concentrations between 0.1–0.5 mM, providing a promising tool for monitoring copper in drinking water [13]. These advancements highlight the effectiveness of biosensors in detecting cadmium and copper while emphasizing specificity, sensitivity, and environmental adaptability.

Biosorption and Bioaccumulation of heavy metals

Biosorption and Bioaccumulation are distinct processes used for heavy metal remediation. Biosorption involves passive binding of metals to biological substrates via physical or chemical interactions, while bioaccumulation is an active, metabolism-driven process in living cells. Both methods have been explored extensively using synthetic biology to enhance their efficiency and specificity. For biosorption, engineered microbes expressing metal-binding peptides or proteins have shown significant improvements. For instance, E. coli modified to produce metallothioneins (MTs) or phytochelatins on its surface demonstrated up to a 20-fold increase in metal-binding capacity. Challenges include short biosorbent lifespan due to fouling and pH sensitivity in wastewater applications [14]. In bioaccumulation, microbes engineered with specific genes, such as those coding for heavy metal transporters and MTs, exhibited enhanced tolerance and metal uptake. For example, E. coli expressing both MTs and a nickel transporter showed a threefold improvement in nickel bioaccumulation [15]. Similarly, cadmium-specific systems combined transporters and synthetic constructs to optimize intracellular accumulation, achieving substantial efficiency gains.

Advances include integration of engineered microbes into gene circuits for metal detection and immobilization. A notable study employed Ralstonia. metallidurans expressing mouse MTs for in-situ cadmium immobilization, protecting plants from contamination [16]. Another innovation used E. coli with MerR-based circuits and biofilm-forming nanofibers to absorb mercury, reducing intracellular toxicity [17]. Despite these successes, challenges such as cytotoxicity, cell viability, and plasmid stability limit large-scale application. Technological improvements, including CRISPR-based systems and cell-free approaches, and ethical considerations surrounding GMO release, remain critical for broader adoption. Addressing these issues may unlock the potential of synthetic biology in sustainable bioremediation.

Future Perspectives

Synthetic biology offers efficient and eco-friendly solutions for remediation and detection but commercializing genetically modified organisms (GMOs) faces technological and ethical challenges. Technological hurdles include variability in sensitivity, competition with wild strains, environmental limitations, and inefficiencies in current detection methods. Innovations like CRISPR-based devices, cell-free systems, and enhanced reporter genes show promise in addressing these issues. Ethical concerns revolve around biosafety and biosecurity risks, such as the unintended spread of artificial organisms and horizontal gene transfer. Addressing these challenges requires collaboration among scientists, regulators, and policymakers to ensure safe and responsible biotechnology development. Transparent discussions can accelerate the acceptance and application of synthetic biology for global development and heavy metal remediation.

Conclusion

Heavy metal pollution demands urgent action, and synthetic biology (SynBio) offers promising solutions for detection, bioaccumulation, and bioremediation through microbial biosensors and engineered organisms. Despite its potential, SynBio's implementation raises concerns due to unknown implications and biosafety risks. To address these challenges, technology development must align with robust biosafety and biosecurity frameworks. Emphasis should be placed on responsible research, open dialogue, and stakeholder education rather than restrictive measures. Transparent discussions about risks can foster innovative solutions and highlight SynBio's transformative potential for environmental remediation and global development.

References

1. Hao, X., Zhu, J., Rensing, C., Liu, Y., Gao, S., Chen, W., ... & Liu, Y. R. (2021). Recent advances in exploring the heavy metal (loid) resistant microbiome. *Computational and structural biotechnology journal*, *19*, 94-109. [google scholar]
2. Liao, V. H. C., & Ou, K. L. (2005). Development and testing of a green fluorescent protein-based bacterial biosensor for measuring bioavailable arsenic in contaminated groundwater samples. *Environmental Toxicology and Chemistry: An International Journal*, *24*(7), 1624-1631. [google scholar]

3. Gupta, S., Sarkar, S., Katranidis, A., & Bhattacharya, J. (2019). Development of a cell-free optical biosensor for detection of a broad range of mercury contaminants in water: A plasmid DNA-based approach. *ACS omega*, *4*(5), 9480-9487. [google scholar]
4. Somayaji, A., Sarkar, S., Balasubramaniam, S., & Raval, R. (2022). Synthetic biology techniques to tackle heavy metal pollution and poisoning. *Synthetic and Systems Biotechnology*, *7*(3), 841-846. [google scholar]
5. Wang, X., Zhu, K., Chen, D., Wang, J., Wang, X., Xu, A., ... & Chen, S. (2021). Monitoring arsenic using genetically encoded biosensors in vitro: The role of evolved regulatory genes. *Ecotoxicology and Environmental Safety*, *207*, 111273. [google scholar]
6. Flynn, H. C., Mc Mahon, V., Diaz, G. C., Demergasso, C. S., Corbisier, P., Meharg, A. A., & Paton, G. I. (2002). Assessment of bioavailable arsenic and copper in soils and sediments from the Antofagasta region of northern Chile. *Science of the Total Environment*, *286*(1-3), 51-59. [google scholar]
7. de Mora K, Joshi N, Balint BL, Ward FB, Elfick A, French CE. A pH-based biosensor for detection of arsenic in drinking water. Anal Bioanal Chem May 2011;400(4): 1031–9. [google scholar] [DOI]
8. Bereza-Malcolm, L., Aracic, S., & Franks, A. E. (2016). Development and application of a synthetically derived lead biosensor construct for use in gram-negative bacteria. *Sensors*, *16*(12), 2174. [google scholar]
9. Nourmohammadi, E., Hosseinkhani, S., Nedaeinia, R., Khoshdel-Sarkarizi, H., Nedaeinia, M., Ranjbar, M., & Sadeghizadeh, M. (2020). Construction of a sensitive and specific lead biosensor using a genetically engineered bacterial system with a luciferase gene reporter controlled by pbr and cadA promoters. *BioMedical Engineering OnLine*, *19*, 1-13. [google scholar]
10. Corbisier, P., Van Der Lelie, D., Borremans, B., Provoost, A., De Lorenzo, V., Brown, N. L., ... & Mattiasson, B. (1999). Whole cell-and protein-based biosensors for the detection of bioavailable heavy metals in environmental samples. *Analytica chimica acta*, *387*(3), 235-244. [google scholar]
11. Guo, Y., Hui, C. Y., Zhang, N. X., Liu, L., Li, H., & Zheng, H. J. (2021). Development of cadmium multiple-signal biosensing and bioadsorption systems based on artificial cad operons. *Frontiers in bioengineering and biotechnology*, *9*, 585617. [google scholar]
12. Hui, C. Y., Guo, Y., Wu, J., Liu, L., Yang, X. Q., Guo, X., ... & Yi, J. (2021). Detection of bioavailable cadmium by double-color fluorescence based on a dual-sensing bioreporter system. *Frontiers in microbiology*, *12*, 696195. [google scholar]
13. Zhou, T., Li, R., Zhang, S., Zhao, S., Sharma, M., Kulshrestha, S., ... & Li, X. (2021). A copper-specific microbial fuel cell biosensor based on riboflavin biosynthesis of engineered Escherichia coli. *Biotechnology and Bioengineering*, *118*(1), 210-222. [google scholar]
14. Romeyer, F. M., Jacobs, F. A., Masson, L., Hanna, Z., & Brousseau, R. (1988). Bioaccumulation of heavy metals in Escherichia coli expressing an inducible synthetic human metallothionein gene. *Journal of biotechnology*, *8*(3), 207-220. [google scholar]

15. Krishnaswamy, R., & Wilson, D. B. (2000). Construction and characterization of an Escherichia coli strain genetically engineered for Ni (II) bioaccumulation. *Applied and environmental microbiology*, *66*(12), 5383-5386. [google scholar]
16. Wuana, R. A., & Okieimen, F. E. (2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *International Scholarly Research Notices*, *2011*(1), 402647. [google scholar]
17. Tay, P. K. R., Nguyen, P. Q., & Joshi, N. S. (2017). A synthetic circuit for mercury bioremediation using self-assembling functional amyloids. *ACS synthetic biology*, *6*(10), 1841-1850. [google scholar]