

Innovative methods using bacteria for removing heavy metals from water^{*}

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*Heavy metal contamination in drinking water is a significant public health concern worldwide. A study by the Environmental Monitoring Assessment has shown that over 140 million people globally are exposed to drinking water containing unsafe levels of heavy metals such as lead, arsenic, cadmium, and mercury (Peterson et al., 2021). Chronic exposure to these contaminants has been linked to neurological disorders, developmental delays, kidney failure, and increased cancer risk. Conventional water treatment technologies, such as chemical precipitation, ion exchange, and activated carbon adsorption, suffer from limitations due to cost, loss of efficiency, and negative environmental impact, particularly in low-resource environments. To address these limitations, we developed a bioengineered filtration system that utilizes genetically modified bacteria to enhance the removal of heavy metals from contaminated water sources. The system employs the following microbial strains: *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas putida*, each engineered to optimize distinct mechanisms of metal removal: biosorption, bioaccumulation, and bioprecipitation. These processes were integrated into a modular biofilter, allowing for scalability and effective purification. This approach offers a promising alternative to conventional filtration technologies by leveraging the adaptability and efficiency of engineered biological systems. It is low-cost with a high removal efficiency, and a modular design that makes it especially suitable for deployment in underserved communities and areas with limited access to advanced water treatment infrastructure. With further development, bioengineered filtration could play an extremely large role in global efforts to ensure safe and sustainable access to clean drinking water.*

Keywords: Heavy metal removal, biofiltration system, biosorption, bioaccumulation, water purification



Heavy metals, such as lead, arsenic, mercury, cadmium, chromium, nickel, copper, zinc, iron, manganese, and aluminum, are recognized as toxic contaminants that pose significant risks to both human health and the environment. These contaminants are released from factories and industrial runoff, causing major

issues in underdeveloped areas without water treatment infrastructure. Even at low concentrations, these metals can cause numerous health issues. Developmental and cognitive impairments in children are associated with lead and arsenic exposure. Mercury and cadmium have been found to damage the nervous system and organs, while

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chromium and nickel can cause respiratory and skin problems (Marshall et al., 2019; Pacini et al., 2012; Iskandar et al., 2024; Gelardi et al., 2017). Excessive exposure to copper and zinc has been shown to affect the liver and kidneys, and iron has been observed to disrupt the balance of essential minerals in the body (Gembillo et al., 2022; Allen & Masters, 1985; Goyer, 1997). Manganese toxicity has been linked to neurological disorders, and aluminum has been associated with Alzheimer's disease and bone health issues (Budinger et al., 2021; Mjöberg et al., 1997). Given these harmful effects, safely removing these metals from drinking water is crucial to ensuring maximum public health. A study published in *Archives of Toxicology* discusses how heavy metals interfere with cellular processes. The article further states, “heavy metals are known to interfere with signaling pathways and affect a variety of cellular processes, including cell growth, proliferation, survival, metabolism, and apoptosis” (Rehman et al., 2024). Despite such an awareness, the current studies on removal methods are limited to basic aspects of the filtration process and have not been effectively scaled. Improvements in scalability and design versatility are aimed at being achieved by the proposed filter. The system will use controlled flow rate pumps to maximize filtration efficiency and provide a way to calibrate the system. By integrating synthetic biology and modular design, this biofiltration system introduces a targeted approach to heavy metal pollution in water.

Systems level

The biofiltration system is made to remove nearly all heavy metal pollutants from water. Yet, it is also small enough to be transported easily, making it a much more versatile and less expensive option compared to traditional systems. The filter is comprised of three targeted chambers, as shown in Figure 1, through which various heavy metal pollutants are removed. The system can be scaled for different applications. For example, smaller models can be made for residential solutions, and larger models can be created for community-wide solutions. A solar-powered pump is used to push water through the

biofiltration mediums, which use the genetically modified bacteria to filter out heavy metals in the water. At the system's core, three main biofiltration chambers are used, each holding genetically modified bacteria: *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas putida*. Encoded plasmids produce metal-detoxifying enzymes, metal-binding proteins, and efflux pumps, which enhance each strain's ability to biosorb, bioaccumulate, and bioprecipitate heavy metals. These microbes are immobilized in a porous biochar medium, which supports the bacterial cells and serves as a physical filtration mechanism. The biochar medium includes protection from environmental stressors and can keep nutrients for the bacteria in its porous structure. Contaminated water is directed to flow through the chambers sequentially: metals partially removed by one strain are further targeted downstream, resulting in a higher overall removal rate for the targeted heavy metals.

The filter is also designed to ensure no bacteria can escape the system or cross-contaminate the chambers, as UV water treatment is applied between filtration pods. This treatment preserves the biosystem inside each pod and keeps it isolated. The system's modularity allows for easy modification and adjustment based on the current application. The filter represents an innovative approach to biologically treating water, enabling the simultaneous removal of multiple heavy metal pollutants. This versatility makes it a promising solution for large-scale water purification, especially in regions affected by diverse environmental contaminants.

Yellow arrows show the direction of water flow.

Device level

The filter uses three genetically engineered bacterial strains: *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas putida*. These bacteria were chosen for this filter due to their natural affinity for removing certain heavy metals. For example, *Escherichia coli* contains PbrA and PbrB proteins, which contribute to detoxifying lead in the cell. Each strain identified similarly targets a specific type of heavy metal from

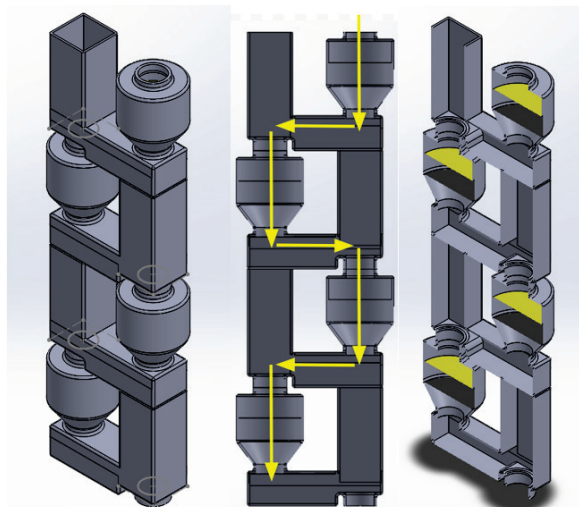


Figure 1. Proposed Model of the Water Filtration System.

contaminated water. These strains are modified by inserting plasmids containing genetic codes for heavy metal filtration from other species.

The *Escherichia coli* is engineered to express metal-binding proteins such as metallothioneins, which naturally chelate metal ions like copper. These proteins bind heavy metals intracellularly and enhance the bacteria's ability to filter out heavy metals such as copper and zinc through biosorption and bioaccumulation.

The *Bacillus subtilis* is engineered to overproduce extracellular polymeric substances (EPS), sticky polymers secreted by cells. EPS is a biofilm matrix in functional groups that provide binding sites for heavy metal ions. This enables the *B. subtilis* bacteria to capture iron, lead, and mercury from the water.

The *Pseudomonas putida* is genetically engineered to express the mer operon, which includes the merA and merB genes. These genes code for the enzyme responsible for transforming mercury ions into elemental mercury, which is non-volatile. This process is known as bioprecipitation and is effective for mercury, cadmium, chromium, and lead.

These bacteria target a wide range of heavy metals, making the system adaptable to different types of water and diverse environments.

Parts level

Each bacterial strain will be genetically engineered using plasmids with synthetic operons designed to enhance its characteristics and aid in removing heavy metals. The non-pathogenic *E. coli* strain will be modified with a constitutive promoter gene, which helps promote the expression of metallothionein genes such as MT-1 and MT-2. These proteins then bind intracellularly to heavy metals like zinc and copper, allowing for biosorption and bioaccumulation within the cell. The *B. subtilis* strain will be engineered to increase the production of extracellular polymeric substances (EPS). The process includes a promoter that controls the genes dictating the production of EPS, such as espA-O operons. The overproduction of EPS helps with biofilm production and metal binding. These genes will thus help with the biosorption of heavy metals like lead, mercury, and iron.

P. Putida will be engineered to express the mer operon (merA and merB), key enzymes for filtering out mercury through bioprecipitation. The merA operon reduces mercury to a less toxic elemental mercury, and the merB operon breaks down organic mercury compounds.

Safety

Safety is a crucial central focus throughout the development and usage of our biofiltration system. All genetic modifications are to be performed using biosafety level 1 (BSL-1) organisms and will follow all institutional biosafety guidelines and NIH Recombinant DNA Advisory Committee standards to prevent accidental exposure or environmental release. The genetically engineered bacteria are to be immobilized within a biochar environment, which would reduce the risk of environmental escape. All experiments will be conducted in controlled lab environments with proper containment and disposal procedures for all materials during testing. Personal protective equipment (PPE), including gloves, lab coats, and masks, will be used while adhering to the training

protocols to minimize all risks. We will also be using a biosafety cabinet, decontaminating our work area with bleach, and ensuring proper disposal of any hazardous materials to ensure safety. In the real-world application, the filtration system will be designed as a closed system to prevent direct human contact with the bacteria and other potentially hazardous components. Post-filtration water would be tested to ensure all safety standards are met before release or use.

Discussions

The biofiltration system presents a promising solution as it addresses the widespread issue of heavy metal contamination in drinking water. According to *Frontiers in Environmental Science*, “synthetic biology tools can be engineered to detect and degrade environmental contaminants with high precision and specificity,” which supports the use of synthetic biology combined with environmental engineering to develop a more cost-effective, sustainable, and scalable solution (Chen et al., 2020). This system can be utilized across underdeveloped, developing, and developed regions.

One of the main advantages is the use of genetically modified bacteria. The bacteria is tailored for efficient heavy metal removal through biosorption, bioaccumulation, and bioprecipitation. These strategies, as reviewed in *ScienceDirect*, “offer higher specificity and a lower environmental footprint than traditional chemical treatments.” Additionally, using biochar ensures bacterial stability and extended filtration performance. Research shows that “biochar-immobilized microbes demonstrate enhanced degradation capacity and prolonged operational stability”. The modular design can then be further enhanced by allowing different communities to scale the system to their needs, providing ease of maintenance.

However, there are challenges present in the system. Working with genetically modified organisms (GMOs) necessitates strict containment and biosafety protocols, which could limit real-world application in certain areas. As discussed by the Carnegie Endowment, “biosafety concerns regarding

GMOs must be addressed through strict adherence to international protocols such as the Cartagena Protocol (Glenna & Cahill, 2021). Another challenge can be maintaining long-term bacterial viability and activity within the filter itself. Accurate detection and quantification of heavy metals would also require access to advanced lab equipment such as Atomic Absorption Spectrometry (AAS) or Inductively Coupled Plasma Mass Spectrometry (ICP-MS), which may not be viable in all settings.

To enhance the system’s performance, the following steps are to explore multi-strain co-culturing strategies, which can help to further improve metal specificity and removal efficiency. Genetic circuit tuning can also be used to boost bacterial resilience and metal uptake capabilities. Development of a compact filter is being explored for use in rural areas. Incorporating real-time biosensors, which “allow for autonomous monitoring of water quality and immediate feedback” (Rodriguez-Mozaz & Barcelo, 2020) can further improve flow control and user trust. Through continued testing and development to enhance the system, the hope is to refine the design and bring this synthetic biology solution closer to a real-world impact.

Next steps

Over the next semester, the team will focus on transforming this design into a bench-scale prototype that can be used for lab testing. First, a biocompatible flow-through module will be designed and 3D printed using SolidWorks computer-aided design software. These three chambers will be connected, and dye tests can test the seals. In parallel with this, each bacterial strain will be prepared by modifying it using the plasmids encoded for heavy metal filtration as specified in the design. Once cultured, these bacteria will be put into a biochar slurry and cast into the printed chambers.

With this assembled unit, bench-scale filtration trials will be conducted using water with known high concentrations of the targeted heavy metals. Flow rates and contact times will be varied, and various setups for filtration chambers will be tested. The

effluent water will be analyzed using colorimetric test kits or available spectroscopy methods to quantify removal efficiency. Several tests at different input settings will be run to maximize removal efficiency. This rigorous testing will create a viable and scalable final prototype.

Author contributions

The Alliance Academy for Innovation BioBuilder team was split into two teams: the engineering team and the biology team. The engineering group consisted of HM, TR, SS, and SW. The biology group consisted of HG, SG, DG, SK, and TB. AG was the Biobuilder team captain, and she organized the team meetings, delegated the tasks, and arranged meetings with our mentor. HG was the biology team captain, and SW was the engineering team captain. The team often met on Monday afternoons and shared progress on last week's research assignments while delegating new tasks to be completed before the following Monday's meeting based on the next steps in research. DG worked on lead, HM worked on arsenic, HG worked on mercury, SS worked on cadmium, SK worked on chromium, SW worked on nickel, SH worked on copper, TB worked on zinc, TR worked on iron, AG worked on manganese, and SD worked on aluminum.

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