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by Sanaul Haque

Submission date: 13-Oct-2025 09:53PM (UTC+0700)
Submission ID: 2779906145
File name: IARCO_2025_Research_Proposal_Sanchita_Rahman_-_Sanchita_Rahman.pdf (385.65K)
Word count: 2346
Character count: 13270

RESEARCH PROPOSAL

on

Arsenic Bioremediation in Rural Bangladeshi Groundwater Using Engineered Microbial–Biochar Filter

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Date of Submission

30/09/2025

1. INTRODUCTION

Under the classification of Group-1 carcinogens, arsenic (As) is critically responsible for widespread groundwater contamination across the Ganges Delta [1], [2]. In Bangladesh, the crisis is much more severe, with concentrations exceeding the country's safety threshold in 62 out of 64 districts [1]. The health is also under threat, as 97% of the rural population relies on contaminated tube wells and crops containing high levels of inorganic arsenic [3], [4]. Now, standard prevention methods are not yet profoundly feasible for the rural scale due to the high maintenance costs and complex logistics [5]. Consequently, this research mainly focuses on locally produced alternatives. Primarily including enhanced bioremediation via microbial biosorption and biomineralization [5], [6]. Also, the usage of biochar, more specifically low-cost rice husk biochar (RHB), shows promising results as a modified adsorbent for heavy metals like arsenic (As) [7], [8]. However, previously, all coexisting microbial-biochar systems were limited by a lack of predictable performance and genetic control in field conditions, often relying on the inconsistent native strains [6].

This research seeks to address these limitations by developing synthetic biology with materials engineering. The proposed solution leverages the Engineered Microbial–Biochar Filter. Notably, this system will utilize genetic tools, including the hyperthermoactive Cas9 system, to genetically enhance the specific microbial strains for maximum, controllable arsenic detoxification [9].

2. RESEARCH OBJECTIVES

2.1 PRIMARY OBJECTIVES

- The initial aim is to design and implement a Dual-strain Microbial Consortium that is supported by a biochar matrix for the effective and low-cost extraction of arsenic from drinking water in neglected communities of Bangladesh.

2.2 SPECIFIC OBJECTIVES

- **2.2.1 Dual-strain Microbial Consortium:** The pre-marked strain-1 will be modified genetically to overexpress arsenite oxidase (aioBA genes), and strain-2 will have its genes altered to express highly selective arsenate transporters (arsB).
- **2.2.2 RHB support matrix:** This matrix will be customized for porosity, microbial adhesion, and hydraulic flow to ensure the high efficiency of the microbial consortium.
- **2.2.3 Point-of-use (POC) filter unit:** For six months this system will be installed in three preselected rural sites under actual real-world conditions, to evaluate contaminated removal rates, microbial stability, and overall water quality.
- **2.2.4 Final review:** The engineered biofilter performance will be compared to standard adsorption filters. Here, notable factors will be under consideration, which may include cost-effectiveness, arsenic removal efficiency, and waste generation.

3. RESEARCH QUESTIONS

This study aims to evaluate the efficiency and accuracy of the engineered microbial–biochar filter for arsenic bioremediation in Bangladesh. In this respect, the following research question has been addressed:

- How well can a genetically modified dual-strain microbial consortium along with rice husk biochar (RHB) excrete arsenic from groundwater?
- Do the biochar properties (pyrolysis temperature, particle size) have the ability to improve microbial adhesion and arsenic removal?
- In a rural setting, what is the long-term performance and viability of the filter system?

4. LITERATURE REVIEW

Global drinking water safety is under severe threat due to arsenic contamination. Around thirty-five million people in Bangladesh are chronically exposed to contaminants like arsenic, leading to an estimated 40,000 fatalities annually [10]. Numerous arsenic mitigation strategies have emerged that are prohibitively costly and logistically complex for rural areas, making low-cost solutions like Subsurface Arsenic Removal (SAR) a promising alternative [11]. Notably, biological oxidation of arsenite (As(III)) is a promising and eco-friendly method due to its potential specificity and cost-effectiveness [12]. An existing study confirms that microorganisms capable of the arsenite oxidation process are prevalent in contaminated Bangladeshi aquifers and exhibit a higher removal capacity than abiotic methods, leading to a strong potential for possible bioremediation [13].

Despite this upward trend, current bioremediation efforts have their limitations. Previous research studies have struggled to isolate and characterize individual high-efficiency strains from native microbial enrichments, resulting in inconsistent outcomes [13]. For instance, some microbial communities containing arsenite-oxidizing genes cannot demonstrate the expected arsenite oxidation activity [13]. Conversely, modified biochar greatly improves heavy metal absorption, but most existing filter systems combine standard biochar with non-engineered bacteria, which lacks the genetic control required for consistency and optimality in arsenic removal across different arsenic valence states [6].

More specifically, this study emphasizes the demand for developing a selective and cost-effective filter system for arsenic removal in rural settings. Therefore, resulting in the approach for the design of an engineered microbial–biochar filter. The proposed method specifically utilizes individual genetically engineered bacteria, for instance, *P. putida* with a constitutively active *aioBA+* operon, to offer more controlled genetic capacity. Furthermore, incorporating these modified bacterial strains with locally sourced rice husk biochar (RHB) will significantly increase the absorbency and provide a reliable solution for arsenic mitigation in affected regions.

5. METHODOLOGY

The methodology seeks to employ synthetic biology and materials engineering to develop a sustainable microbial–biochar filter system (MBFS) targeting reduction of arsenic levels consistently below 5 µg/L in groundwater, substantially lower than the Bangladesh standard of 50 µg/L. The system mostly utilizes engineered microbes to oxidize arsenite (As(III)) to arsenate (As(V)) onto a specialized biochar matrix.

5.1 Biocatalyst Engineering and Stability (Phase 1) As here, the core component is an engineered microbial biocatalyst, designed to enhance arsenic extraction by the conversion of highly mobile As(III) to easily adsorbable As(V).

- **5.1.1 Genetic Construct Design:** As chassis strains, either *Pseudomonas putida* or *Bacillus subtilis* are selected due to their durability. Following that, the arsenite oxidase gene (*aioBA*) is placed under the strong constitutive promoter *Pveg* to ensure continuous and non-selective expression.
- **5.1.2 Chromosomal Integration:** The *aioBA* genetic construct is integrated into the host's chromosome by the usage of suicide vectors (e.g., pK18mobsacB, pMUTIN4) to maintain further genetic stability and prevent plasmid loss.
- **5.1.3 Biosafety Measures:** Antibiotic resistance genes that are used for selection are deleted after integration using the FLP/FRT site-specific recombination system. The target is to comply with regulatory requirements for Living Modified Organisms (LMOs).

5.2 Materials Engineering and Immobilization (Phase 2) This phase mainly focuses on the biochar media to ease the structural support and adsorption capacity for MBSF.

- **5.2.1 Biochar Production:** Locally collected rice husk is initially pyrolyzed at high temperatures (600°C–650°C) to increase the carbon content and mechanical strength. Followed by chemical activation (e.g., using K_2CO_3 or H_3PO_4), it expands the overall surface area and porosity.
- **5.2.2 Functionalization:** The activated biochar is later processed via wet impregnation with iron (Fe) and manganese (Mn) salts to create the hydrous oxide sites, which maximize the capture of adsorbable arsenate As(V).
- **5.2.3 Immobilization:** Engineered cells from phase 1 are fixed onto the functionalized biochar using Glutaraldehyde (GA) crosslinking. Moreover, this modification can ensure the maximum cell retention (target >108 CFU/g) while preserving the microbe's in-built catalytic activity.

5.3 Performance Validation and Environmental Safety (Phases 3 & 4) Phases 3 and 4 primarily focus on validating the performance against the realistic groundwater conditions and safety protocols.

- **5.3.1 Competitive Ion Testing:** Arsenic excretion can interfere with Bench-scale tests as it mimics household use with synthetic groundwater with high phosphate (PO_4^{3-}) and silicate (SiO_4^{4-}). The MBFS uses Iron and Manganese dosing to form Fe precipitates that collect arsenate (As(V))
- **5.3.2 Arsenic Speciation:** Employment of High-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) to detect a distinct shift from As(III) in the influent to As(V) in the effluent.
- **5.3.3 Biosafety and Leaching:**

- **5.3.3.1 HGT Monitoring:** Horizontal Gene Transfer (HGT) along with Quantitative Real-Time PCR (qPCR) is applied to detect the engineered gene construct (*aioBA*) in native water microbes.
- **5.3.3.2 Chemical Safety:** Glutaraldehyde leaching is used for microbial immobilization and then monitored using Headspace Solid-Phase Microextraction Gas Chromatography–Mass Spectrometry (HS-SPME GC-MS) with a detection limit of 0.1 µg/L to ensure safety. Following that, the filter housing materials, such as PVC are tested for phthalate absorption.

5.4 Sustainability and Waste Management (Phase 5)

- **5.4.1 Testing:** Spent biochar will be tested using the sequential Extraction Procedure (SEP) to maintain the arsenic stability in landfills.
- **5.4.2 Stabilization:** Following the previous process, biochar (arsenic-laden) is mixed with cement or polymer to form a renewable, low-cost construction material.
- **5.4.3 Life Cycle Assessment (LCA):** LCA with SimaPro software will be implemented to evaluate environmental impacts. Next, Cost Per Liter (CPL) is calculated to confirm affordability.

7. EXPECTED OUTCOMES

The MBFS is designed to expect high efficiency in the excretion of arsenic from the ground-level water in rural Bangladesh. Also, emphasizing the usage of locally manufactured materials to ensure key factors, including affordability, efficiency, and scalability, for a long-term adaptation and overall public health improvement.

8. IMPACT AND SIGNIFICANCE

- **8.1 Public Health:** A low-cost approach can provide safe drinking water to millions of citizens while preventing cancerous skin diseases and saving lives.
- **8.2 Environmental and Economical Aspects:** Rice husks from local sources are mainly used in the MBFS system. Profoundly, it is considered to be an agricultural waste product just to create the biochar filter. Now, the proposed system significantly reduces both the production costs and environmental impact.
- **8.3 Global Value:** While the primary focus is on the Bangladesh perspective, the technology is quite flexible and adaptable and can also be applied to other regions in South Asia, Latin America, and Africa that deal with similar challenges.

9. POTENTIAL LIMITATION

- **9.1 Genetic Stability:** Newly adapted bacterial strains may drift genetically over time, which might affect the filter's quality.
- **9.2 Material Quality:** Locally collected low-quality rice husk biochar may change the overall outcome.

10. TIMELINE

- Phase 1 (Months 1–3): Strain design, biochar refinement, and lab test conduction.
- Phase 2 (Months 4–6): Development of filters and creation of user manuals.
- Phase 3 (Months 7–12): Installation of filters in three villages for six months and monitoring performance.
- Phase 4 (Months 13–15): Data analysis and comparison with a standard bar chart.
- Phase 5 (Months 16–18): Publish the substantial findings.

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