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# Bioremediation of Fracking: Novel Hybrid Biofilm System Using Synthetically Engineered Curli Fibres

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**Abstract**

Hydraulic fracturing, a popular mining technique, generates heavy metal contamination in nearby freshwater aquifers. This poses a threat to both the surrounding ecosystems and human health if exposed. Existing methods of heavy metal removal can produce additional hazardous byproducts. This proposal presents the use of a hybrid biofilm filter containing graphene and curli fibres with metal binding sites. Curli fibres are amyloid fibrils found on the extracellular biofilm of *Escherichia coli* (*E. coli*). Through the use of plasmid vectors, *E. coli* will be engineered to produce secreted curli fibres with metal-binding residues. The stability and cohesive properties of the curli fibres augments the adherence to the graphene scaffolding, thus allowing for generation of a hybrid biofilm. With the filtration design and various experimental controls proposed, this model is ready for empirical proof of concept and subsequent quantitative optimization.

**Keywords:** synthetic biology; bioremediation; heavy metal; ecoli; curli fibers; plasmid; filtration; metal binding

## Introduction

Among leading global concerns, water pollution is perhaps the most pertinent to ecological survival. Industrial developments such as natural gas extraction can present detrimental consequences to the environment. Hydraulic fracturing, also known as fracking, extracts natural gas by pumping fracturing fluid into unconventional reservoirs such as shale formations, thus forming a hydraulic fracture [1]. Although fracking has reinvigorated the oil industry, this technique raises several environmental concerns. With the large production of flowback water containing fracturing fluid, freshwater aquifers overlying shale formations may be contaminated [2]. In a study conducted by Mchugh et al., freshwater samples were screened near fracking sites and various heavy metals such as arsenic and barium were discovered, warranting further investigation [2].

Heavy metals can negatively impact human health by binding to enzymes, thus displacing the original metal cofactors and reducing or blocking enzymatic activity. The resulting cell malfunction and toxicity can severely impact neurological function and promote pathogenesis [3]. A study investigating heavy metal contamination near large industrial complexes in Bangladesh found excessive arsenic accumulation in water and vegetation. Environmental exposures to this heavy metal has been related to increased cardiovascular mortality in these regions [4]. In another instance, gold mining practices in China have resulted in an augmented chromium contamination of nearby soil [5]. Chromium toxicity can result in increased onset of pulmonary diseases such as asthma and lung cancer [6].

While measures can be taken to remove heavy metal contaminants from freshwater reservoirs, these methods may adversely impact the environment and are limited in their effectiveness. For instance, chemical precipitation, the current gold standard for metal filtration, results in the production of toxic fume and poses harmful threats to wildlife and human health [7]. Novel methods of water decontamination are necessary to regulate heavy metal concentration in freshwater.

Synthetic biology provides a novel avenue through which contaminated water can be safely purified. The natural ability of *Escherichia coli* (*E. coli*) to produce curli fibres, a type of amyloid fibril, can be utilized to target metal contaminants in freshwater aquifers [8]. Curli fibres are a major protein component of *E. coli* extracellular pellicle biofilms and are produced naturally. Thus, their production is reliable and relatively inexpensive compared to analogous synthetic biomaterials [8]. Curli fibres are non-selectively adhesive, thereby highlighting the effectiveness of this method in treating contaminated water. Curli fibre aggregates can generate a biofilm, a thin layer of microorganisms whereby cells can adhere to each other or to a surface [8].

While effectiveness of a curli fibre biofilm has been demonstrated in external literature due to their highly adhesive properties, additional use of a scaffolding material can enhance the filtration design [8]. With an established capacity to clear heavy metals, activated carbon can be added to genetically engineered curli fibres to strengthen and support the filter model. A specific methodology for this process

will be detailed in the sections below. This hybrid biofilm has demonstrated an increased capacity to filter heavy metals in laboratory research studies [9].

*E. coli* can be genetically engineered to secrete curli fibres with increased affinity to heavy metals contaminants. Specific metal binding residues can be incorporated into curli fiber proteins through plasmid transformation. If a subsequent generation of a biofilm with an activated carbon scaffolded filtration system is introduced to contaminated freshwater, heavy metal concentrations can be reduced.

## Methods

A three-part methodology, discussed extensively below, will be utilized to examine the capacity of curli fibres in decontaminating freshwater resources. *E. coli* will be genetically engineered to produce curli fibres with increased affinity to heavy metal pollutants. Curli fibre secretions will then be isolated from the bacteria, and a hybrid biofilm containing activated carbon and curli fibres will be generated and used in conjunction with a novel filtration system which will be outlined in Part 3 of this methodology. The aforementioned techniques will be monitored using various tests at each stage of the experimental procedure.

### 1. *Engineering E. coli*

To create the proposed biofilm, specific bacterial components must be genetically engineered (see Figure 1). The strain E. Coli K12 will host the engineered plasmid and produce the curli fibres [10, 11]. Curli fibres, which will form the biofilm, are produced due to the presence of the operons CsgBA and CsgDEFG. These sequences will be isolated from the E. Coli K12 genome then transformed into the selected E. coli plasmid strain, pBbE1a [10]. The plasmid will accommodate the insertion of a metal-binding protein gene sequences to increase affinity to heavy metals found in water aquifers. A His-tag is required to incorporate metal binding residues on the fibres; the tag will be adjacent to operon sequences [12]. Copies of the recombinant plasmid will be generated using PCR and inserted using various techniques involved in DNA transformation.

In order to select for transfected bacteria, an antibiotic cassette will be cloned into the plasmid [13]. Antibiotic cassettes code for a mobile site-specific recombination system and can be integrated within a genome or transferred between organisms [13]. To incorporate cassettes within a plasmid, site-specific recombination systems known as integrons are required to mobilize and insert gene sequences [13]. The proposed experimental design will utilize integron fragment ln4, which contains the cassettes aacC1-orfE-aadA2 [13]. The genes aacC1 and aadA2 code for antibiotic-resistant against gentamicin and streptomycin, respectively [13]. Cloning will be performed using DNA transformation techniques and successful incorporation will be confirmed using DNA sequence analysis [10]. Successfully transfected bacteria will be collected using YESCA-CR plates supplemented with growth media and streptomycin (10, 13).

### 2. *Isolating Curli fibres from E. coli*

In order to avoid the introduction of potentially harmful bacteria to freshwater sources, the curli fibres must be separated from the *E. Coli*. The isolation of curli fibres from *E. Coli* cell membranes is required to create the filter and will be achieved using a sodium chloride solution (NaCl) [14]. *E. coli* colonies will be cultivated on YESCA agar plates for 72 hours, after which they will be suspended in 1.5 M of NaCl for 10 minutes [14]. The plate media will be supplemented with streptomycin to only select for curli fibers from the engineered bacteria [13]. Following the centrifugation of the solution, the curli fibres can be harvested [14].

### 3. *Hybrid Biofilm Generation and Filtration System*

The extracted curli fibre solution will be mixed with activated carbon and vacuum filtered using a cellulose filter [15]. A hybrid composition adsorption membrane can consequently be formed due to the extreme adhesiveness and stiffness of curli fibres [15]. As activated carbon can act as a scaffold for the hybrid biofilm, the overall structural support for the system is enhanced, thus strengthening the filter model [16]. Several hybrid biofilms will be organized orthogonal to water flow within the body of the filter in order to maximize rate of filtration. The proposed filtration system is shaped into a curving spiral to increase path traveled by water within a specific length, thus augmenting the surface area of the filter that a given unit of water is exposed to (see Figure 2). This curving filter model additionally introduces turbulence to water flow, amplifying metal filtration quality. Gravity is sufficient to direct the flow of water.

Several positive and negative controls will be implemented at the various stages of the methodology to ensure reliability and validity of collected results during experimentation.

## Results

There currently lacks empirical data regarding the binding rate of His-tag for different heavy metal concentrations and biomass of curli fibres. However, existing studies under analogous experimental conditions provide gross parameters for what to expect as saturation rates are investigated in future steps, beyond the scope of this methodology. Laboratory experiments can be conducted to determine vital information regarding curli fibre behaviour. Previous studies have demonstrated that unaltered curli fibres on *E. Coli* have higher absorption thresholds for heavy metal Hg than the negative control: curli-deficient derivative - PHL628 csgA [17]. While there will be higher concentrations and a variety of heavy metals found in fracking water compared to laboratory heavy metal conditions, we expect quasi-steady-state concentrations of heavy metals within the filter to approximate the steady concentration seen in this study,

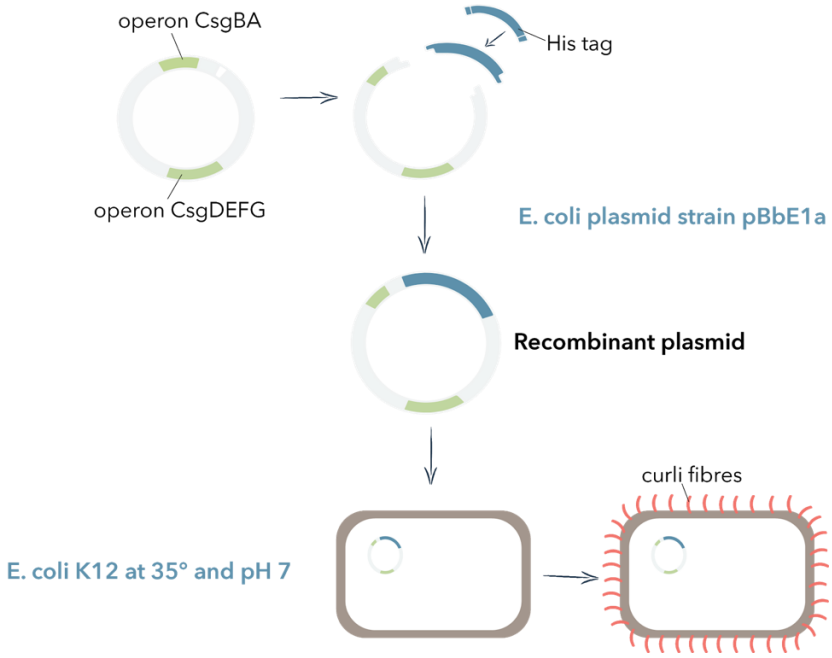


**Figure 1.** Flowchart Portraying *E. Coli* Engineering and Curli Fibre Production

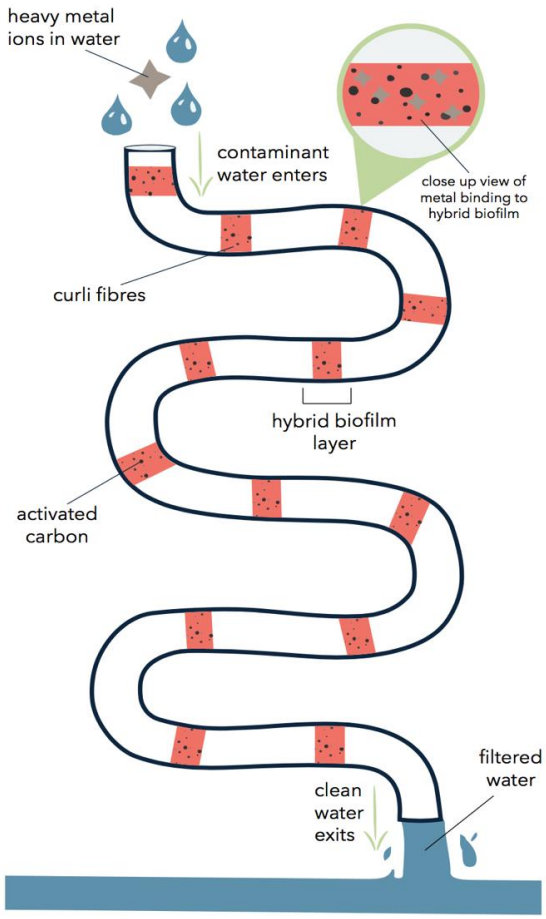


**Figure 2.** Displaying Flow of Water through the Proposed Filter


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**Table 1.** Controls and Expected Results for Experimental Phases

| Phase | Dependent Variable | Test and expected results | +/ - Control and expected result |
|---|---|---|---|
| Part 1 - Curli Production | His-Residues in altered curli on E.coli | Colony PCR | +: gel electrophoresis will show His-Tag fusion protein bands<br>-: No protein bands will be present |
| Part 2 - Curli Isolation | Curli presence in isolated curli mass | Congo Red Dye: Significant signal | + : Amyloid produced from milk: Significant signal<br>-: E.coli growth medium: insufficient signal |
| | E. coli in isolated curli mass | E. Coli Assay:<br>No presence | +: E.coli in growth medium: presence detected<br>-: E.coli growth medium with curli presence: no presence |
| Part 3 - Filtration | Heavy Metal Concentration in wastewater | ICP- Mass Spectrometry: Decreased detected concentration after filtration | +: Water with known Heavy metal concentration: decrease in signal<br>-: Tap water: insufficient change in signal |
| | Curli presence in filtered water | Congo Red Dye: No presence | + : Amyloid produced from milk: Significant signal<br>-: E.coli growth medium: insufficient signal |

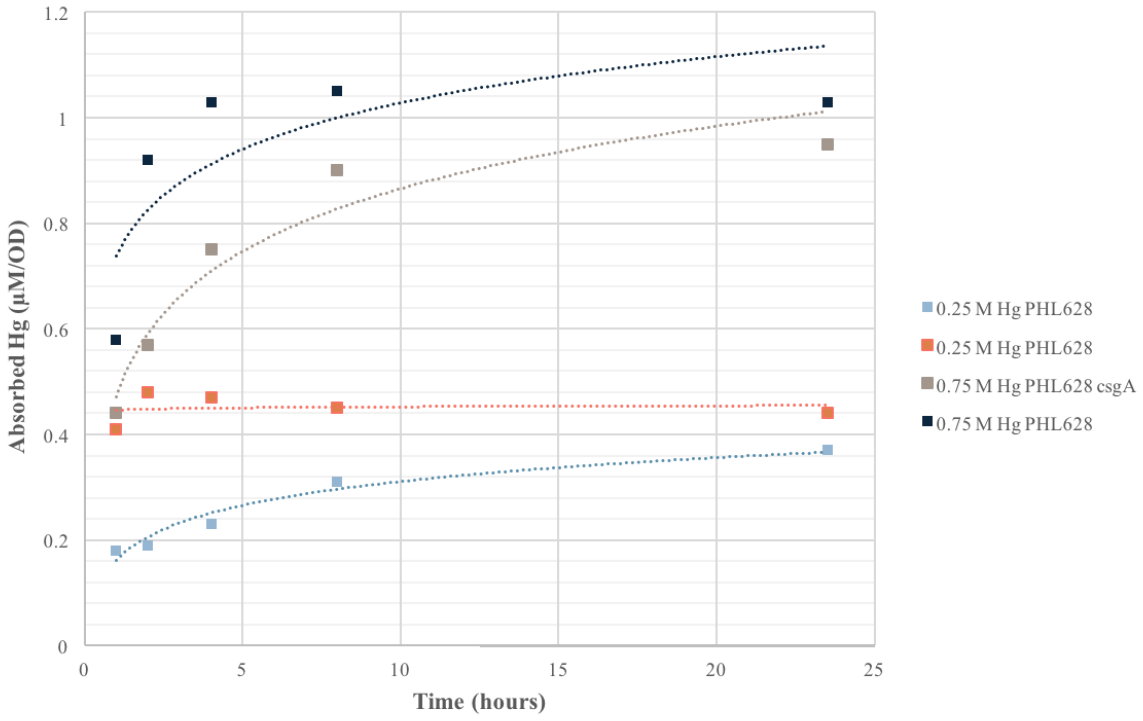


**Figure 3.** Graph Displaying Hypothesized Absorption Thresholds of Heavy Metals in the Proposed Filter Based on External Literature Conducted [17]

with absorption characteristics following a similar model to reach equilibrium.

Saturation was reached in 10-15 hours, faster for absorbents of higher affinity given the same Hg concentration, and faster overall for lower Hg concentrations (see Figure 3) [17]. As wastewater has significantly higher heavy metal concentrations than the conducted study we expect longer wait-times to reach full saturation before the filter needs to be replaced [17]. Ultimately, wastewater concentration of heavy metals would require constant monitoring to determine saturation of filter, as per protocol of current water filtration systems used today [17].

## Discussion

It is estimated that several million people are exposed to heavy metals chronically throughout the world, and hydraulic fracturing is augmenting this number [4]. Heavy metal contamination poses a plethora of threats to human health, including a lower birth weight, onset of childhood cancer, cardiovascular disease and overall increased mortality rates.

This filtration system proposed in this article may mitigate health consequences by eradicating heavy metal contaminants in freshwater supplies. The generation of curli fibres is reliable and relatively inexpensive compared to other synthetic biomaterials [8]. In addition, this technique is environmentally friendly and does not pose any harmful effects to other organisms. To control of E. coli and curli fiber contamination, several controls and environmental precautions will be put in place, as discussed in Table 2. As a further precautionary measure, E. coli contamination will be detected using standard methods and the water will be treated with 1.5 mg/L of chlorine, a potent disinfectant [18].

While the experimental design is rigorously tested and controlled, there are several confounding variables which may influence the quality of the results. For instance, the presence of other bacteria and organic compounds in the contaminated water may influence interaction between curli fibre and heavy metals. Additionally, in the graphene-based filtration system, there is a potential for the escape of curli fibres. Further experimentation will determine how these factors may impact study results and quality of filtration.

## Conclusions

The scope of this particular methodology was limited to whether genetically modified curli fibres can significantly reduce heavy metal contamination. As a result, further laboratory experiments can identify valuable and specific information regarding saturation points of curli fibres and curli lifespan. A fracking-based filtration design can also be implemented to investigate methods of flowback water filtration prior to freshwater contamination. Although this study was restricted to consequences arising from hydraulic fracturing specifically, ideas from this methodology can be globally applied in other populated areas with polluted water source concerns.

While profitable in natural gas extraction, fracking poses a plethora of detrimental effects to the environment. As a solution, the introduction of the proposed hybrid graphene-curli biofilm can significantly reduce heavy metal concentrations in freshwater reservoirs. This hybrid biofilm system aims to transform current methods of heavy metal filtration by providing an environmentally-friendly alternative. The suggested system's contributions to bioremediation promotes the sustenance of water systems and local ecology.

## List of Abbreviations

E. Coli: Escherichia coli
PCR: Polymerase Chain Reaction

## Conflicts of Interest

All authors declare that they have no conflict of interest

## Ethics Approval and/or Participant Consent

This study did not require ethics approval or participant consent as it was a proposal and no human participants were used.

## Authors' Contributions

KP: made contributions to the design of the study, created all figures and graphic artwork, drafted the manuscript, and gave final approval of the version to be published
HR: contributed to study design, investigated controls for methodology, drafted the manuscript, and gave final approval of the version to be published
MH: made contributions to study design, proposed and extrapolated study results based current literature, and gave final approval of the version to be published
JL: contributed to design of the study, discussed implications of study idea, and gave final approval of the version to be published

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