Fighting Mercury Pollution in a Sustainable, Environmentally-friendly, and Cost-effective manner

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Abstract:

Mercury (Hg) , defined as a considerably toxic heavy metal, has been exhibited to cause substantial risks to aquatic environments through contamination, particularly through bioaccumulation in seafood. The current remediation methods for mercury are limited, driving the study of sustainable biomaterials. This theoretical study delves into the design of nature-based bacterial biofilms, integrating curli proteins in embedded amyloid fibril matrices, to effectively sequester mercury. Mercury ions are captured by the biofilm optimised with high specificity, while the introduction of mutant *merR* gene serves to enhance sensitivity to fluctuating mercury concentrations. The system is suggested to be validated through transcriptional activation, protein aggregation, and mercury quantification, while the optimum parameters are suggested to be confirmed through mathematical modelling. The approach adheres to sustainable bioengineering principles by offering alternative bacterial hosts and genetic safeguards minimising risks associated with genetically modified organisms (GMOs) release.

Introduction:

The Problem:

The continuing emission of heavy metals into aquatic environments has led to contamination of organisms at every trophic level, particularly mercury, which shows toxicity at even low levels (Kimáková et al., 2018). In recent years, therefore, the level of mercury contamination in seafood has become a major human health problem. This has resulted in a significant number of publications investigating the bioavailability of mercury pollutants and the cycles surrounding its presence in the oceans (Jinadasa et al., 2021). Since the Minamata Bay disaster of 1956, in which over 2000 people died after eating contaminated seafood following a release of methylmercury into the bay by a local chemical factory, a global effort has been underway to mitigate the threat posed by mercury pollution (Kimáková et al., 2018).

Why use Biomaterials for sequestering Mercury?

This theoretical approach scrutinises the employment of biomaterials to seclude mercury. Efficient binding and immobilisation of contaminants have been demonstrated by biomaterials such as plant based-amyloid fibrils and biofilms, granting a sustainable approach to heavy metal remediation (Li et al., 2023). This in accordance with secondary sources evoking effective binding capabilities of fungi-derived functional amyloids (Soon et al., 2022), as well as the potential of fungal hydrophobins and amyloids for mercury sequestration (Wu et al., 2022), emphasising their promise in sustainable remediation. This investigation explores functionality optimisation of the biomaterials executed through the inclusion of genetic alterations targeting enhanced mercury retention and sequestration.

Methodology:

Engineered system:

To mitigate mercury contamination concerns in marine ecosystems, the suggested approach leverages novel biomaterials with genetic manipulation to augment captured mercury. The Hg-contaminated water would flow through a biofilm which would be directly engaged with mercury uptake from the functional amyloid fibrils integrated within the matrix. The curli proteins in these fibril structures create an extracellular, sponge-like functionality in the amyloids, allowing the entrapment of mercury ions through highly specific and strong affinity binding (Tay et al., 2017). A novel gene circuit governs the mercury sequestration process. The incorporation of mutant meR genes and mercury transporter elements would implement a dynamic circuit towards fluctuating mercury concentrations for sensitivity optimisation, effectively secluding mercury and maximising resource usage.

Plasmid design:

The plasmid design would be carried in SnapGene once sequences are retrieved in UniProt. Mutations would be integrated into the meR gene altering the transcriptional activator *merR* which is designed to sustain a repressor conformation in the absence of Hg, and activator conformation in the opposite case. This follows the methodology of PARKHILL et al. (1998), in which the most strongly activated phenotype was identified with a triple mutation, attaining 86 ± 17% of induced wildtype

activity. This mutant *merR* exhibited lower binding affinity to the wild-type operator, promoting reactivity to mercury. Additionally, the binding affinity of the *MerR* variants to the operator was further refined to advance responsiveness to mercury. The inclusion of mutant *merR* gene with mercury transported in the plasmid design would enable to increase sensitivity and efficacy of sequestration (Hakkila et al., 2011). The combined optimisation of binding affinity and transcriptional activation establishes efficient response to mercury contamination.

Proof of Concept/ Validation Experiments - How we will test that it works:

The gene circuit would be validated from exposing the bacterial host with the integrated mutant *merR* to ambivalent mercury contractions and certifying the responsiveness of the circuit by measuring transcriptional activation through the *16S* reporter genes (Tay et al., 2017). Mercury exposure would be conducted from the addition of mercury to media for varying lengths of time to investigate its effects. Transcriptional analysis of engineered *E. coli* would be carried out, involving the use of Congo Red to study protein aggregation.

Moreover, the efficacy of the biofilm would be confirmed through the quantification of mercury prior and subsequent to contamination using atomic absorption spectroscopy, with the difference affirming the competence of the system. For visualisation, scanning and transmission electron microscopy (SEM and TEM) would be utilised to assess the structure and functionality of the biofilm. Lastly, inductively coupled plasma mass spectrometry (ICP-MS) would serve as the quantification of mercury binding, validating a holistic verification of the designed system.

Mathematical modelling/Simulation experiments:

The dynamic genetic network would be modelled using ordinary differential equations simulating varying parameters to identify the optimum promoter strength, binding affinity and mercury concentration and establish a compatible configuration that would enable optimal performance. Competence of the designed biofilm within the aquatic environment would be simulated to predict its capability to seclude mercury. For optimisation of a durable and robust system, the model should be simulated under variable environmental parameters through tools like HADDOCK and ClusPro for the examination of protein interactions while Phyre2 would assist in structural predictions of the biofilm system. Finally, an *in vitro* simulation experiment should be carried out by testing the biofilm filter in a small tank system, introducing mercury in controlled quantities to test uptake by material. Mercury uptake could be tested in both freshwater and seawater

to evaluate the durability and robustness of the engineered construct across diverse aquatic environments.

Biosafety/ Ethical concerns of engineered E.coli:

The objective of this research project is founded on sustainable bioengineering principles and ethical mercury decontamination. The amyloid fibril-embedded material proposes a sustainable, nature-based remediation method adjusting to mercury concentrations, enhancing heavy metal sequestration over chemical methods.

Biosafety concerns, however, would include the possible horizontal gene transfer of altered genes, through a transfer to wild-type bacteria in the environment. Furthermore, accidental leak of genetically modified microorganisms from the biofuel could become disruptive to ecosystems and non-target organisms (Lensch et al., 2024). Solutions to minimise these risks could include extending the experiment by handling alternative expression hosts *Shewanella spp.* and *Bacillus spp,* which are better adapted to aquatic ecosystems and mercury resistance than E.coli. These aquatic-adapted bacteria could diminish the potential risks posed to other species while reducing the excessive use of E.coli from their stronger resilience to mercury (Joshi et al., 2021).

Additionally, a genetic safeguard system could be implemented (Moe-Behrens et al., 2013), comprising a circuit designed to monitor mercury retention and prompt cell death, thereby preventing the escape of manipulated organisms. Self-destructive enzymes would be generated in reaction to predetermined mercury concentrations, ensuring meticulous control over gene expression and regulated termination of modified cells (Xue et al., 2022).

For a refined application of the biofilm system, one of the common fish farming techniques requires nets in open water which could be adapted for applying the biofilm material to be suitable in these environmentss. Tests extracting mercury from biofilm for reuse purposes of the filter could be further conducted for a sustainable system. Continued testing of *merR* mutations through direct evolution would ensure usage of a long-term resilient system.

Conclusion:

This theoretical study proposes a sustainable approach to minimise mercury contamination issues in aquatic environments through the design of a bacterial biofilm with enhanced mercury sequestration functionality. The selection of nature-based materials and integration of genetic alterations allow for a sustainable methodology to to effectively seclude mercury. The combined effect from curli proteins and mutant *merR* genes strengthens mercury binding through an increased specificity and sensitivity, delivering a competent yet eco-friendly substitute to traditional remediation techniques. For a real-life application of the designed system, further experimental validation should be conducted to enable its use at a large scale.

References:

Jinadasa, B.K.K.K., Jayasinghe, G.D.T.M., Pohl, P. and Fowler, S.W. (2021). Mitigating the impact of mercury contaminants in fish and other seafood—A review. *Marine Pollution Bulletin*, 171, p.112710. doi:https://doi.org/10.1016/j.marpolbul.2021.112710.

Joshi, G., Meena, B., Verma, P., Nayak, J., Vinithkumar, N.V. and Dharani, G. (2021). Deep-sea mercury resistant bacteria from the Central Indian Ocean: A potential candidate for mercury bioremediation. *Marine Pollution Bulletin*, 169, p.112549. doi:https://doi.org/10.1016/j.marpolbul.2021.112549.

Kaisa Hakkila, Nikander, P., Sini Junttila, Urpo Lamminmäki and Virta, M. (2011). Cd-Specific Mutants of Mercury-Sensing Regulatory Protein MerR, Generated by Directed Evolution. 77(17), pp.6215–6224. doi:https://doi.org/10.1128/aem.00662-11.

Kimáková, T., Kuzmová, L., Nevolná, Z. and Bencko, V. (2018). Fish and fish products as risk factors of mercury exposure. *Annals of agricultural and environmental medicine: AAEM*, [online] 25(3), pp.488–493. doi:https://doi.org/10.26444/aaem/84934.

Lensch, A., Lindfors, H.A., Duwenig, E., Fleischmann, T., Hjort, C., Kärenlampi, S.O., McMurtry, L., Melton, E.-D., Andersen, M.R., Skinner, R., Wyss, M. and van Kranenburg, R. (2024). Safety aspects of microorganisms deliberately released into the environment. *EFB Bioeconomy Journal*, [online] 4, p.100061. doi:https://doi.org/10.1016/j.bioeco.2023.100061.

Li, T., Zhou, J., Peydayesh, M., Yao, Y., Massimo Bagnani, Kutzli, I., Chen, Z., Wang, L. and Raffaele Mezzenga (2023). Plant Protein Amyloid Fibrils for Multifunctional Sustainable Materials. *Advanced Sustainable Systems*, 7(4). doi:https://doi.org/10.1002/adsu.202200414.

Li, Z., Ren, Y., Li, Z., Zhang, J., Fan, Y., Jiang, G., Xu, D., Gu, T. and Wang, F. (2023). Engineered Living Biofilm with Enhanced Metal Binding Ability for Corrosion Protection in Seawater. *Advanced Functional Materials*, 34(13). doi:https://doi.org/10.1002/adfm.202313120.

Moe-Behrens, G.H.G., Davis, R. and Haynes, K.A. (2013). Preparing synthetic biology for the world. *Frontiers in Microbiology*, 4. doi:https://doi.org/10.3389/fmicb.2013.00005.

*PARKHILL, J., LAWLEY, B., HOBMAN, J. L. & BROWN, N. L. 1998. Selection and characterization of mercury- independent activation mutants of the Tn501 transcriptional regulator, MerR. Microbiology, 144, 2855-2864.

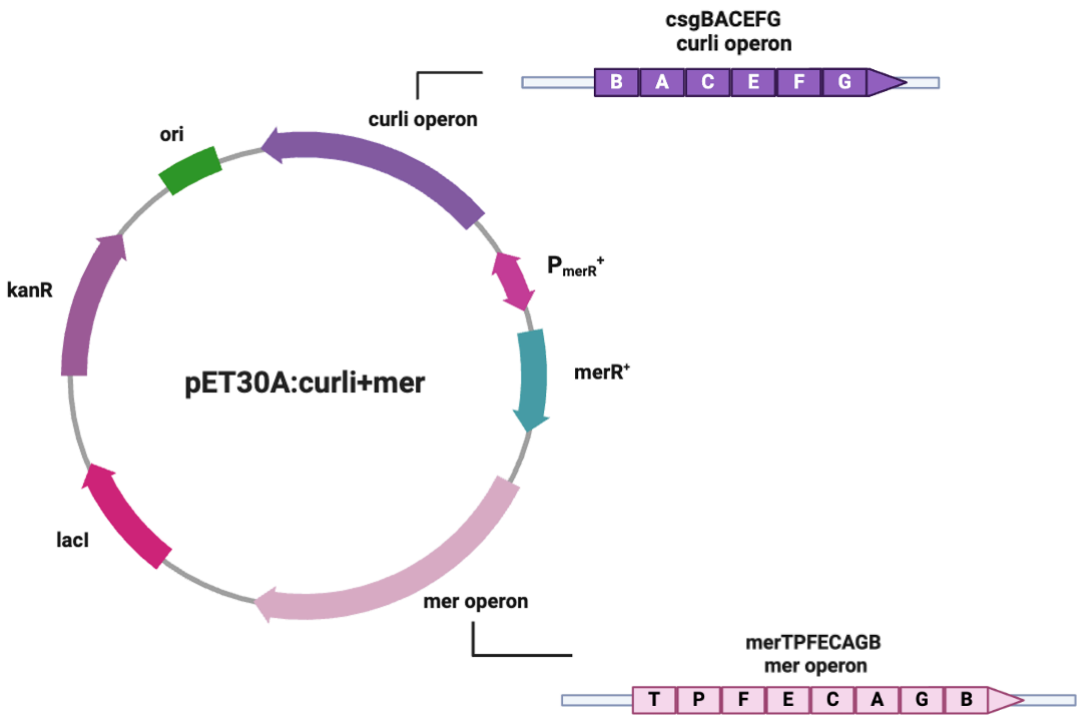
Soon, W.L., Peydayesh, M., Mezzenga, R. and Miserez, A. (2022). Plant-based amyloids from food waste for removal of heavy metals from contaminated water. *Chemical Engineering Journal*, 445, p.136513. doi:https://doi.org/10.1016/j.cej.2022.136513.

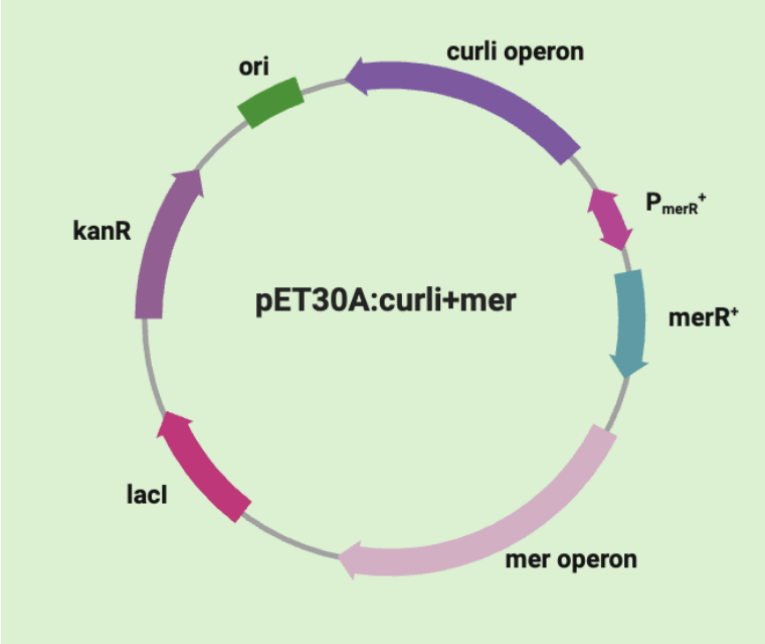
Tay, P.K.R., Nguyen, P.Q. and Joshi, N.S. (2017). A Synthetic Circuit for Mercury Bioremediation Using Self-Assembling Functional Amyloids. *ACS Synthetic Biology*, 6(10), pp.1841–1850. doi:https://doi.org/10.1021/acssynbio.7b00137.

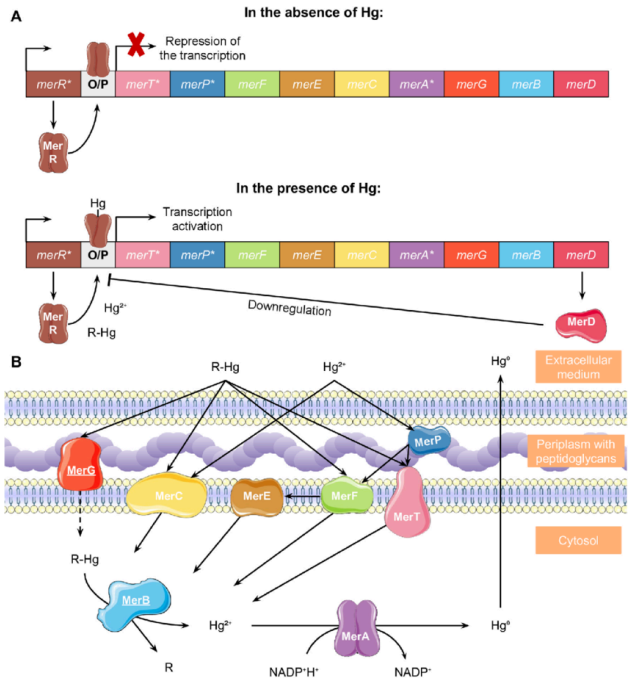
Wu, C., Tang, D., Dai, J., Tang, X., Bao, Y., Ning, J., Zhen, Q., Song, H., St. Leger, R.J. and Fang, W. (2022). Bioremediation of mercury-polluted soil and water by the plant symbiotic fungus *Metarhizium robertsii*. *Proceedings of the National Academy of Sciences*, 119(47). doi:https://doi.org/10.1073/pnas.2214513119.

Xue, Y., Du, P., Ibrahim Shendi, A.A. and Yu, B. (2022). Mercury bioremediation in aquatic environment by genetically modified bacteria with self-controlled biosecurity circuit. *Journal of Cleaner Production*, 337, p.130524. doi:https://doi.org/10.1016/j.jclepro.2022.130524.

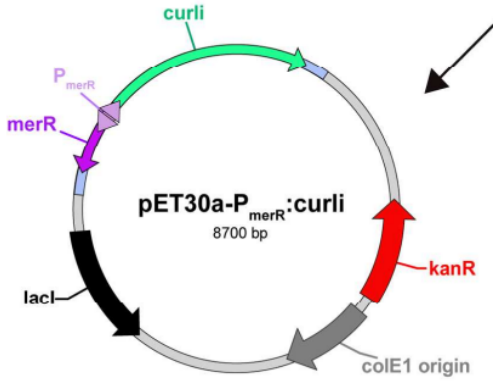
Supplementary material:







https://pubs.acs.org/doi/suppl/10.1021/acssynbio.7b00137/suppl_file/sb7b00137_si_001.pdf



Uniprot entries:

MERP_SHIGELLA: https://www.uniprot.org/uniprotkb/P04129/entry

MERA_SHIGELLA: https://www.uniprot.org/uniprotkb/P08332/entry

MERB_Escherchia coli: https://www.uniprot.org/uniprotkb/P77072/entry