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Bioremediation of lead using spore surface displayed proteins

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Bioremediation of lead using spore surface displayed proteins

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Abstract

Lead is a toxic pollutant very harmful to human health since it accumulates in the body and affects the brain, liver, kidney, and bones. Fetuses can be exposed to lead during pregnancy, which can cause problems with learning later on in the baby's life. The purpose of this project is to display a lead binding protein on bacterial spore surface for the bioremediation of lead from water. Spores from *Bacillus subtilis* are very robust and resistant to various harsh environments. Genetically fused to a spore surface protein, the displayed proteins demonstrate enhanced robustness and can be easily produced through sporulation without the need of further purification. PbrR is a regulatory protein that modulates the lead resistance in bacteria. In this project, we fused seven variants of PbrR to the spore surface protein CotC (CotC-PbrRs). The lead binding affinity and specificity of these PbrR variants on the spore surface will be characterized. The robustness of these spores with PbrR will be also evaluated using wastewater samples.

Introduction

Environmental remediation of contaminants in drinking water, soil, and wastewater is very important to human health and public safety. Bioremediation may be the solution to this because it is a relatively inexpensive remediation technique that does not require the introduction of outside chemicals. This research focuses on bioremediation of lead using spore surface displayed proteins. The objectives of this research were to:

- Genetically fuse 7 variants of PbrR to the spore surface of *B. subtilis*
- Measure each variant's binding affinity to lead
- Compare the binding affinity of spore surface displayed proteins to that of purified proteins and spore surface displayed proteins with a his-tag
- Study the effect of temperature and pH on the lead binding affinity
- Design a reversible process for lead recovery and reuse of the spores

Background

Heavy Metals and Lead

Heavy metals are naturally occurring and are widely used in industrial, domestic, agricultural, medical, and technological applications. They are toxic metals that damage organs, can cause cancer, and can accumulate in the body [1]. Lead, for example, is a harmful heavy metal because it accumulates in the human body. Fetuses can be exposed to lead in the womb and can detrimentally affect their development. Lead is widely used in daily life in lifting weights, protection against radiation, batteries, nuclear reactor coolant, and semiconductors, among many more. Figure 1 outlines several ways in which lead can affect the human body.

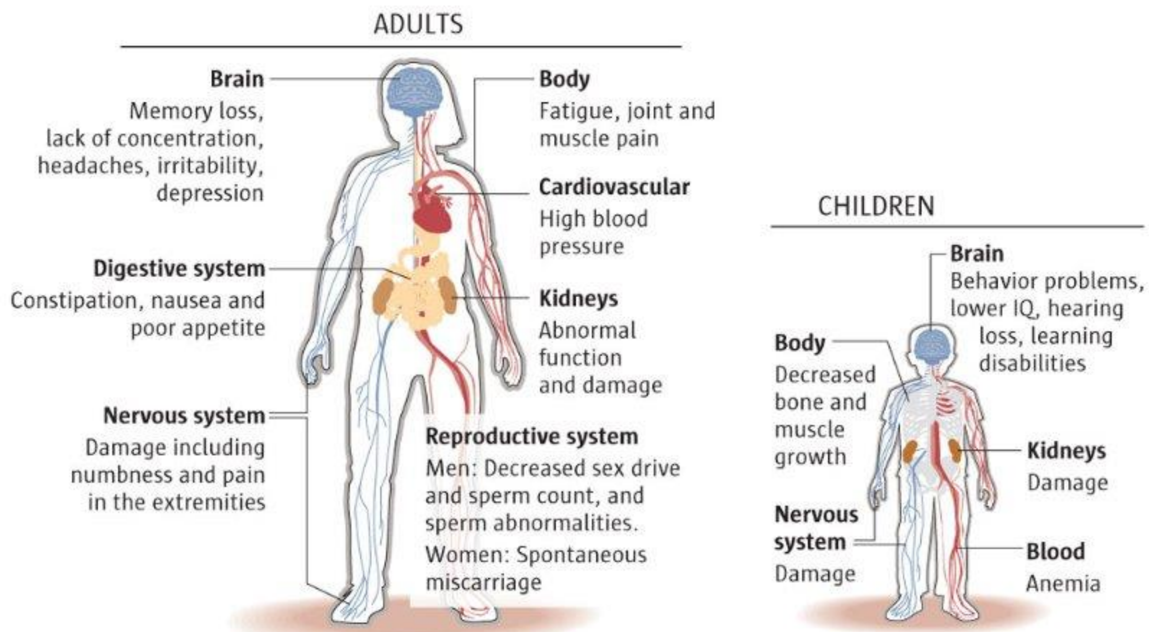

Figure 1.Ways that lead can affect the human body [2].

Remediation Techniques

There are several ways to remove pollutants from the environment. For example, chemical treatment, physical treatment, and biological treatment are all commonly used remediation techniques. Some examples of chemical treatment include chemical precipitation, ion exchange, and carbon absorption. Some examples of physical treatment include pump and treat and air sparging. Some examples of biological treatment include bioventing, biofilters, and bioaugmentation.

To expand on bioremediation, it uses organisms to break down pollutants in the environment or other contaminated areas. For example, some bacteria help clean up oil spills while maggots help clean up infectious wounds. Below is an example of the algae bioremediation cycle (Figure 2).

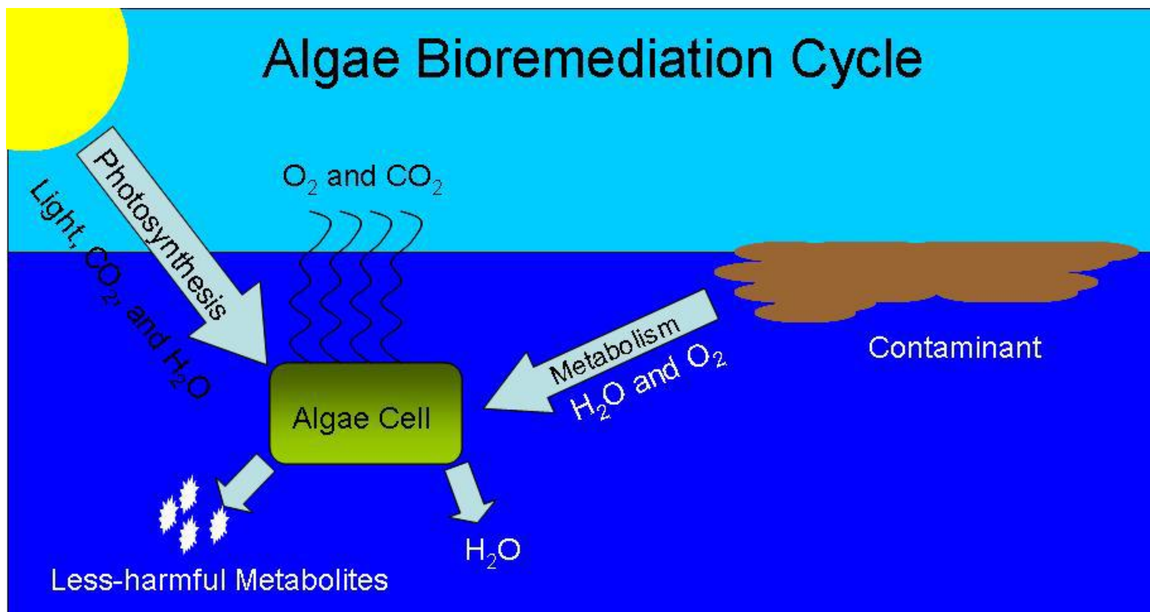

Figure 2. The algae bioremediation cycle [3].

Bioremediation was used in this project because it is relatively inexpensive compared to the other techniques, it does not introduce outside chemicals to the environment, and it can potentially be reused.

Spores

B. subtilis spores are very robust in the sense that they can survive a wide range of temperatures, a wide range of pH levels, in the presence of radiation, and in the presence of organic solvents. Spores are the dormant form of vegetative cells meaning they do not require nutrients to survive. In theory, they are able to survive for several years with no attention. Spores are robust because of their protective protein coat around the DNA (Figure 3).

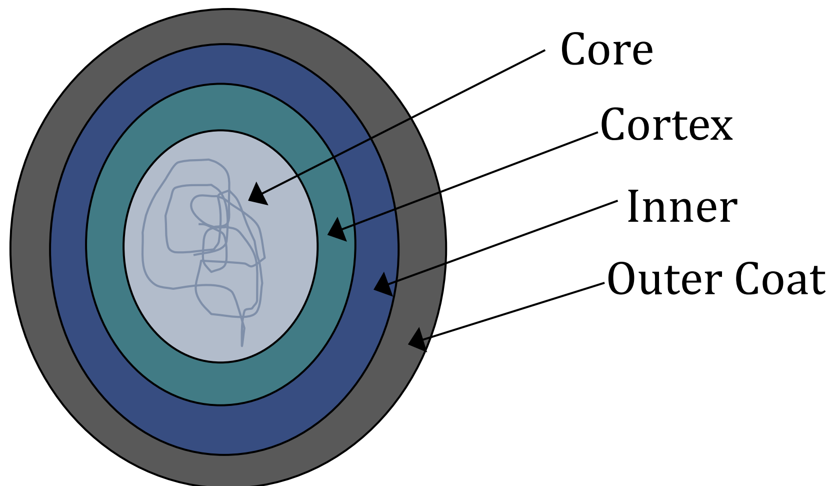

Figure 3.Schematic of a spore.

Spores are formed through sporulation when the environment is unfavorable to the vegetative cell (Figure 4). When a vegetative cell feels threatened, it packs up its DNA and forms a strong, protective protein coat around the DNA, which makes up the spore. The spore is then released from the remains of the cell into the environment. In contrast to fungi, sporulation of *B. subtilis* is a survival mechanism as opposed to a means of reproduction.

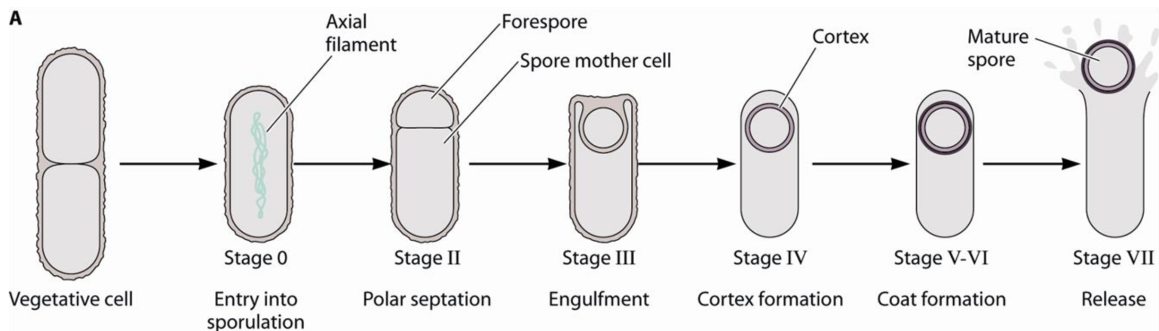

Figure 4. Sporulation cycle of cells [4].

Using spores for bioremediation purposes in place of purified proteins or regular cells has several advantages. For example, spores are more robust and easily created through sporulation. There is no necessity for further purification, which makes them less expensive than purified proteins. Another advantage is that spores can potentially be reused after recovering the heavy metals.

Lead Binding Protein PbrR

PbrR is the regulating protein for the lead resistance operon [5]. The protein is silent without lead. However, in the presence of lead, lead binds to the dimer and activates the DNA-binding and lead-binding genes in the protein (Figure 5).

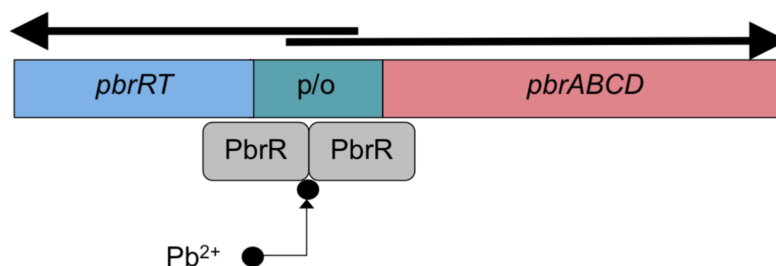

Figure 5.Figure of PbrR showing that in the presence of lead, both the lead binding and DNA binding genes are activated.

PbrR has a very similar amino acid sequence as MerR, which is a protein whose mercury-binding gene activates in the presence of mercury. MerR has been extensively studied, and its 3D structure is known. We are able to predict the structure of PbrR based on the structure of PbrR, which can be seen in Figure 6. This figure was produced using the PHYRE2 program.

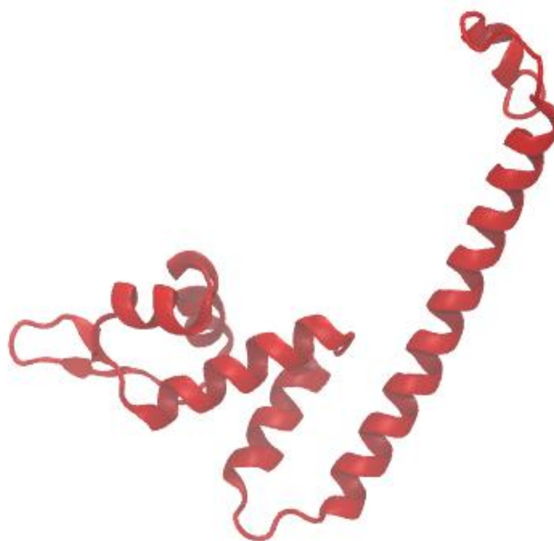

Figure 6.Structural prediction of PbrR based on the known structure of MerR using PHYRE2 program.

Experimental Design

Three variants of PbrR and four truncated variants of PbrR with and without a c-terminal his-tag were constructed. These variants include PbrR, PbrR2, PbrR3, PbrRΔ42, PbrRΔ60, PbrRΔ76, and PbrRΔ76Δ126-145. Each variant with the his-tag were expressed in *E. coli* using the plasmid pPROTet.E for purification of the protein. Each PbrR variant with and without his-tag was genetically fused to the anchor protein CotC in *B. subtilis* [6]. Then the lead binding affinity of the PbrRs displayed on the spore surface and the purified soluble PbrRs will be compared. This can be visually seen in Figure 7.

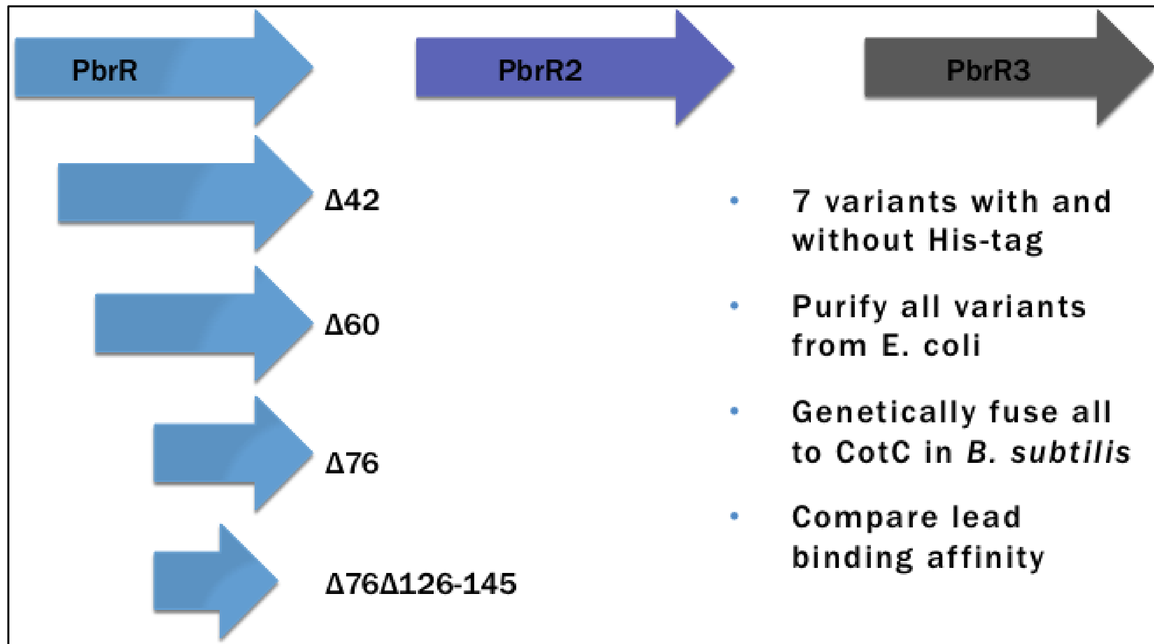

Figure 7. Visual representation of the experimental design.

Each PbrR-CotC variant was constructed using Gibson Assembly. The resulting protein can attach to the spore surface and has a lead-binding capability (Figure 8).

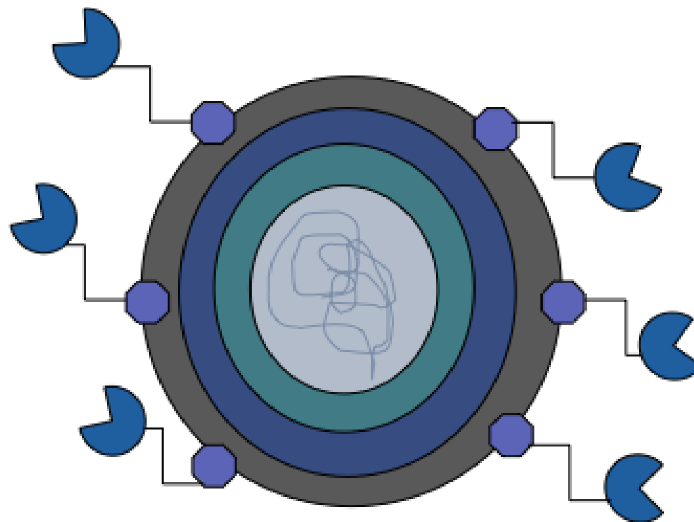

Figure 8. CotC-PbrR geneticallyfuses to spore surface during sporulation.

Results

Plasmids for the expression and purification in *E. coli* were successfully constructed (Figure 9-10).

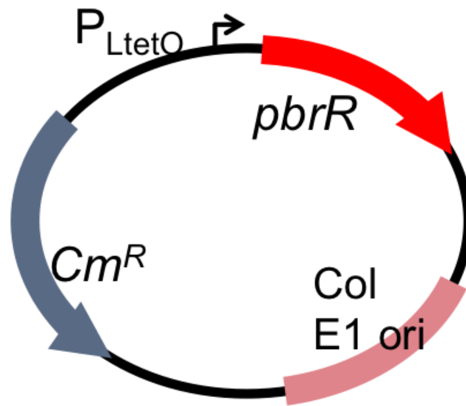

Figure 9. E. coli plasmid example.

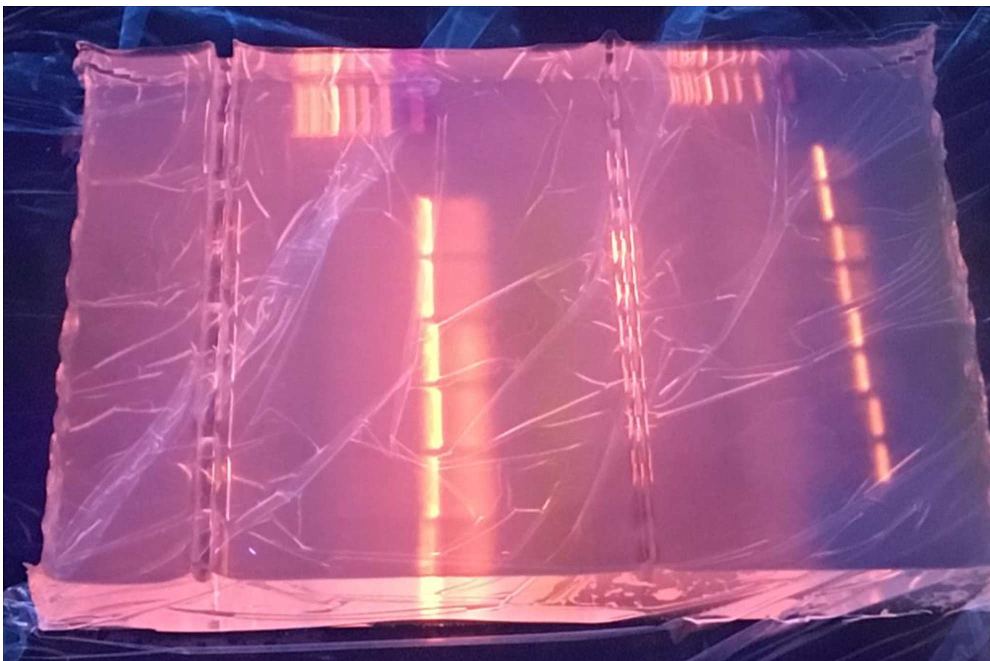

Figure 10.Gel electrophoresis results showing that the E. coli plasmid construction was successful.

Linear DNA containing cotC-pbrRs with and without His-tag were successfully assembled (Figure 11).

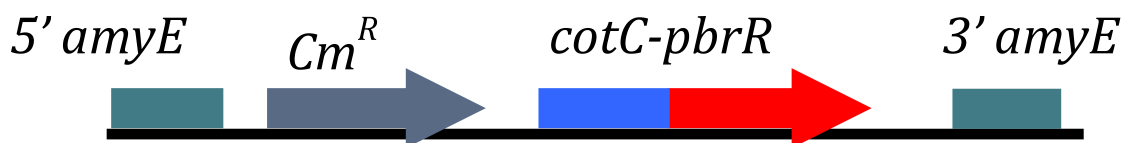

Figure 11. Linear DNA fragment example.

The transformation of the linear DNA into *B. subtilis* was unsuccessful.

Conclusions

The standard of cloning DNA is to use *E. coli*, and the process takes about three weeks [7]. However, our lab is working on shortening this process by performing the cloning out of cells and using methods such as PCR and Gibson Assembly. This whole process takes about one week, which is significantly shorter and allows for more testing. It is inevitable that we will run into issues with this process since it is such a new protocol. We suspect that the CotC-PbrR DNA chains are too short and therefore are unable to successfully integrate into *B. subtilis*. We have designed new DNA fragments that will be tested.

Future Work

Since the construction of plasmids for the expression and purification in *E. coli* was successful, the next step is to purify the seven PbrRs using a His-tag purification column. Next, each cotC-pbrR will be integrated into *B. subtilis* using new primers to make the DNA inserts longer in length. Once a successful integration is achieved, the concentration of PbrR on the spore surface will be quantified using ELISA. The lead binding affinity will be measured using a wild type spore at room temperature and neutral pH as the control. The effect of temperature and pH on the lead binding affinity will be tested. Once these steps are successful, we will work on designing a reversible lead binding process using spores displaying PbrRs for lead recovery (Figure 12) [8].

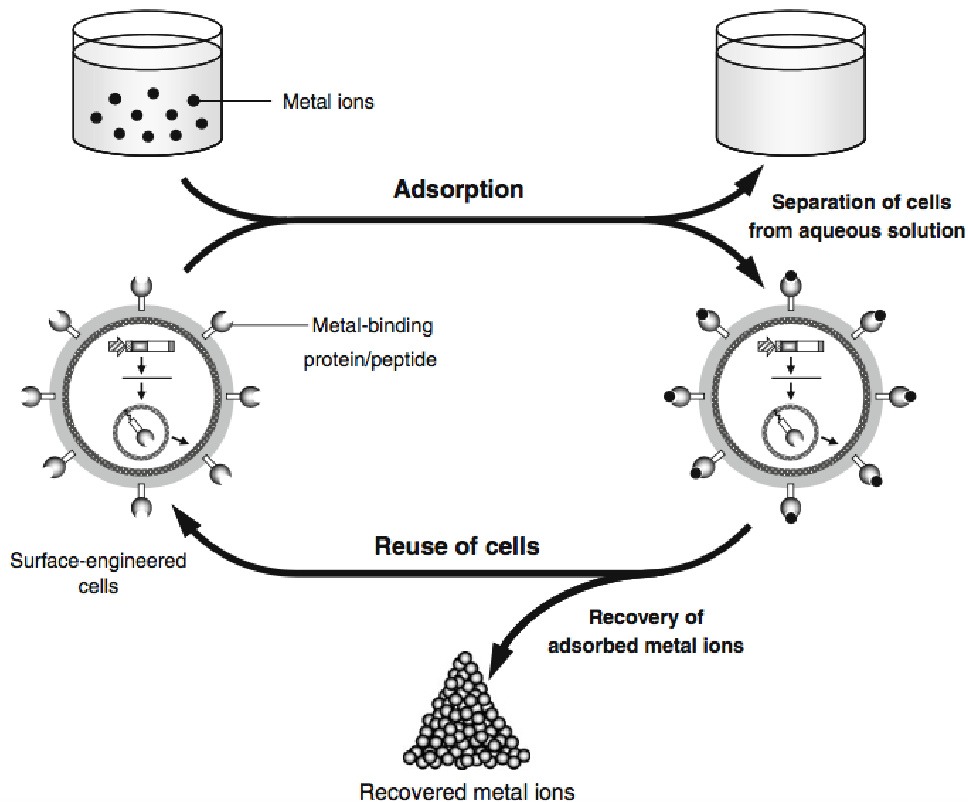

Figure 12. Process of reversible lead binding.

Acknowledgements

- Dr. Kang Wu
- Erin Drufva
- Department of Chemical Engineering
- University of New Hampshire

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