Dry Lab Outline

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Background

Common pollutants such as heavy metals and metalloids are often found in mining effluents. Certain species of bacteria are reported to biosorb pollutants and have been genetically engineered to express better pollutants capture systems. Despite past success, biosorption has seen limited commercialization; part of the issue is the biomass separation process. iGEM Toronto proposes coupling this bioremediation technique with gas vesicle expressing systems, offering a more cost-effective separation technique for industrial scales. Upon uptake of pollutants, these gas vesicles will be expressed and cause the cells to float up to the surface of given filtration containers, allowing for skimming and removal of the pollutant-harboring bacteria. Our project focuses on characterizing the different components of the gas vesicle system.

Our project focuses on optimizing flotation of E. Coli using gas vesicles. Homology in gas vesicle proteins (GvPs) from A. flos-aquae and B. megaterium will be modeled to reduce the size of current gas vesicle producing operons. Previous iGEM teams have been able to show gas vesicle production and flotation in mammalian and yeast cells using only GvP A and GvP C from different bacterial strains, but results were inconsistent in E. Coli. Shapiro et al engineered a gas vesicle producing plasmid optimized for ultrasound imaging which also enabled E. Coli to float, using this construct as a positive control permutations of secondary GVPs will be done to optimize for flotation.

If the wet-lab has time (which I doubt) they want to over-express certain genes that are responsible for the production of metal binding peptides that will allow metal to 'stick' to our E. coli cells. The dry lab will mainly explore the viability of this idea.

Aim

The dry lab will,

1. Describe the E. coli growth dynamics as a function of the culture medium composite and the bioreactor operative conditions.
2. Describe the buoyancy of the genetically engineered E. coli cells.
3. Characterization of expected system performance based off of binding affinity of particle to be sequestered.
4. Modeling of promoter switching and determination of critical concentration threshold for system response.
5. Describe the efficiency of the bio-reactor, which will be composed of a composition of the previous models.
6. If we have time, we want to estimate and optimize the cost of running such a bioreactor.

Bioreactor Modeling

This model will describe how efficient our floating E. coli cells that have the metal binding peptides on their surface will be at cleaning wasterwater of certain metals. We will run this model on different metals to determine which are the easiest to remove.

The bioreactor model depends on,

- cell numbers and population dynamics;
- temperature of the wastewater;
- pH of the wastewater;
- pressure of the wastewater / pressure of the bioreactor container;
- concentration of metal particles;
- binding affinity of metal binding peptides (would the E. coli cells only bind to certain metal?);
- promoter switching and critical concentration threshold for system response;
- buoyancy of E. coli cells (in contaminated water and in non-contaminated water);
- ratio of buoyant E. coli cells to non-buoyant cells;

- Whether the bioreactor will be continually mixed or static? We should explore having a mixing phase and a separation (static) phase;
- time scale.

We want to find the optimal value for all (or most) of the above points to make the bioreactor as efficient and thus as inexpensive as possible.

An issue to discuss is, cells dying and thus not floating to be skimmed off. We might need an additional screen that filters out the E. coli, or maybe the dead E. coli will drop to the bottom on the container, allowing us the easily remove them. If we cannot use a filtration screen, then we could model how many E. coli we would expect to die, how many we would expect to drop to the bottom, and thus how many we can remove by siphoning off the bottom. Also, is it an active process to keep the gas vesicles intact?

Will the cells coalesce due to simultaneously binding the same metal particle? This should be modelled if so.

It would also be interesting to determine how a change in metal contamination is linked to the pH of the wastewater, and how this differs for different kinds of metals. We should also do a sensitivity analysis of the pH, maybe it's not even worth investigating...

Growth Dynamics

For the bioreactor model, we need to know how the E. coli population will change. This depends on,

- initial population size;
- wastewater pH (what is the possible range of pH of wastewater);
- wastewater temperature;
- wastewater pressure;
- the specific growth rate of the E. coli (the wet lab could measure this). Call this μ;
- The maximum specific growth rate of the E. coli, call this $\mu_{\max}$.
- The concentration of the limiting substrate for growth, call this S.
- The "half-velocity constant", the value of S where $\frac{\mu}{\mu_{max}} = 0.5$. Call this K_S.

$\mu_{\max}$ and K_S are empirical coefficients. They will differ between species and depend on ambient environmental conditions (pH, temp, pressure, etc.). If we have those coefficients, then we can model the growth rate through the monod equation,

$$\mu = \mu_{\max} \frac{S}{K_S + S}$$

For background on Monod equation investigate Michalis-Menton kinetics as they are closely related.

At the moment we are not sure how important this growth model is. It depends on what time-scale our bioreactor will be operating on. Growth might be negligible. This depends on the binding affinity and metal concentration in the waste water.

For more information, an immensely useful resource is, "A Model for Cell Growth in Batch Bioreactors" by Susanna Carcano which I will post in the Dry Lab channel.

Buoyancy Model

Separatory funnel experimental data can be used to estimate average acceleration which can then be used to estimate buoyant force by multiplying this acceleration by mass of E. coli cell (there must exist a literature value for this) and then subtract gravitational force.

Next we want to estimate whether our E. coli cells will be buoyant enough to float while metals are attached to them. We need to find out if wild-type E. coli express 'metal-binding peptides' on its surface, and if so, 'how much'. Then we could repeat the separatory funnel experiment with with wild-type E. coli in a solution with some concentration of metal (perhaps we use some of the sample from the mining company) to see how the buyout force changes due to binding of metals to surface proteins. Assuming a proportionality relationship between mass of metal that binds E. coli and amount of surface proteins, we can then estimate the buoyant force to expect for surface engineered E. coli cells that would be used in the industrial bioremediation platform.

Look into the Stokes-Einstein equation.

Binding Affinity

This is super important. We need to do more research to determine an approach.

Promoter Switching and Critical Concentration Threshold

This is very important. The result of this fundamentally constraints the bioreactor model. We need to do more research of this too.