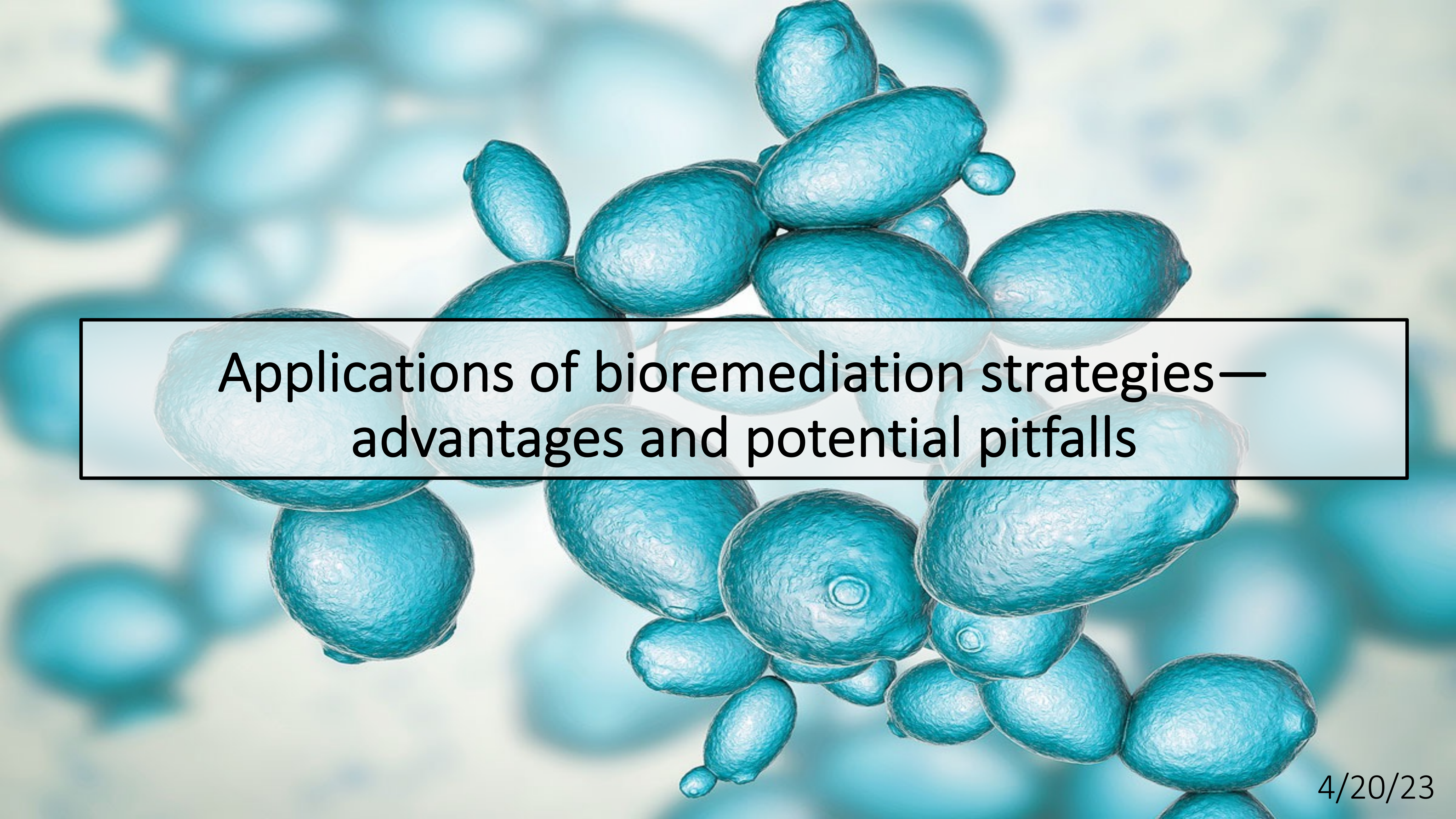


Applications of bioremediation strategies—advantages and potential pitfalls

4/20/23

Module Outline

- **M2D1**: Environmental heavy metal contamination — Intro
- **M2D2**: Model system – target selection and engineering approach — Design
- **M2D3**: Model system – choosing a chassis host — Design
- **M2D4**: Screening a system—assessing features of a bioremediation system — Test
- **M2D5**: Analysis of elemental metals – laboratory and field approaches — Test
- **M2D6**: Engineering a problem-specific bioremediation solution — Apply
- **M2D7**: Applying remediation strategies—advantages and pitfalls — Apply
- **M2D8**: Comm Lab — Review

Lecture overview

- Applying bioremediation approaches for environmental use
  - In situ
  - Ex situ
- Advantages and limitations of bioremediation
- Integrated bioremediation approaches
- How does your work fit into a bigger picture?
- Review

Advantages and limitations of bioremediation

Overall advantages of bioremediation

- More sustainable than physical-chemical remediation
  - Avoids use of potentially hazardous chemicals used in chemical remediation and high energy expenditure of physical remediation
  - Physical remediation approaches like soil washing can remove or destroy organisms and organic matter which damages soil health and productivity
  - Lower cost
  - Increased worker safety
  - Smaller environmental footprint

- Chemicals used to promote microbial remediation are fertilizer components

Advantages of bioremediation

- Microorganisms are small and have high surface area-to-volume ratio that allows them maximum contact with metals in solution

- Microbes are genetic tractable and engineering can enhance specificity
  - Enables the deployment of microbes selective for certain metals or a type of processing

- Can can recover metals through bioaccumulation
  - High yields and purity, they can be reintroduced to the industrial process

Overall limitations of bioremediation

- Slower than physical-chemical methods
- Not all contaminants can be taken up and accumulated or broken down by current systems
- There is a limit to how much metal biological systems can tolerate
- Multiple environmental conditions must align for successful onsite remediation
- Releasing genetic material into the environment can be highly problematic

Applying bioremediation approaches for environmental use

Ex situ bioremediation

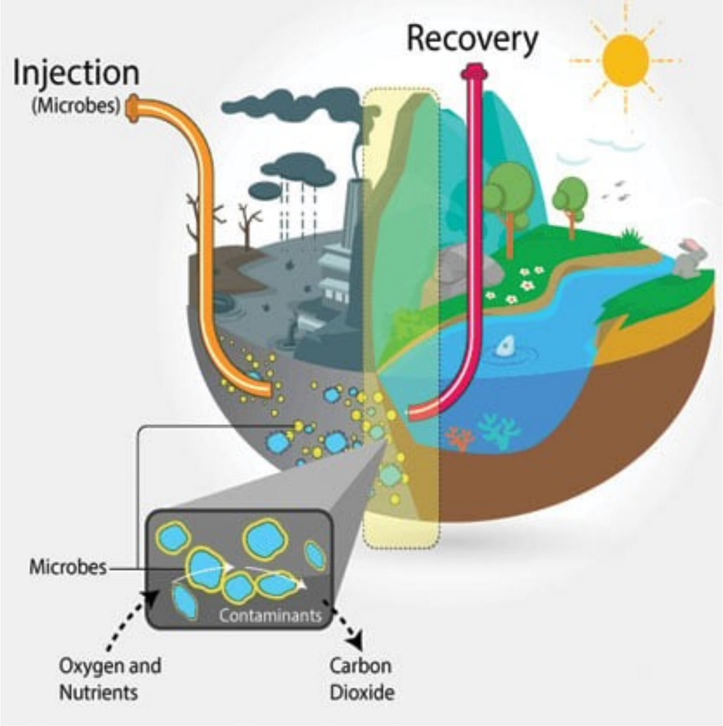
- *Ex situ*: out of place
  - Removal of contaminated soil/water from the ground
  - Take to bioreactor or outdoor facility where you can control some conditions like water and oxygen
  - Incorporate bioremediation microbes and facilitate different phases of clean up by changing conditions

<u>Advantages</u>:
- good control of process
- suitable for a variety of contaminants

<u>Limitations</u>:
- requires transport
- potential for contamination of additional space

In situ bioremediation

- *In situ*: in place
  - Biostimulation: incorporating additional resources to stimulate indigenous microbes capable of remediation
  - Bioaugmentation: incorporate new microbial strains that naturally remediate or are genetically engineered to decontaminate pollution
    - Pro: organisms there may not be capable of degrading pollutants
    - Con: nonindigenous organisms tends not to compete as well with indigenous ones



Advantages:

- cost-effective
- suitable for organic and inorganic contaminants

Limitations:

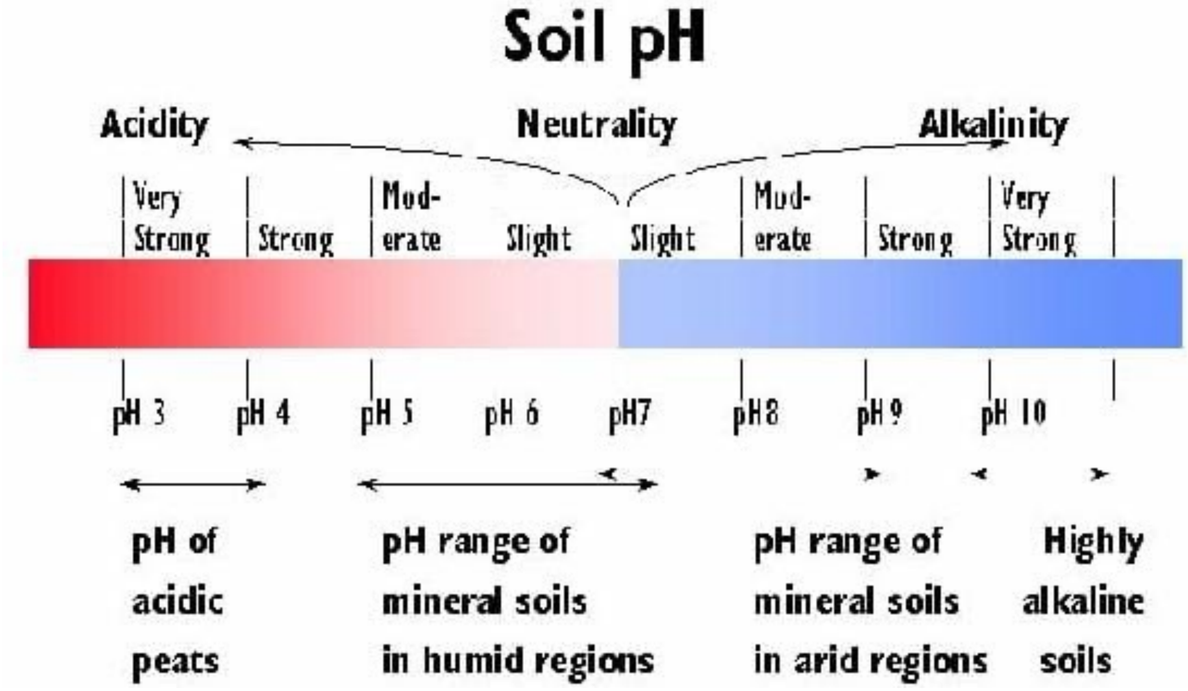
- possible for microbes to transform pollutants to a more toxic form
- remediation limited by contact ability of microbes with pollutants

Environmental conditions to consider for in situ bioremediation

- Condition of soil
  - Permeability and pH
  - Soil must be permeable enough to allow contaminants, nutrients, and air to move through the soil to encounter remediation microbes
- O_2 availability
  - This can be enhanced by venting the soil
- Nutrient content
  - This can be chemically enhanced to promote microbial growth

Special consideration given to pH when considering bioremediation

- pH affects cell physiology and metabolism of microbes
  - microbes that are using enzymatic reactions to transform heavy metals into less toxic forms needs largely neutral pH (6-9) for function
- Extremes in pH decrease the ability of yeast to accumulate heavy metals
- Heavy metal accumulation in soil can result in acidification of soil
- Industrial effluents can have very low pH values (2) which must be corrected before wastewater bioremediation



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Considerations for releasing microbes into the wider environment

- There are other microbes in the environment
  - Promoting the growth of the bioremediation microbes could disrupt the growth of important endogenous microbes
- Release of genetic material into the environment
  - Can have serious unintended and unpredictable consequences
  - Horizontal gene transfer between engineered and native microbes
- Selection pressure
  - Lab genetic engineering uses selection markers to identify modified microbes
  - Need markerless genome engineering
  - Can’t induce expression with traditional laboratory means

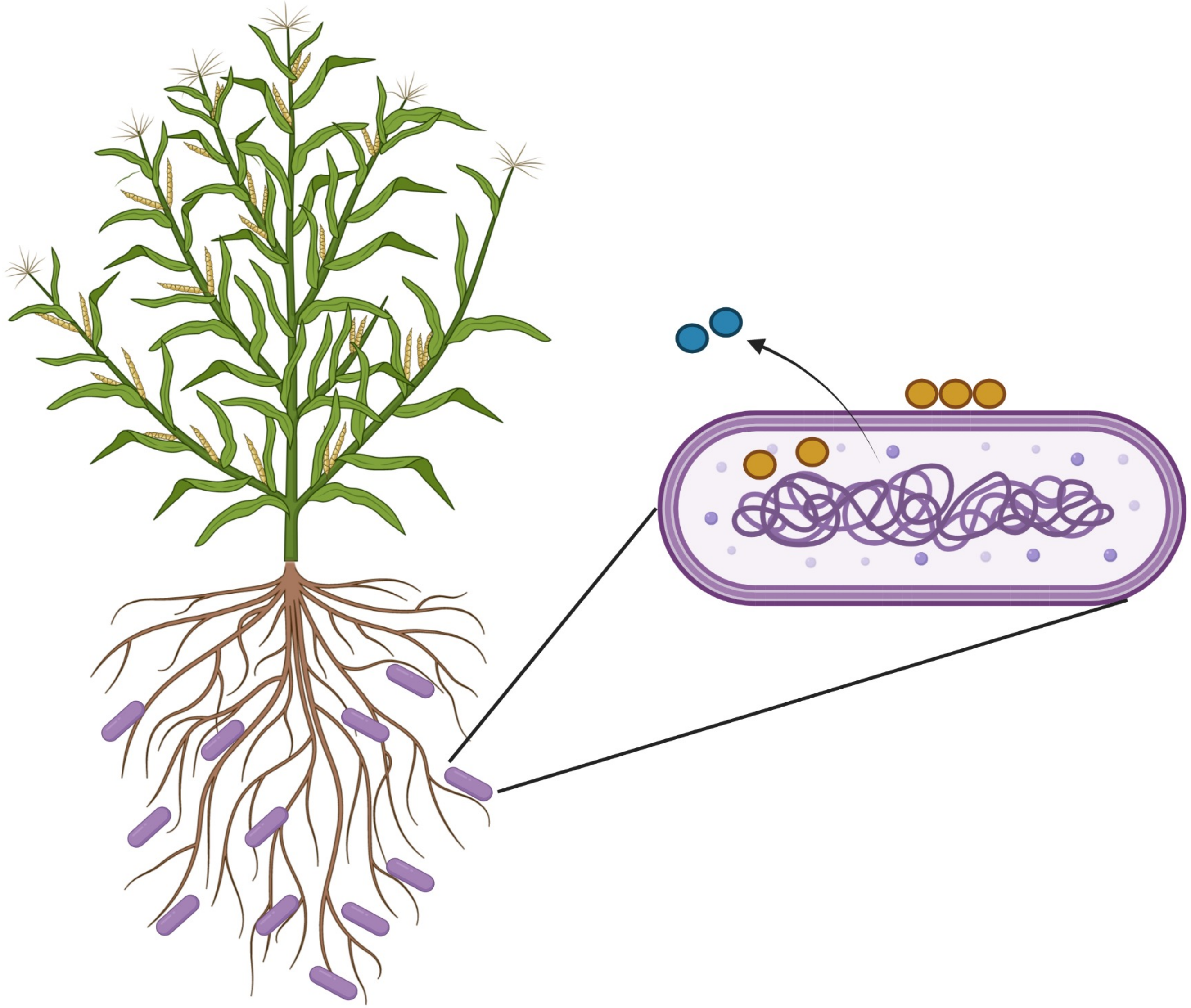
Summary

- Bioremediation has great potential to be a powerful tool for environmental clean up and protection!
- Because of the complexity of biological and environmental factors, much work still needs to be done to make these tools safe and effective

Integrated remediation approaches

Phytobial remediation can be an effective and environmentally friendly combination

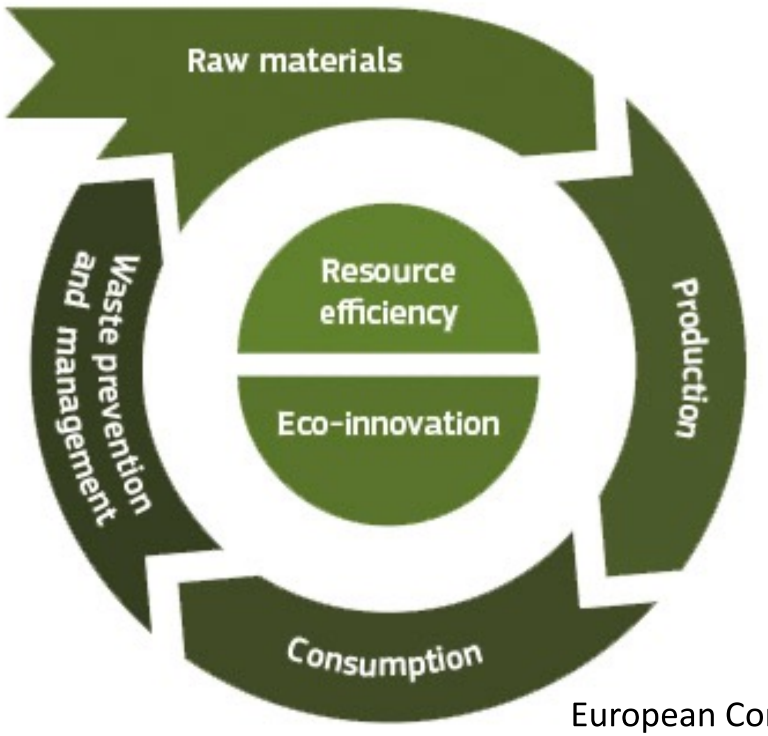
- Use a combination of bacteria and plants as a source of remediation
- Plants will act as metal hyperaccumulators
- Bacteria can serve multiple enhancing roles
  - Bioaccumulation
  - Biosorption
  - Biotransformation
- These roles can make the metal more bioavailable to the plants for remediation or serve as an additional source of remediation



Where are we going with your work?

Circular economy (applied to metals in manufacturing)

- Promote sustainability by keeping resources in circulation for as long as possible
  - Maximize use of precious materials
  - Reduce waste
- Efficiency in production
- Mindful consumption
- Waste management
  - Recycling
  - Containment of waste in production so that material can be reused
    - Closed-loop manufacturing



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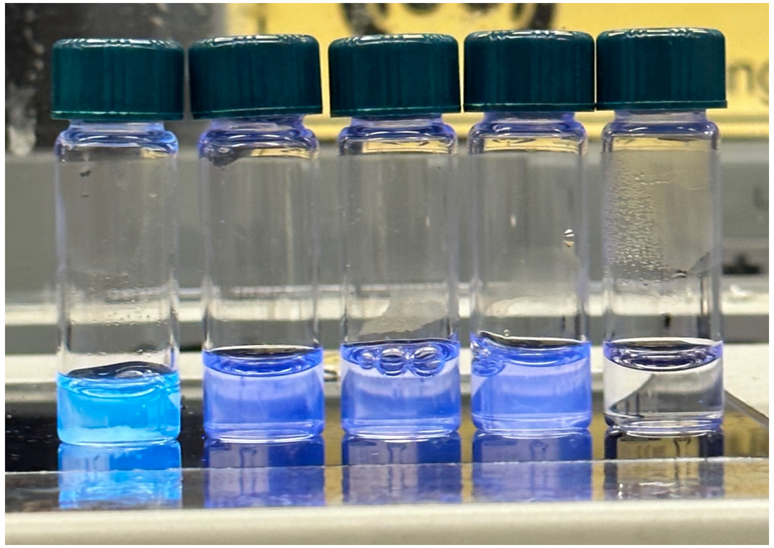
How could your work contribute to this idea?

- Design and create a yeast model system for heavy metal bioremediation
  - Modifications to promote bioaccumulation
  - Lots of opportunities for refinement and expansion to other metals

- What do you do with cadmium that has been accumulated?
  - Extract and recycle it!
  - Cadmium is used in:
    - photoresistors
    - photovoltaic cells
    - quantum dots

Cystathionine γ-lyase enzyme purified from *S. maltophilia* can be used to biosynthesize quantum dots with different metal cores from a variety of metal sulfides



Bill Pinney, BE Teaching Fellow (and former 20.109 student)

Review

So… what IS your project?

Overall idea

- Can we create a new model system for bioremediation that will preferentially take up cadmium from the environment
  - Can we use this genetically engineered organism to separate a dangerous heavy metal from the rest of the space?
  - Can the metal be safely accumulated until it can be recovered?

- What are our possible routes to creating this kind of system?
  - Look at single-cell organisms that are already capable of taking up and tolerating heavy metals and genetically engineer them for specificity and capacity

So… what IS your project?

Why Fet4?

- Talked to the Belcher lab about routes we could take to create a bioremediation system that could be specific for particular heavy metals
  - They found the high-affinity manganese transporter (SMF1) was a great target to modify for cadmium uptake

- One challenge to using SMF1 was the strong preference of the transporter for manganese
  - Difficult to identify metal uptake against that kind of background

Are there other options available that might be able to avoid this issue?

- Cell surface protein
- Low affinity transporter
- Already known to take up other metals

] Fet4

- Bonus: low basal expression in aerobic conditions
  - Less contribution of endogenous protein
  - We get more of a "blank slate" when overexpress our mutants

So… what IS your project?

Why yeast?

- Known model system with genome sequencing information
  - Genetically tractable
- Previously identified ability to remove multiple heavy metals and sequester them
  - survives metal exposure
- Interested in creating a bioaccumulator
  - Want to try and recycle the metal in future 20.109 modules
- Yeast has a number of cell surface transporters that could be manipulated for specificity
  - Could create a system to selectively take up a particular metal
- Yeast flocculate
  - Autoaggregation that allows them to separate from effluent

So… what IS your project?
Prelab edition

- We will spend time in prelab over the final two labs reviewing the project and the research article assignment

- M2D7: Talk about how experiments and data relate to overall project goals

- M2D8: Talk about components of the research article and how to put it all together

What are you doing in lab today?

- Analyze ICP-OES data
  - It's so pretty…
  - There is plenty there to unpack

- Final experiment!
  - Look at cell tolerance of incubation with cadmium
  - Collect luminescence data