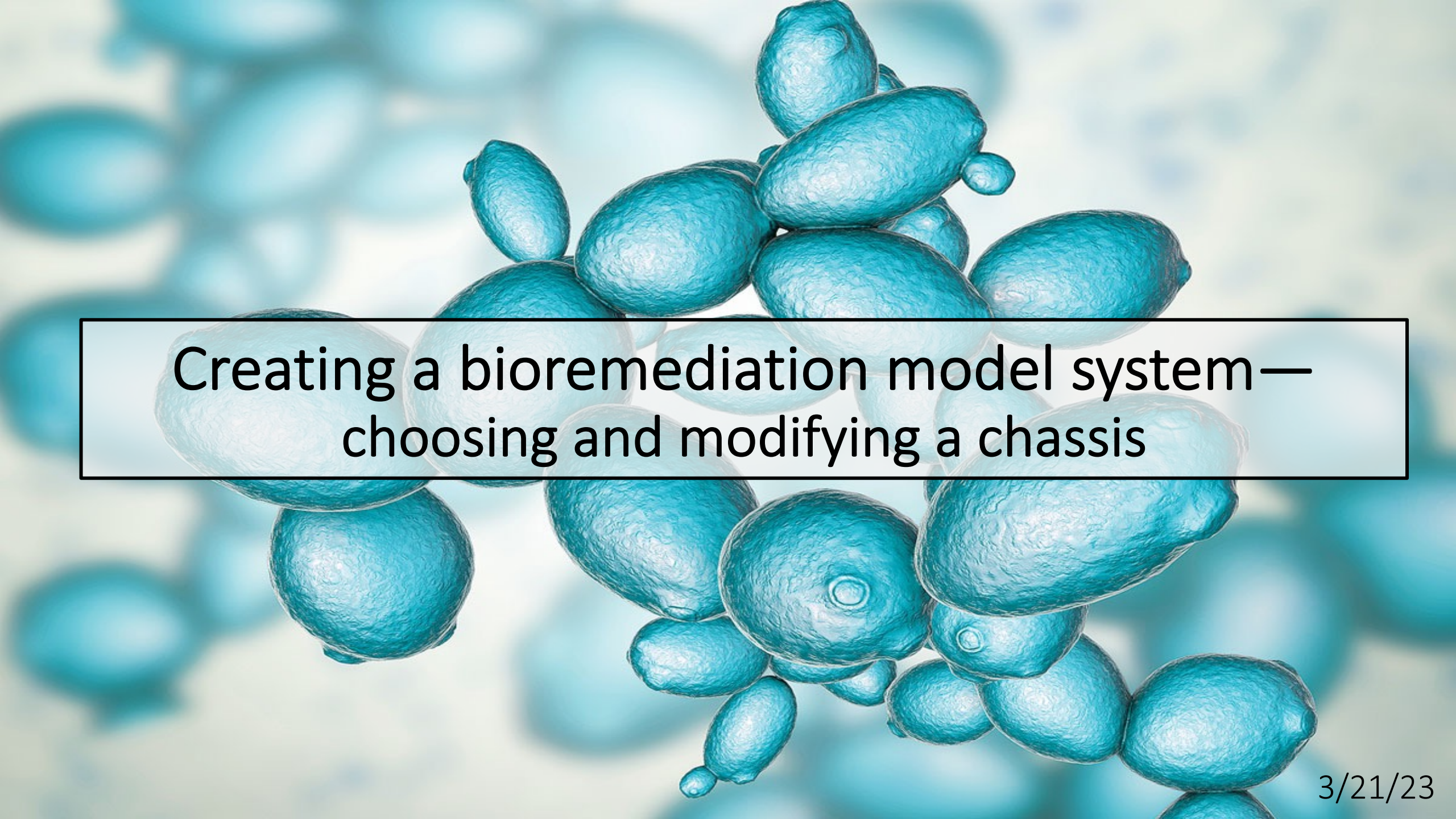


Creating a bioremediation model system—
choosing and modifying a chassis

3/21/23

Module Outline

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

Intro

Design

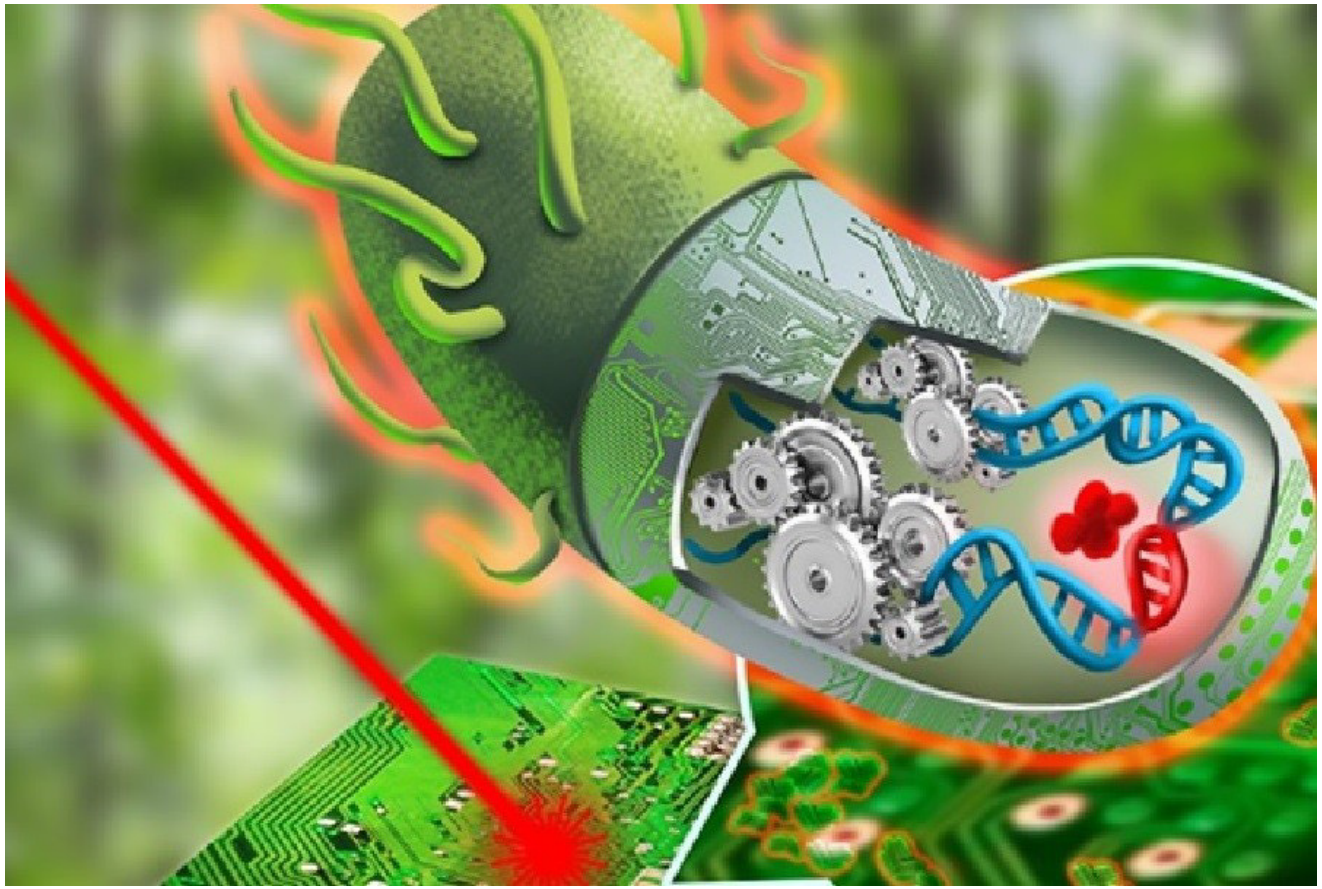
Test

Apply

Review

Lecture overview

- Engineered bioremediation system
  - Modified transporter
  - Host to express transporter
- Chassis is the host cell used to house and support engineered biological material
  - replication machinery
  - metabolic resources
- Common organism hosts for bioremediation
  - Cyanobacteria
  - Microalgae
  - Yeast



What do you want from a chassis?

What do you want from a chassis?

Biological relevance

- Has relevant endogenous machinery

Genetically tractable

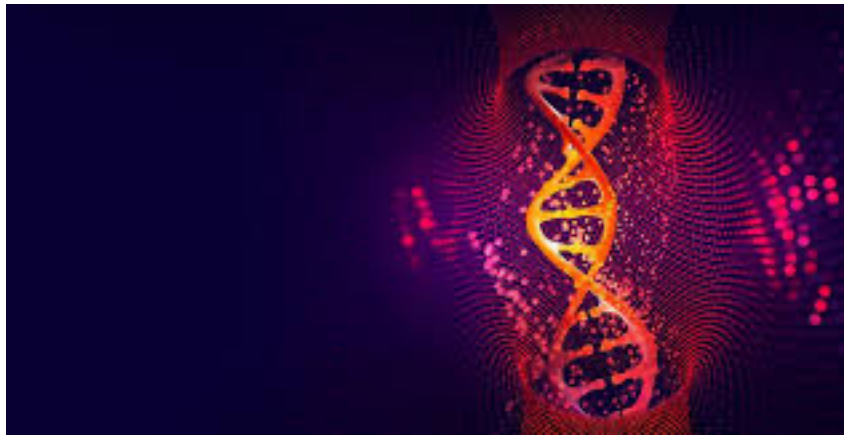
- Straightforward protocols for genetic manipulation
- Laboratory strains of bacteria/cells/animals = highly genetically tractable
  - Environmental strains = less so, but possible

Economically viable

- Scalable
  - Relatively rapid growth
  - Resource usage
- Able to thrive without extensive assistance

Additional useful host features

- Robust growth
  - Support production of non-native proteins
  - Increased survival rates
- Able to operate under non-ideal conditions
  - temperature range
  - pH range
  - low resource availability
- Well characterized
  - genome
  - growing conditions
  - pathogenicity
  - DNA delivery tools



Single-cell organisms for bioremediation

Natural defense mechanisms against heavy metal toxicity that can be utilized for bioremediation

- Defenses most useful to modulate for remediation
  - Metal transformation
  - Metal sequestration

- Good chassis hosts for remediation due to natural defenses
  - Bacteria
  - Microalgae
  - Yeast

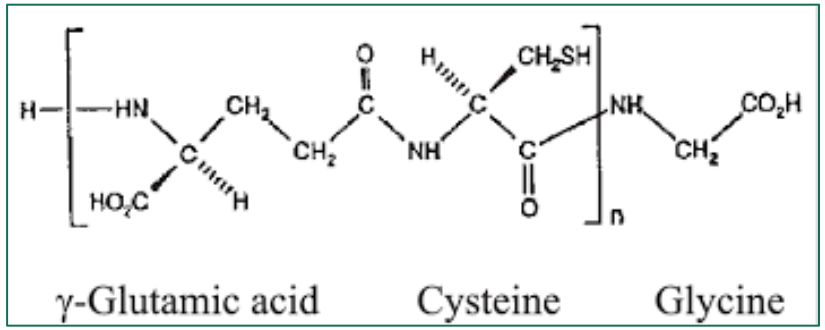
Metal transformation by bioremediation hosts

Oxidation, reduction, methylation of metal to less toxic form

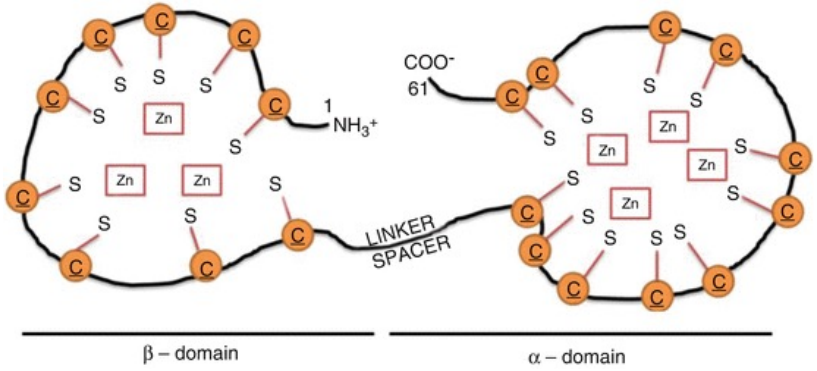
- Example: Cr(VI) and Cr(III) are the most stable oxidation states of chromium
  - Cr(VI) is most toxic but is reduced to Cr(III) by chromium reductase enzyme or interaction with intracellular reducing agents

Chelation by cysteine-rich polypeptides

- Phytochelatins
  - Enzymatically synthesized from glutathione



- Metallothioneins
  - Gene-encoded
  - Contain two cysteine-rich metal binding domains



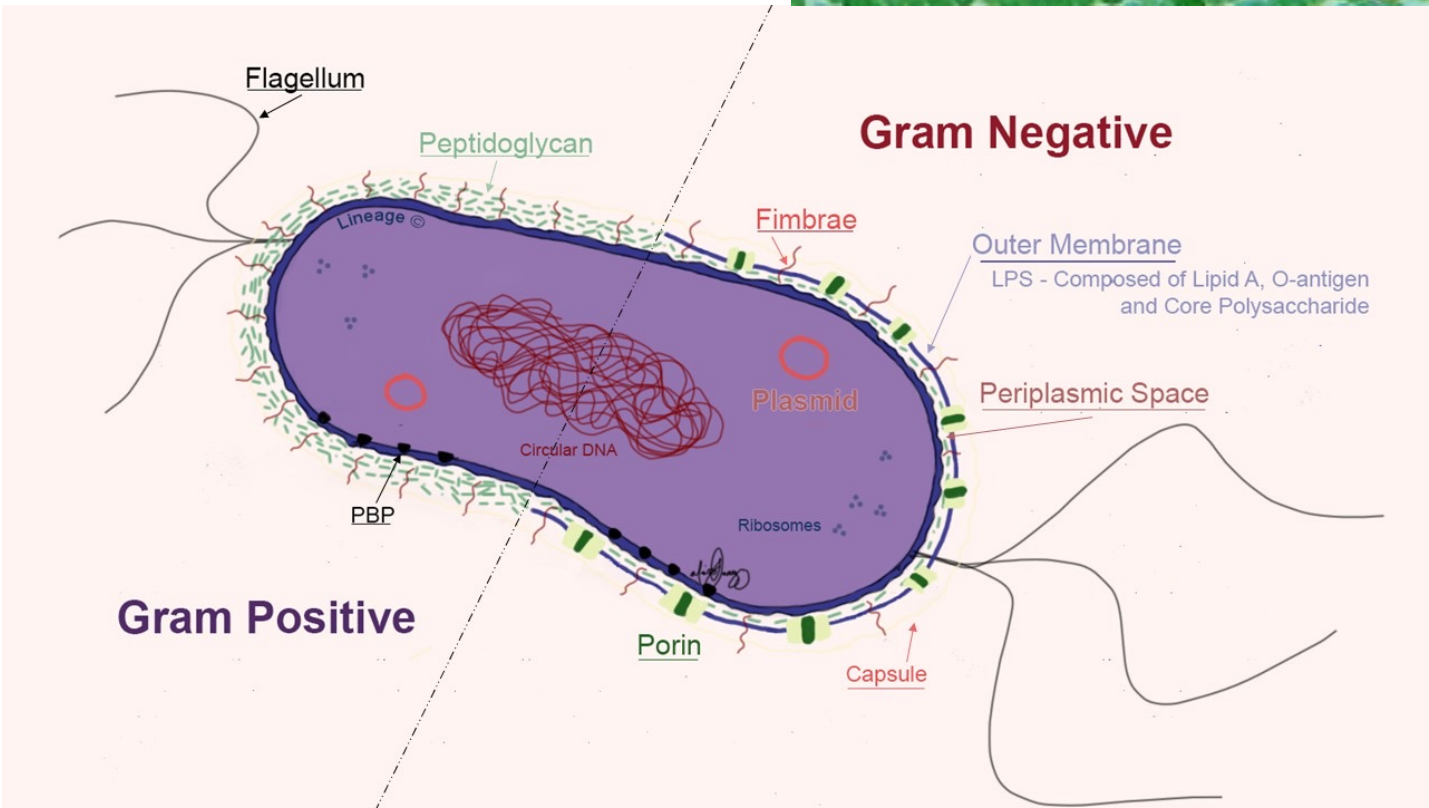
Metal sequestration

- Adsorption to the cell surface can be mediated by
  - Binding of heavy metal cations with negatively charged groups
  - Interactions with polysaccharides
  - Interactions with proteins containing carboxyl, hydroxyl, carbonyl, amide functional groups

- Accumulation in an intracellular vesicle
  - GSH-conjugated metals can be transported into cell vacuoles by ATP-Binding Cassette (ABC) transporters for sequestration

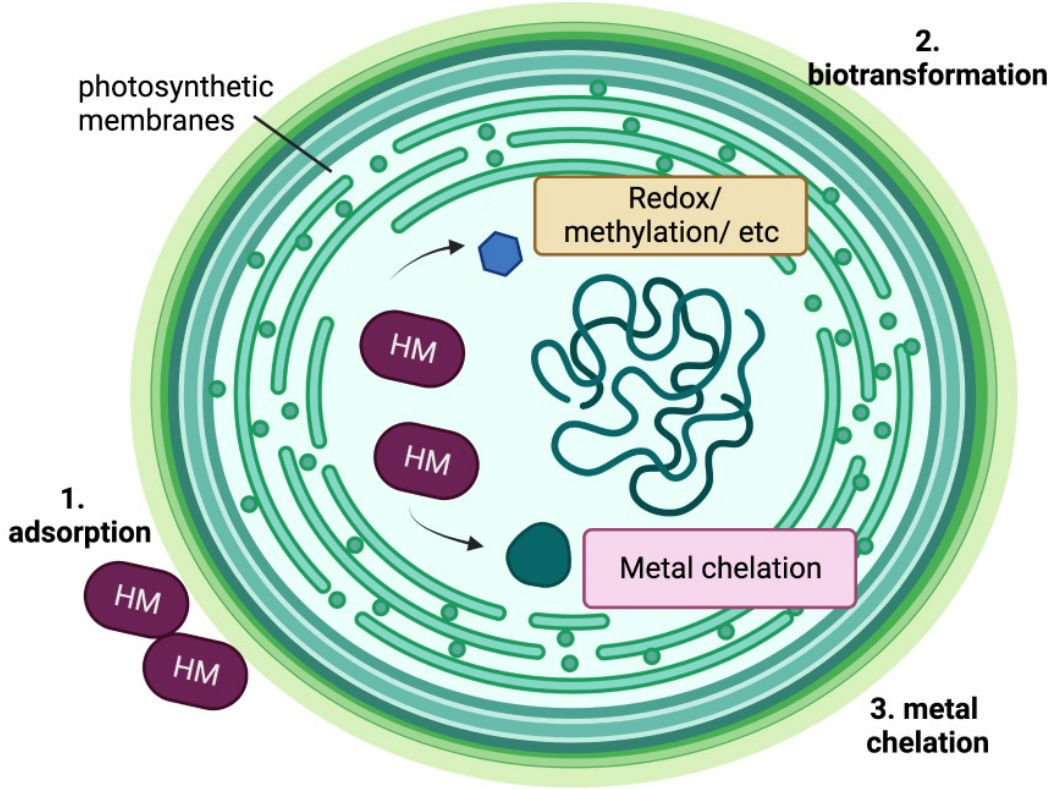
Cyanobacteria

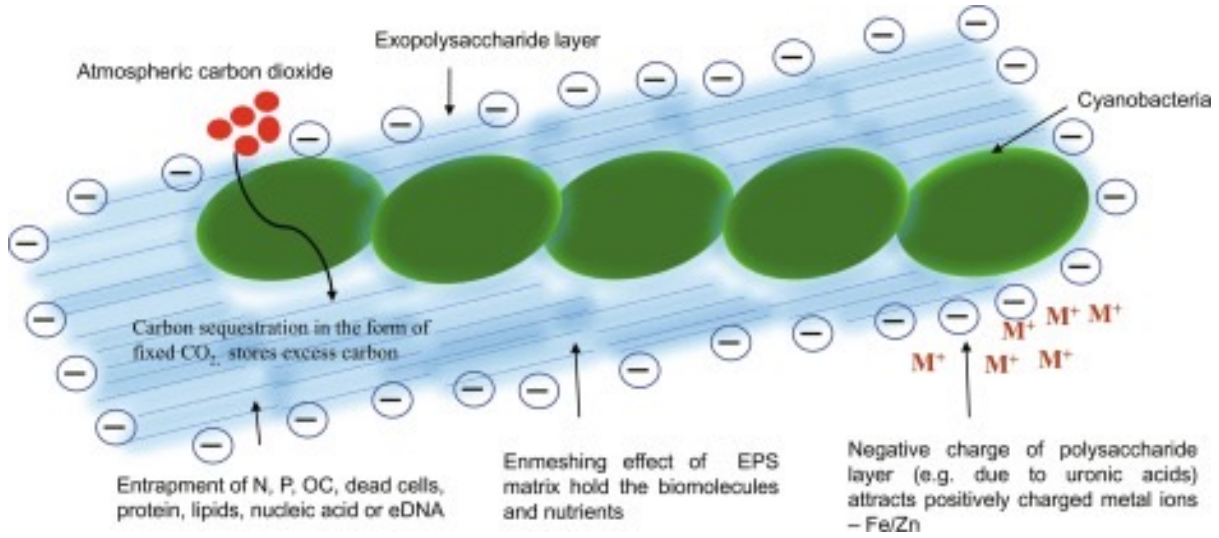
- Gram negative bacteria
  - prokaryotes
- Undergoes photosynthesis
- Grows in soil and water
  - Can produce biofilm matrix to group bacterial cells together or to a surface
- Has metal transporters, but lacks the specificity of other host organisms



Cyanobacteria in bioremediation

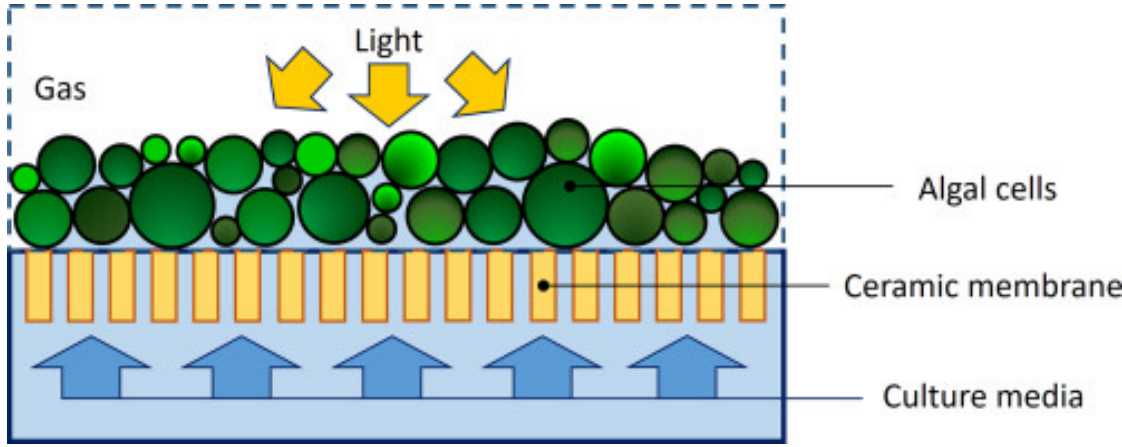
- EPS-mediation adsorption
  - Extracellular Polymeric Substances
  - Can be live or dead for adsorption
- MT and PC
  - metal chelation
- Biotransformation machinery





Microalgae

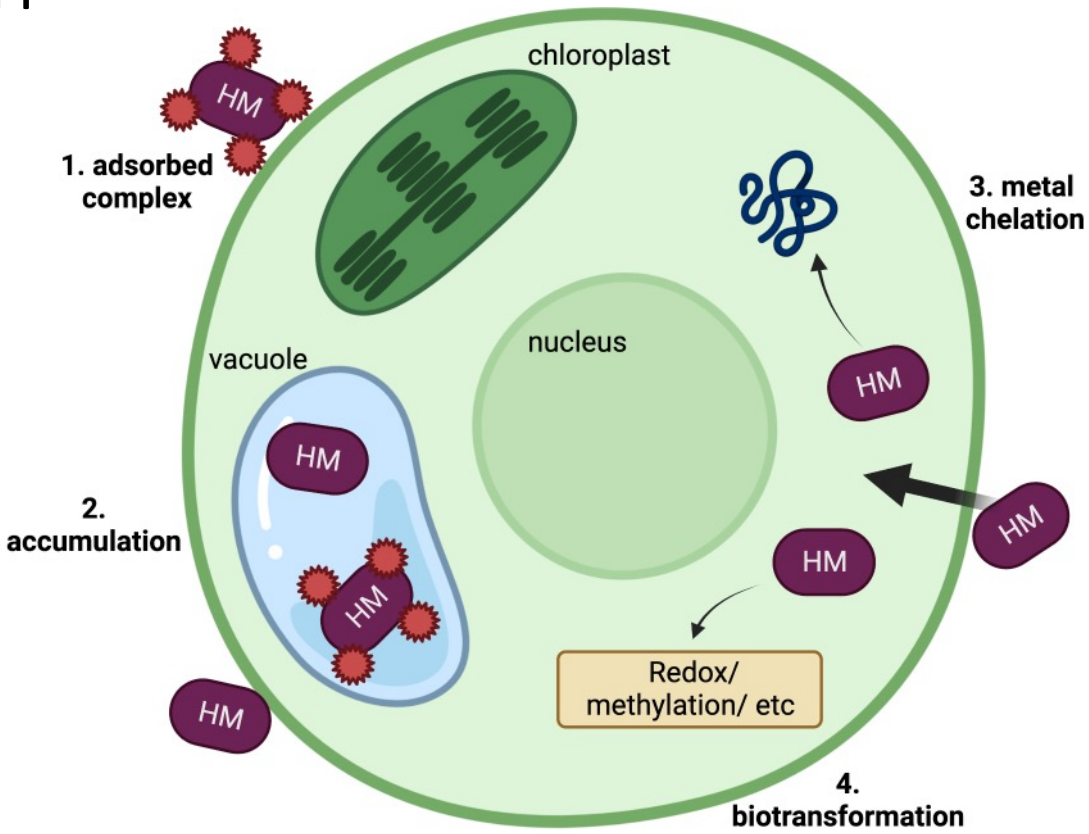
- Single cell eukaryote
- Undergoes photosynthesis
- Can form biofilm to attach to a surface
  - reduces resources requirements for growth



- Some membrane transporters for metals, but not the complexity/diversity of yeast

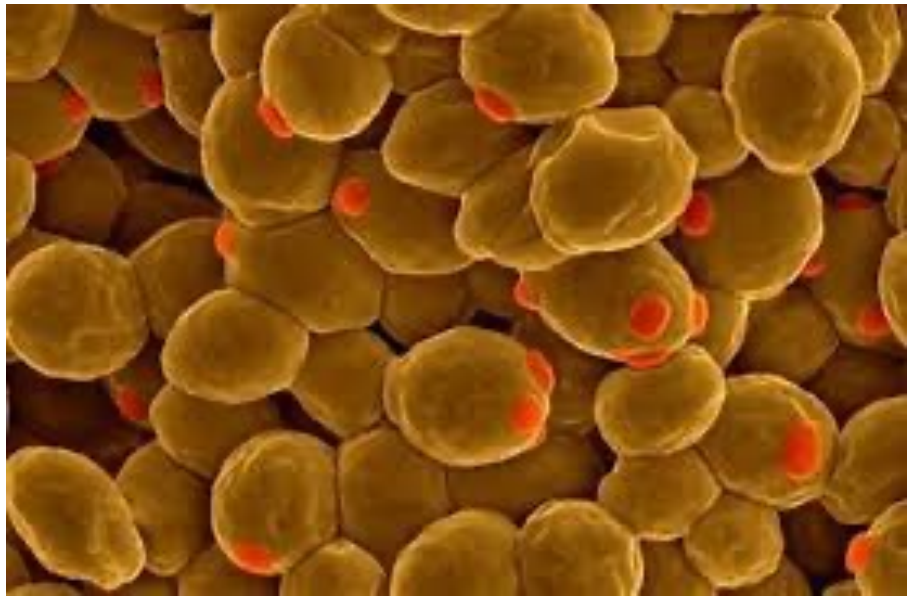
Microalgae in bioremediation

- MT and PC
  - metal chelation
- Vacuole
  - bioaccumulation
- Adsorption
- Biotransformation machinery



- Heavy metals can interact with surface groups to form complexes which can be transported internally where they are accumulated or transformed

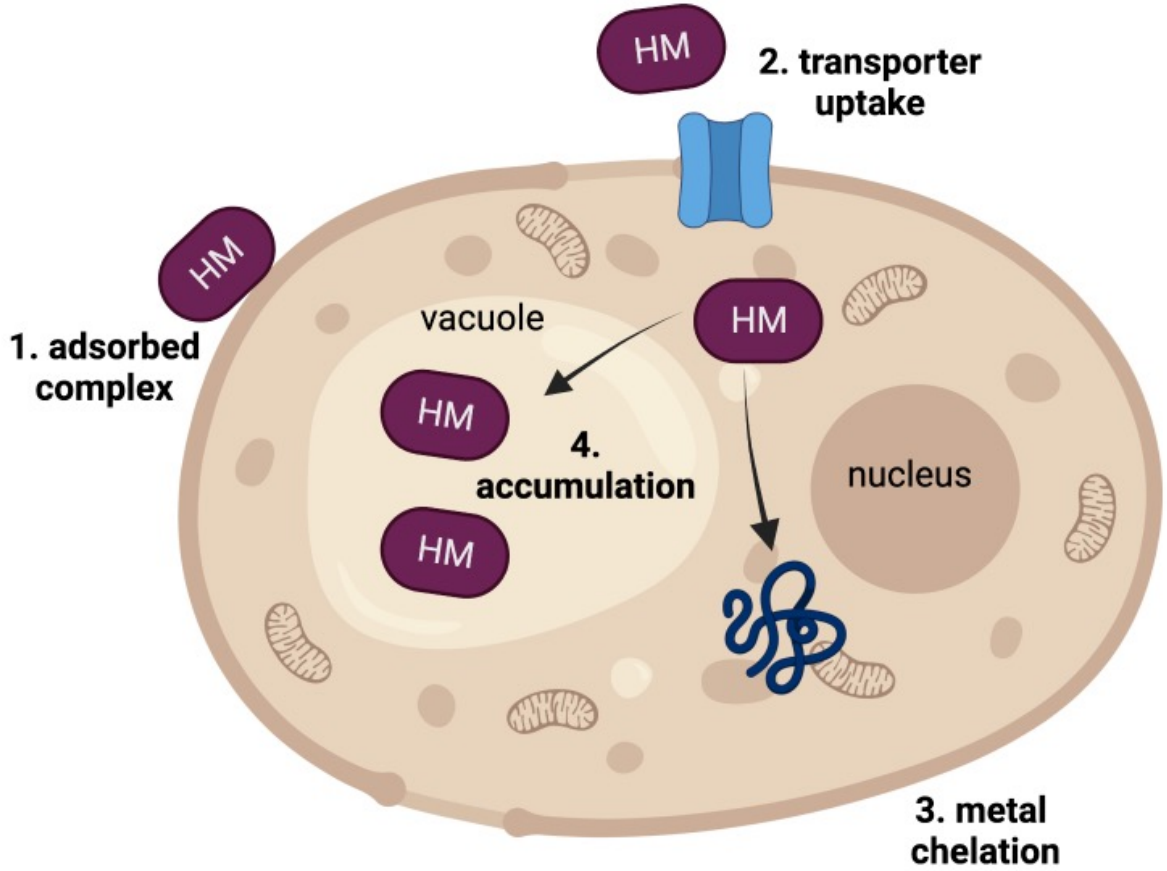
Yeast



- Single-cell eukaryote
  - fungi
  - *Saccharomyces cerevisiae*
- Widely studied and genetically tractable
- Resilient growth under non-ideal pH and osmolarity conditions
- *S. cerevisae* is generally considered non-pathogenic and safe
- Slower growth rate than bacteria or algae

Yeast in bioremediation

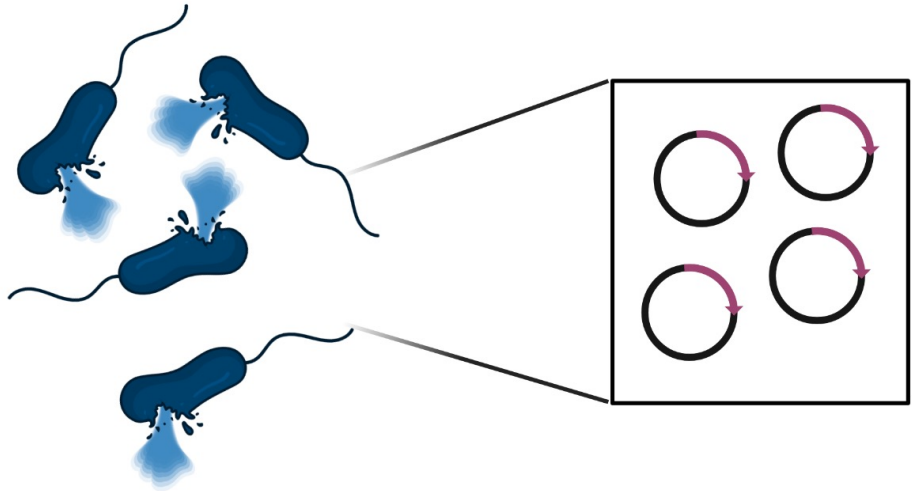
- Adsorption
- Specific metal transporters
- MT and PC
  - metal chelation
- Vacuole
  - bioaccumulation
- Some biotransformation machinery



Take home message: find the right host

- Any of these cell types can be a chassis host for your bioremediation system
  - Depends on the environment you want to work with and the cell machinery you need
- A target for modification is selected to balance desired and undesired effects
- A chassis host needs to be able to support your genetically engineered material while also functioning in the desired environment

What are you doing in lab today

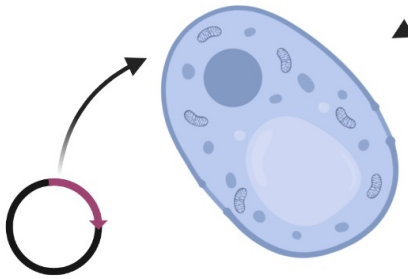


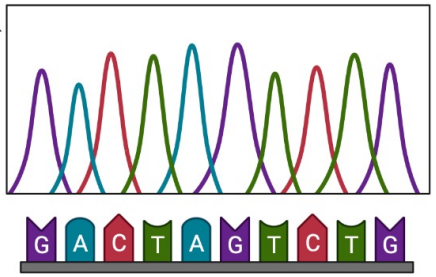
1

Purify (hopefully) mutated plasmid from *E. coli*

2a

Transform putative mutants into W303α *S. cerevisae*





2b

Sequence plasmid to confirm mutation