Plastic Biodegradation Using Genetically Edited Microorganisms

FST 100B Final Project Presentation

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Background Information

- Biodegradation ⟶ sustainable way to fight plastic pollution
  - Degrading organic matter through microorganisms (ScienceDirect-Biodegradation)
- Use genetically engineered microorganisms
  - Fungi (He, Y et al., 2024)
  - Bacteria/Cyanobacteria
    - *Phormidium lucidum* and *Oscillatoria subbrevis* (Sarmah, P., & Rout, J., 2018)
    - *S. pavanii* (Karbalaei, M., et al., 2020) (Martín-González, D et al., 2024)
  - Algae
    - *Uronema africanum* algae, in freshwater, is able to degrade LDPE

(Chia, W. Y et al., 2020), (Moog, D et al., 2019)

- Break down multiple plastics, efficiently

Why is Plastic Pollution an Issue and Why Choose Biodegradation?

- <60% Plastics in Landfills & Environment (Martín-González, D et al., 2024)
- Incineration releases toxic gases into the atmosphere
- Other techniques expensive and/or reuse plastic, don't reduce plastic presence
- Microorganisms degrade different plastics without harming environment

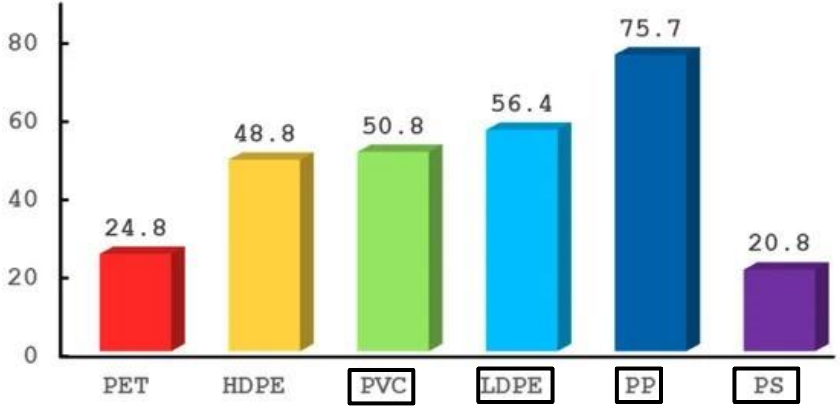


Figure 1. Global production of the 6 commodity plastics (in million metric tons).

- ➢ *S. pavanii (bacterium)* **PET and PLA**
  - (Qing-Song Huang et al., 2024)
- ➢ *Spirulina sp* Isolates (microalgae) **PP**
  - **(**Moog, D et al., 2019**)**
- ➢ *Uronema africanum* (Algae) **LDPE**
  - (Chia, W. Y et al., 2020)
- ➢ *Phormidium lucidum* and *Oscillatoria subbrevis* (Cyanobacteria) **LDPE**
  - (Sarmah, P., & Rout, J. 2018)

Plastic Types 1-7 (RIC)

Most Recyclable:

- Polyethylene Terephthalate
- High Density Polyethylene

Less Recyclable:

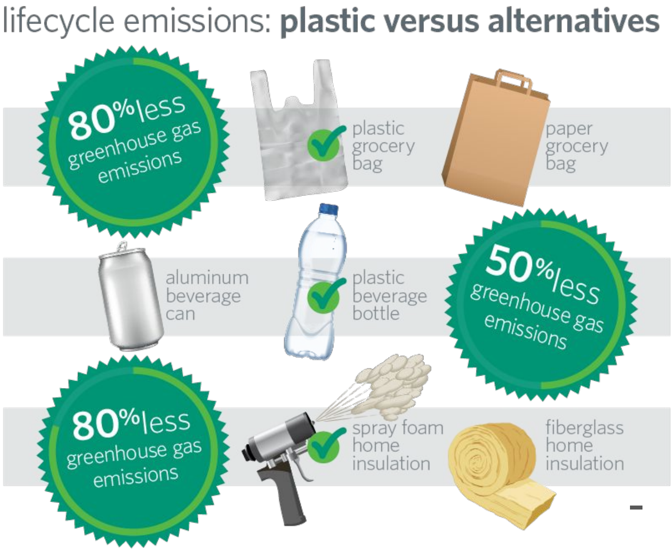
- PVC
- LDPE
- PP
- PS
- Other

\- (Oceanworks.co)

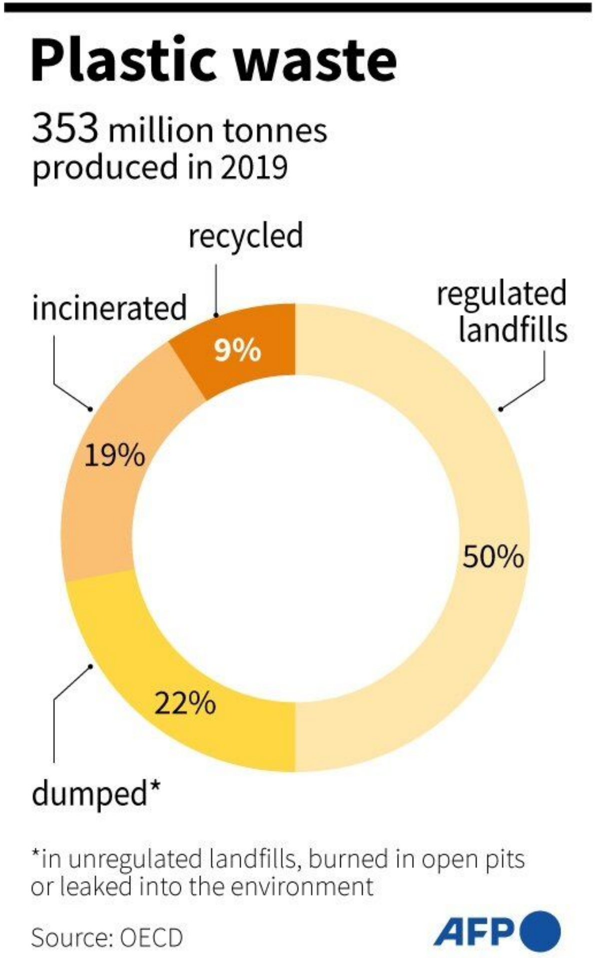
Infinite Recycling?

"About 75% of global plastics produced are thermoplastics that can be melted and molded over and over to produce new plastics, which – in theory – makes all thermoplastics recyclable. The remaining 25% of plastics are thermoset plastics that do not soften when exposed to heat, making them near-impossible to recycle."

- (Ayres. R., 1998)



- (AFT)



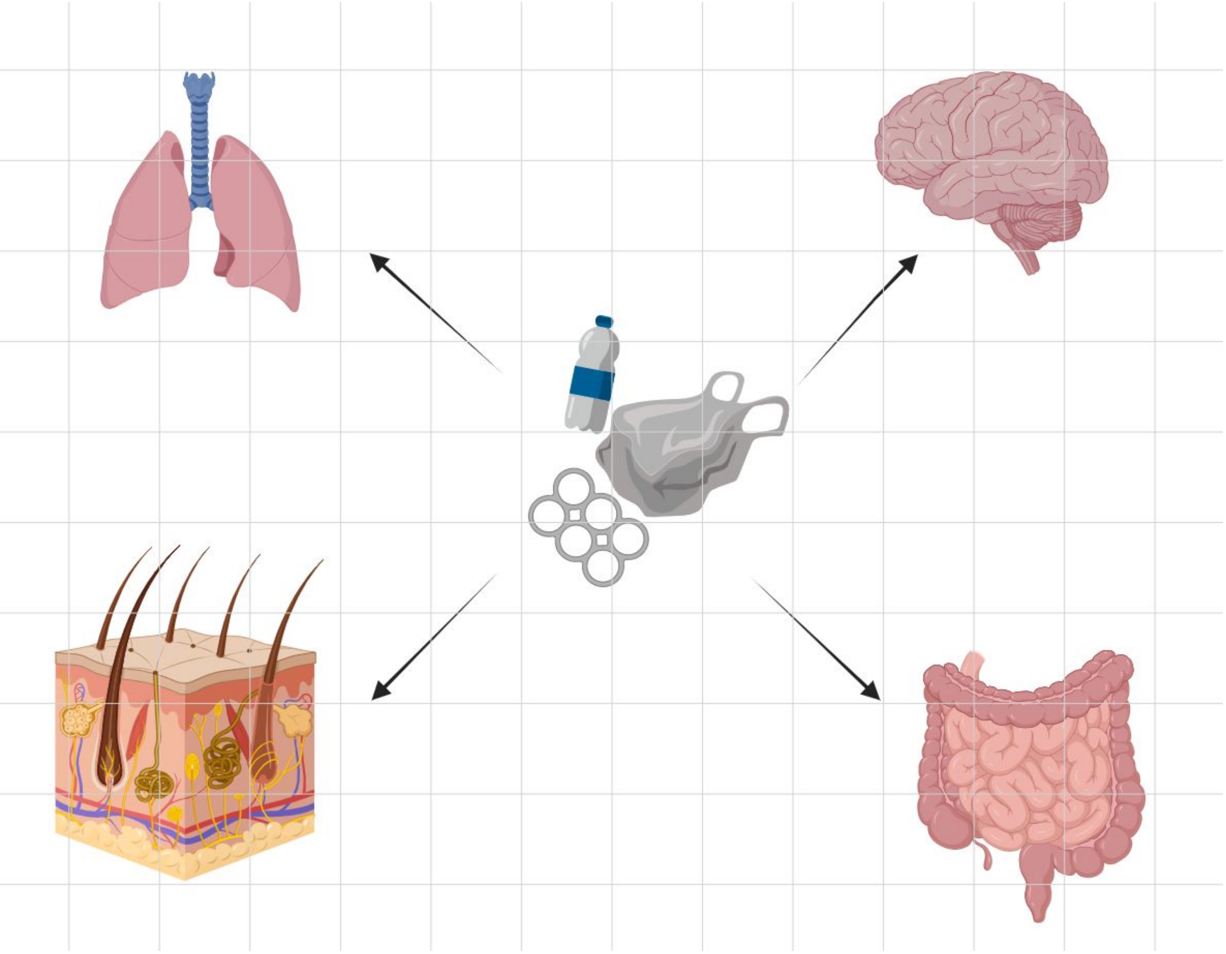
Summary:

- Thermoset vs. Thermoplastics
- Clean Plastics
- Greenwashing
- Biodegradable Plastics
- Decreased Quality

Affects:

- Respiratory System
- Digestive System
- Central Nervous System
- Immune System

(Zhao B et al., 2024)

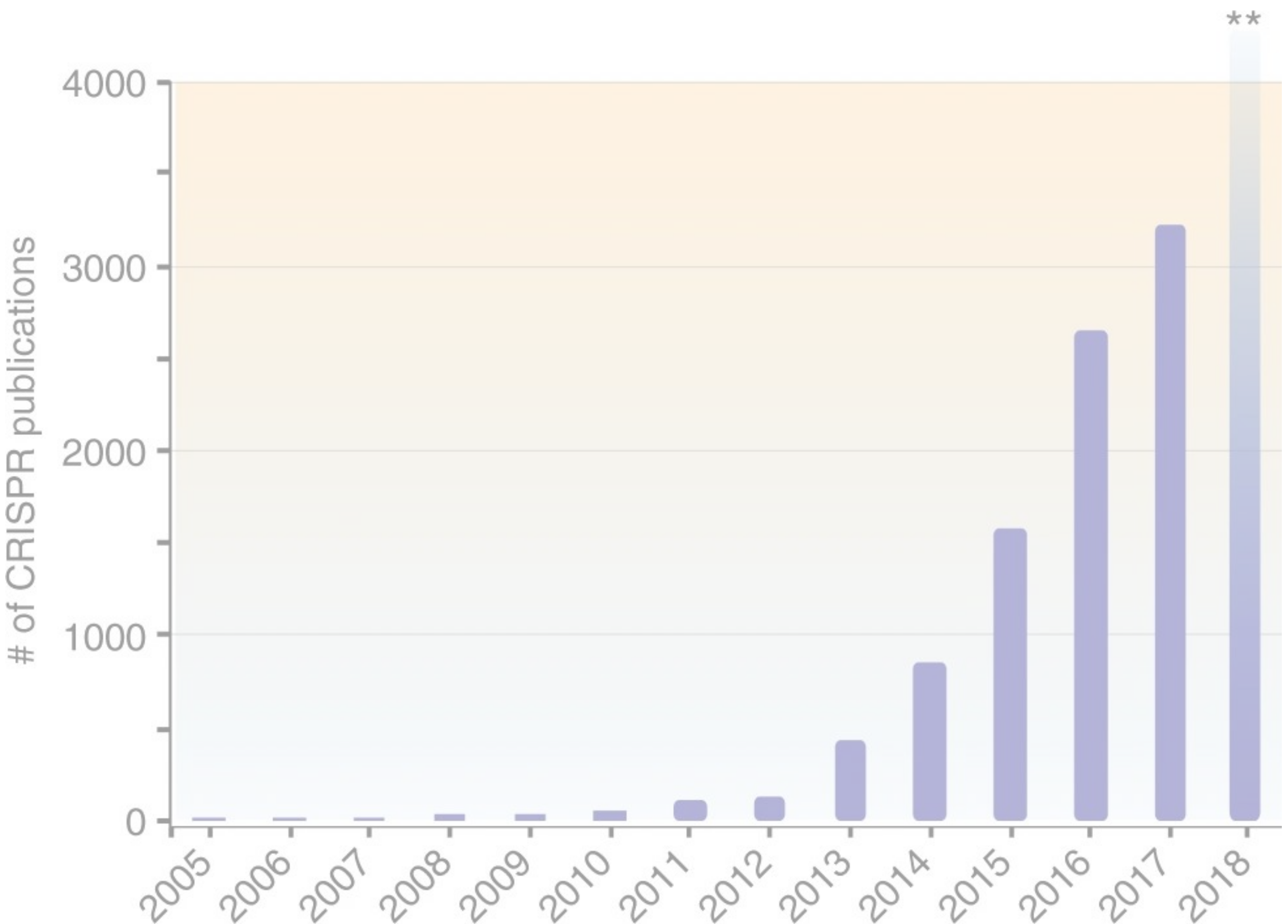


Why do Microorganisms Need to be Modified?

- Microorganism plastic degradation efficiency is generally low
- Different organisms have different enzyme activity and degradation abilities
  - **Example**: By enhancing the expression of PET hydrolases through modifying certain promoters and signal peptides of the bioform making bacterium *S. pavanii*, greater yield of microplastic degradation can be achieved (Qing-Song Huang et al., 2024).
    - pBBR1-P24-SP1-DuraPETase displayed highest microplastic degradation
  - **Example**: Separate chimeric lipase-cutinase enzymes can be synthesized using genes from *Thermomyces lanuginosus* and *Thielavia terrestris*, which can be expressed in *P. pastoris*, a species of fungi that easily expresses heterologous genes (Karbalaei, M., et al., 2020; Martín-González, D et al., 2024).

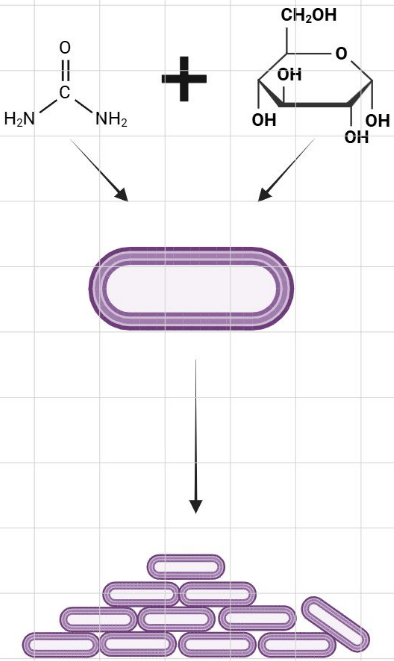
Nature Communications*

- More DNA sequencing needed
- Bioengineering techniques like (CRISPR-cas9) cheaper/available
- Transformation
- Find sustainable solutions to plastic pollution



Methods

Methods of Cultivation and Transformation



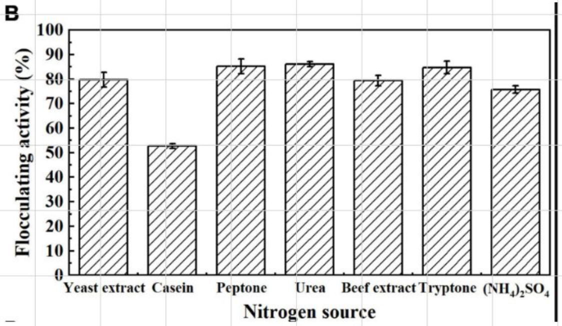


Figure 2: Nitrogen source effect on flocculation (biofilm forming activity)

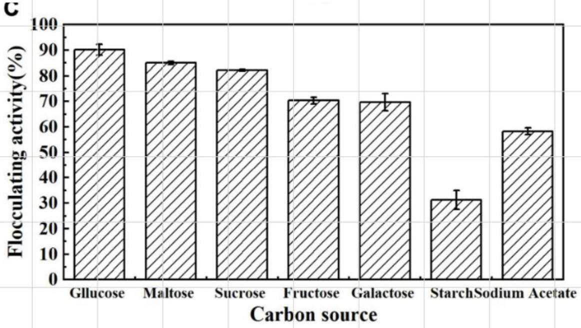
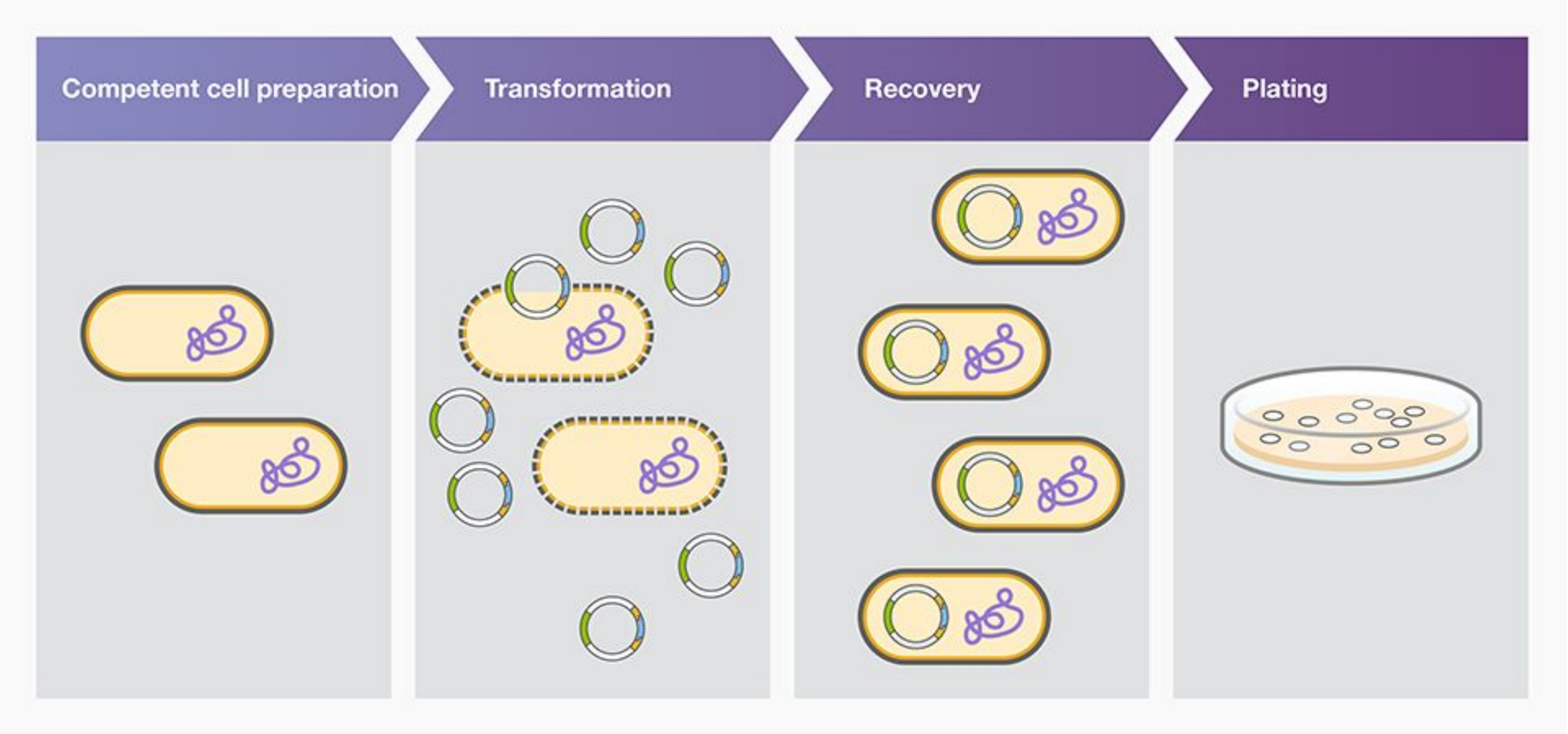


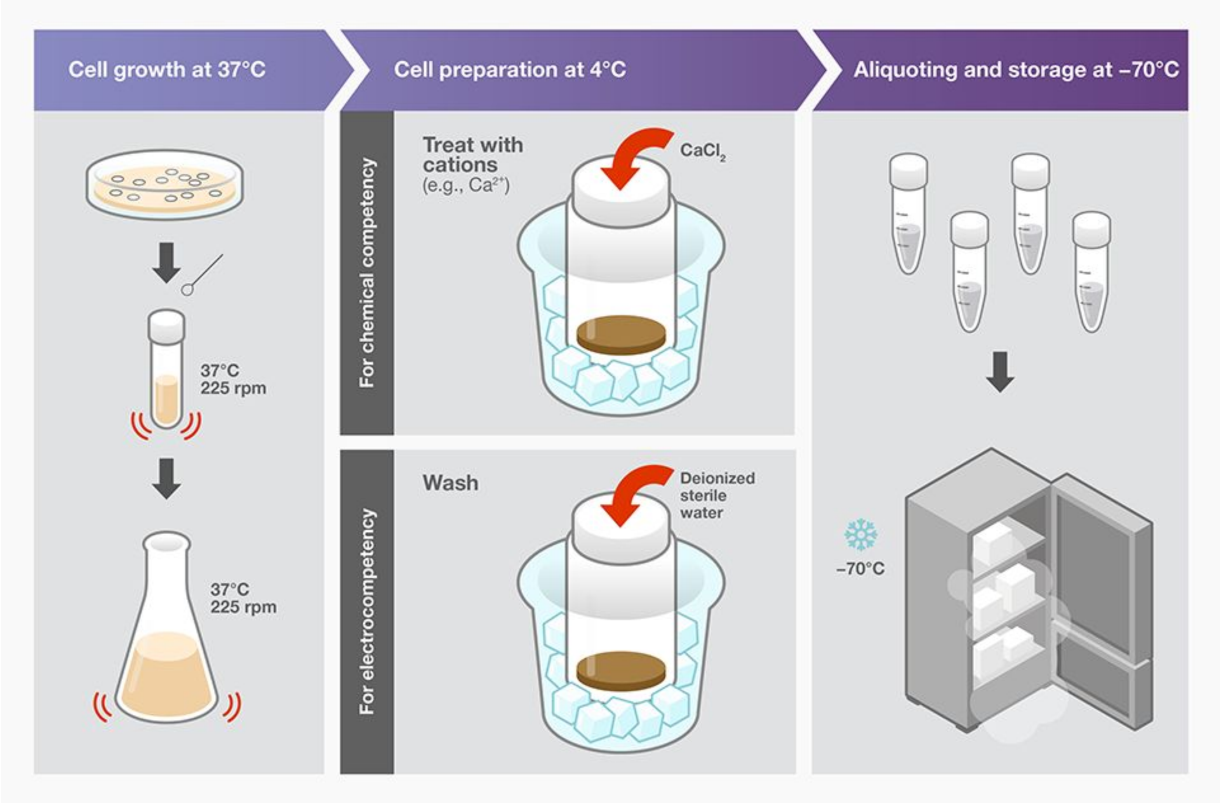
Figure 3: Carbon source effect on flocculation (biofilm forming capability)

(Qin S., et al., 2024)

Transforming Bacteria - Visuals from ThermoFisher



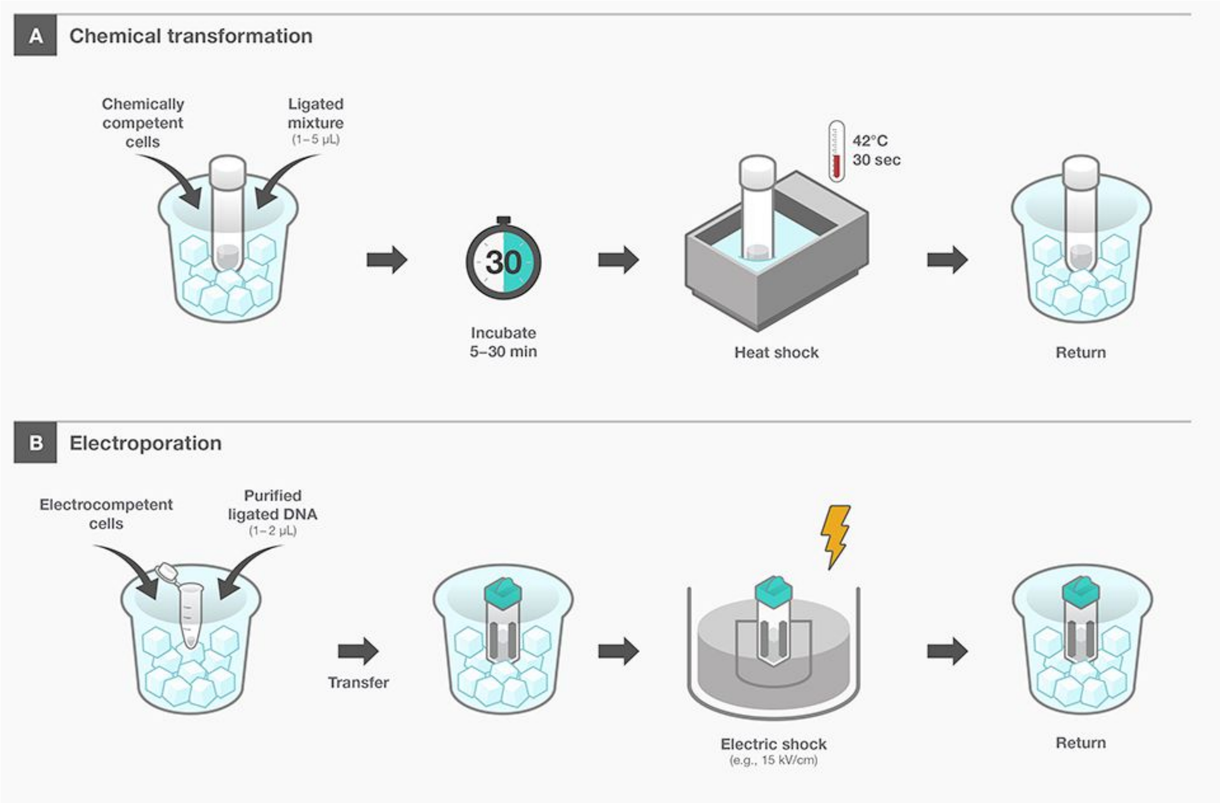
Transforming Bacteria - Visuals from ThermoFisher



First, the bacteria are grown to get a large enough sample to test.
The bacteria S. *pavinii* samples are kept on ice and washed with calcium chloride to neutralize the negative membranes.
The samples are washed and pelleted to remove excess salts.
The samples are aliquoted and stored until needed.
(ThermoFisher)

Transforming Bacteria - Visuals from ThermoFisher

Make sure bacteria and plasmid are kept at cold temperatures to prevent denaturation.
Combine the plasmid and neutralized cells and let them sit for up to 30 minutes before heat shocking them to make the plasma membrane more permeable for the plasmid to go through.
Return to ice, then store until further use. (ThermoFisher)



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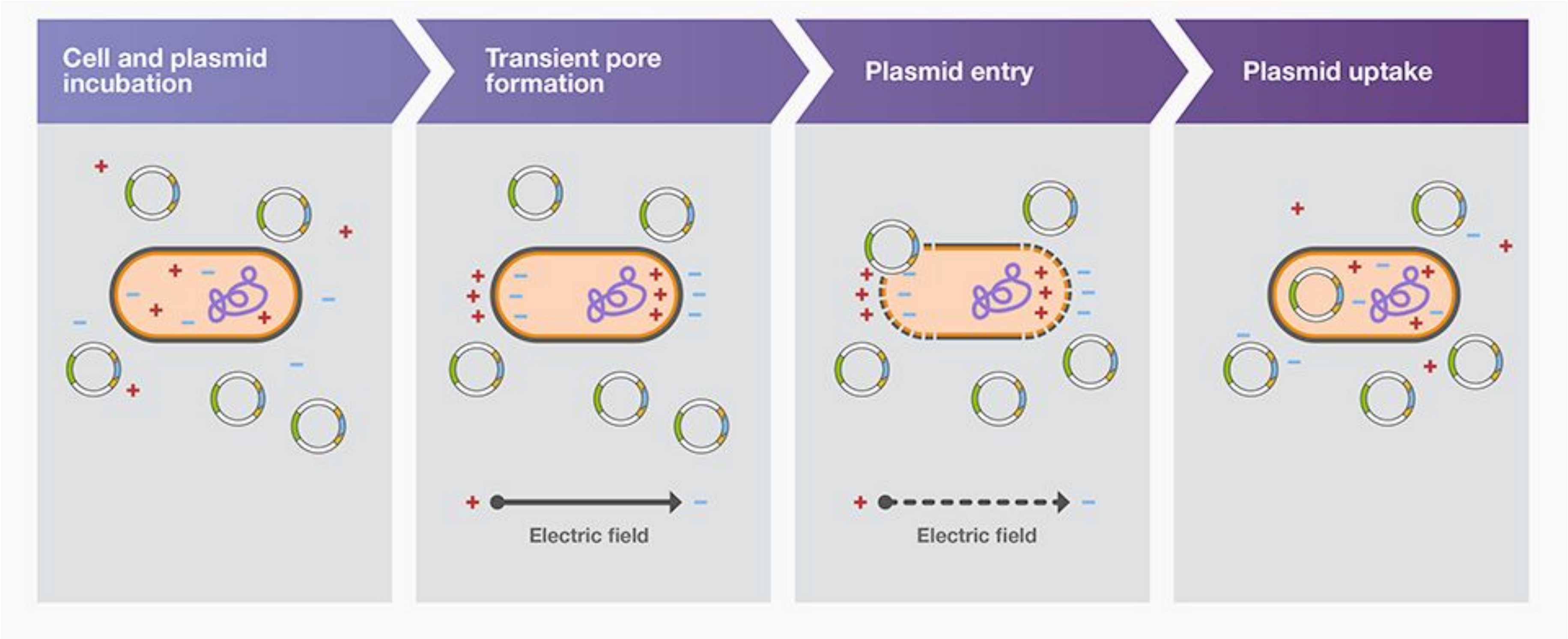
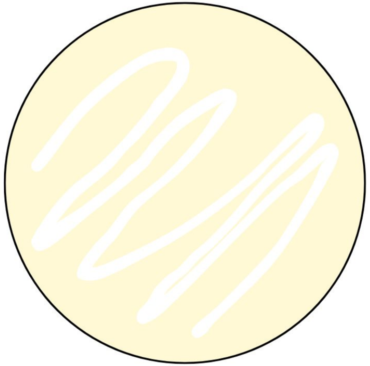


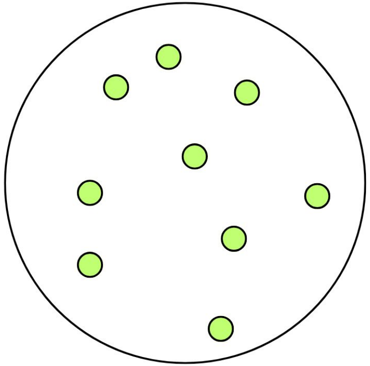
Gene Transforming: Plating

LB Plate



Lawn

LB Plate with Arabinose and Ampicillin



Individual Colonies

The pGLO gene that has been made uses arabinose to express the gene, and is also ampicillin resistant, so we can use selective media to test whether or not bacteria have transformed by plating. You can also do a similar process in broths. (Bio-Rad)

Transforming Bacteria: Gene Overview

Bacterium: *Stenotrophomonas pavanii*

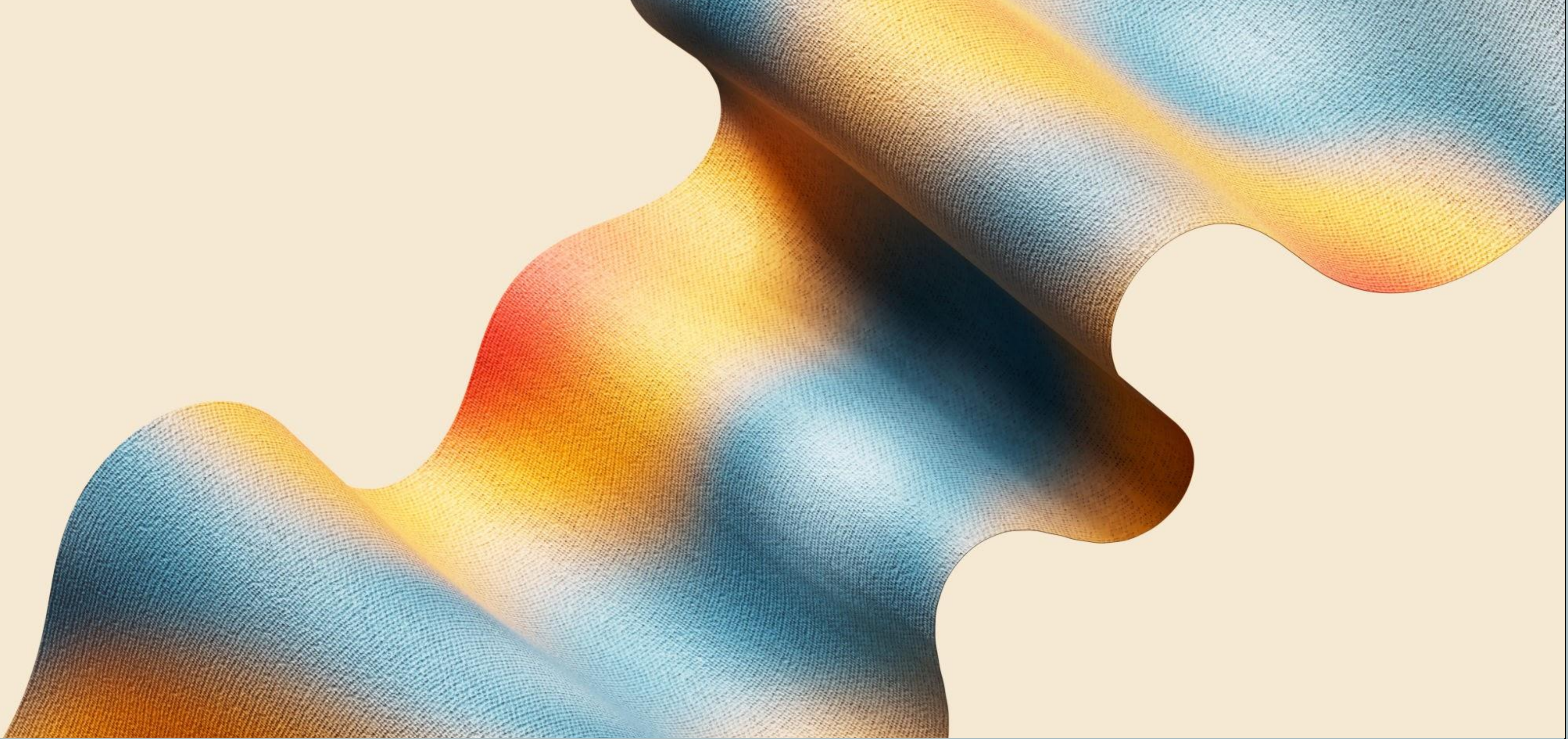
Algae: *Phormidium lucidum* and *Oscillatoria subbrevis*

Genes:

- PETase from I. *sakaiensis*
- Genes from T.*fusca* for PBAT, PLA, and PBS
- Alkane Hydroxylase from Pseudomonas *sp.*
- pGLO gene as an indicator during testing.

Facilities for Controlled Biodegradation

- Build facilities to collect plastics & create ideal environment for microbial growth, without harming surrounding environment ecosystems
- Eventually create a microorganism capable of degrading microplastics on land/in ocean, without disrupting natural ecosystems
- Where?: Near target site (ex. Near pacific garbage patch in California and Hawaii). Reduces the need to travel far distances to process the plastic.

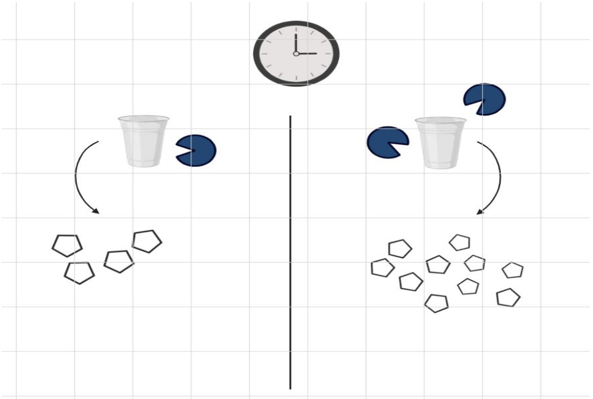


Expected Outcomes

Increased Efficiency of Microplastic Degradation

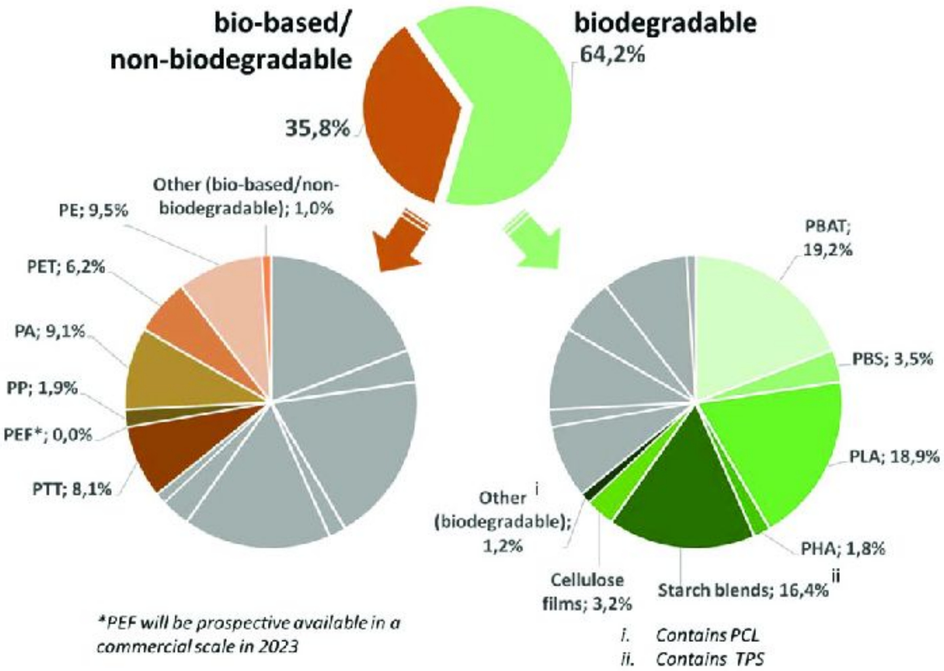
- Post-modification of microorganism should increase the amount of plastic degrading enzymes secreted
- With more enzymes present, more microplastics would be degraded compared to unmodified microorganisms within the same timeframe
- In conditions of 30ºC, in the span of 30 days: without genetic modification, the waste produced is 22.72 micrograms
    - With genetic modification, the waste produced is 38.04 micrograms

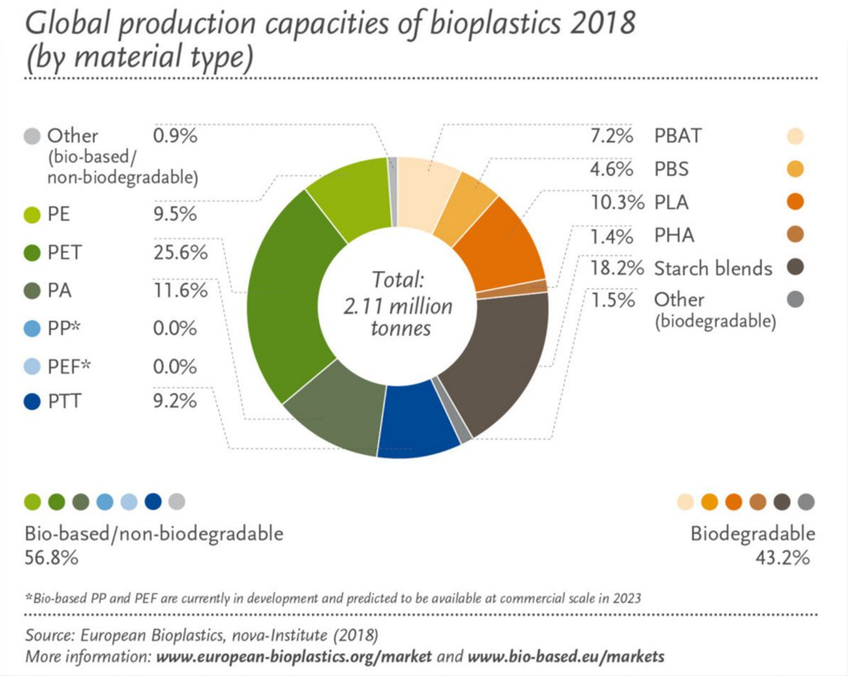
(Gostel & Kress, 2022)



Impact on Environmental Plastic Pollution

- Developing systems better suited to sort and process biodegradable plastics
- Bringing plastic biodegradation to its source





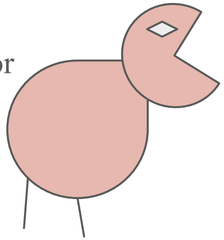
Gathering more microorganism DNA sequences

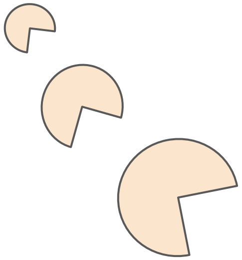
- Experimenting with different microorganisms that can degrade different kinds of plastics automatically expands the existing data available for microorganism DNA sequences
    - This information can be useful for future research/reference
    - For understanding microbial diversity and helping advance research and biotechnology with these microorganisms (Gostel & Kress, 2022).

What To Do With Waste After Use:

- Find ways to recycle ⟶ Bioplastic Products
- Promote further plastic degradation ⟶ Textile & Heavy Metal Use
- Use in crops as fertilizer and as feed

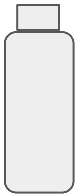
Use as Crops or Feed





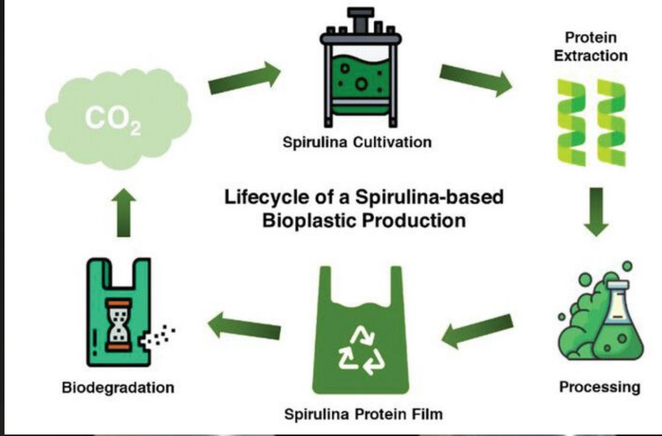
Absorb heavy metals and dyes

Recycle: Bioplastic Use



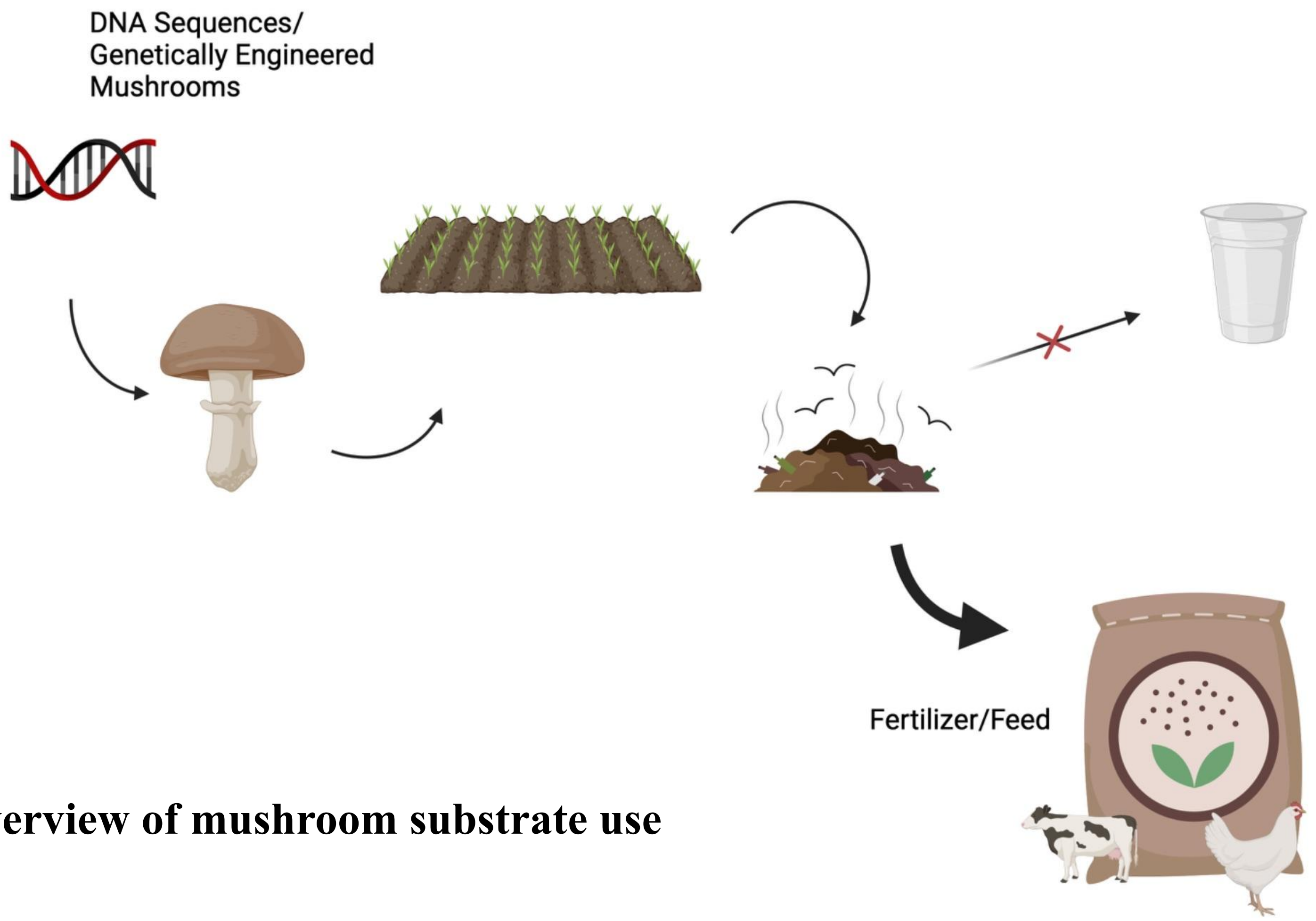
Algae For Bioplastic Use:

- **Spirulina-good for plastic degradation of PP and PE and a top choice in bioplastic creation**
  - (*Chia, W. Y et al., 2020*)



Wiley Advanced*

JONAA*



- **Overview of mushroom substrate use**

Fungi substrate as fertilizer or fertilizer conditioner

- Mushroom compost contains high salt levels, and an alkaline environment, and thus retains water in soil. Although this can be harmful to some crops, plants like tomatoes and corn do very well. (Rajavat, A. S et al., 2022)

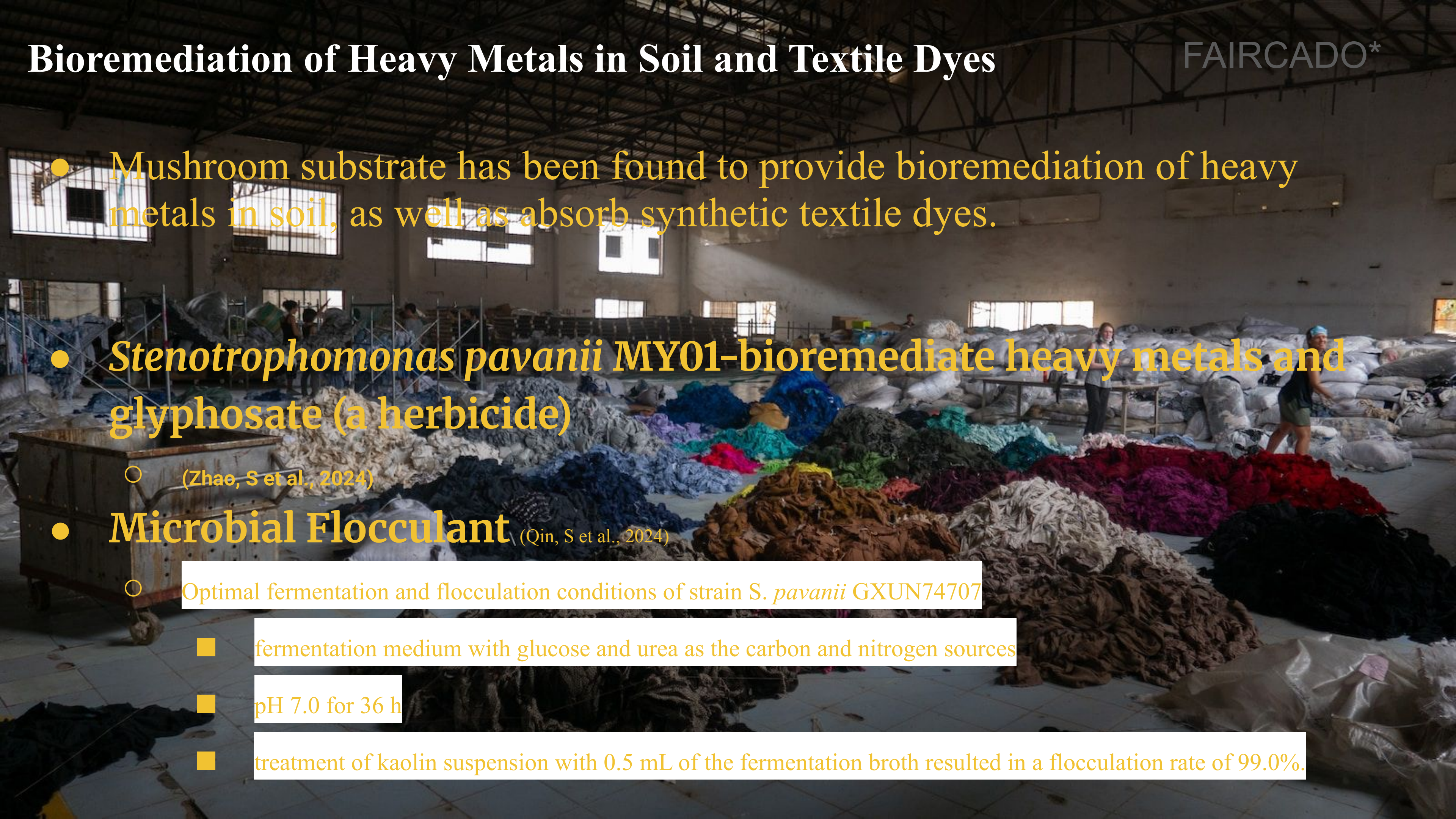
- Corn is one of the top food commodities globally
- Tomatoes are the second most consumed vegetable in the United States (*Tomatoes*. Ag Marketing Resource Center.)

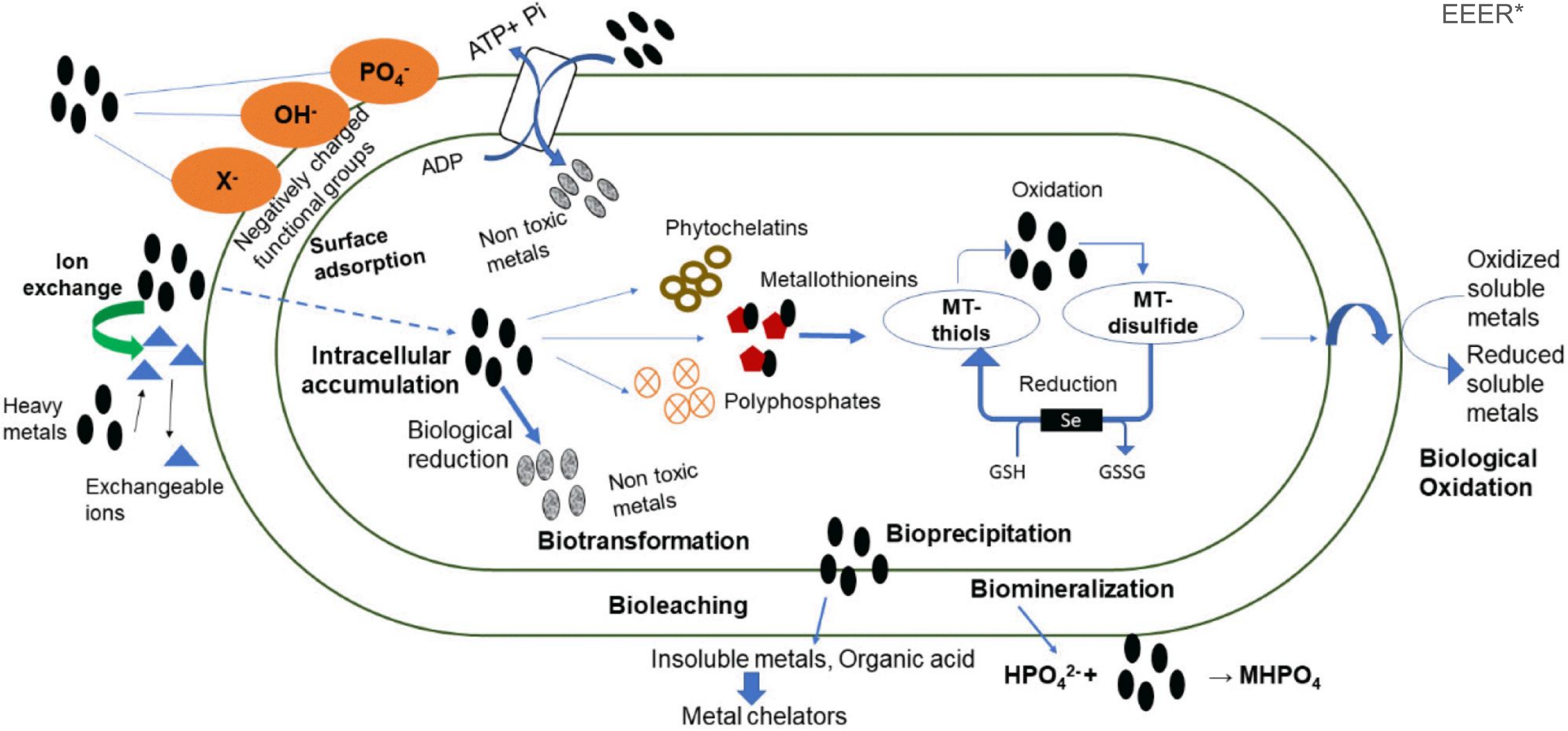
Bioremediation of Heavy Metals in Soil and Textile Dyes

FAIRCADO*

- Mushroom substrate has been found to provide bioremediation of heavy metals in soil, as well as absorb synthetic textile dyes.

- ***Stenotrophomonas pavanii*** **MY01-bioremediate heavy metals and glyphosate (a herbicide)**
  - **(Zhao, S et al., 2024)**
- **Microbial Flocculant** (Qin, S et al., 2024)
  - Optimal fermentation and flocculation conditions of strain S. *pavanii* GXUN74707
    - fermentation medium with glucose and urea as the carbon and nitrogen sources
    - pH 7.0 for 36 h
    - treatment of kaolin suspension with 0.5 mL of the fermentation broth resulted in a flocculation rate of 99.0%.





Oval shapes represents the heavy metals; MT- represents as Metallothioneins; Se-Compound is amplifier of thionin-reduction; GSH-Glutathione; GSSG-Glutathione disulfide

- ★ Expand DNA sequence research base
- ★ Enhance biodegradation of plastics
- ★ Reduce plastic pollution
- ★ Create secondary ways to reduce plastic pollution

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