UCLA Samueli School of Engineering

Enzyme Nanohybrids for Emerging Contaminant Degradation

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UCLA Civil and Environmental Engineering

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- University of Georgia:
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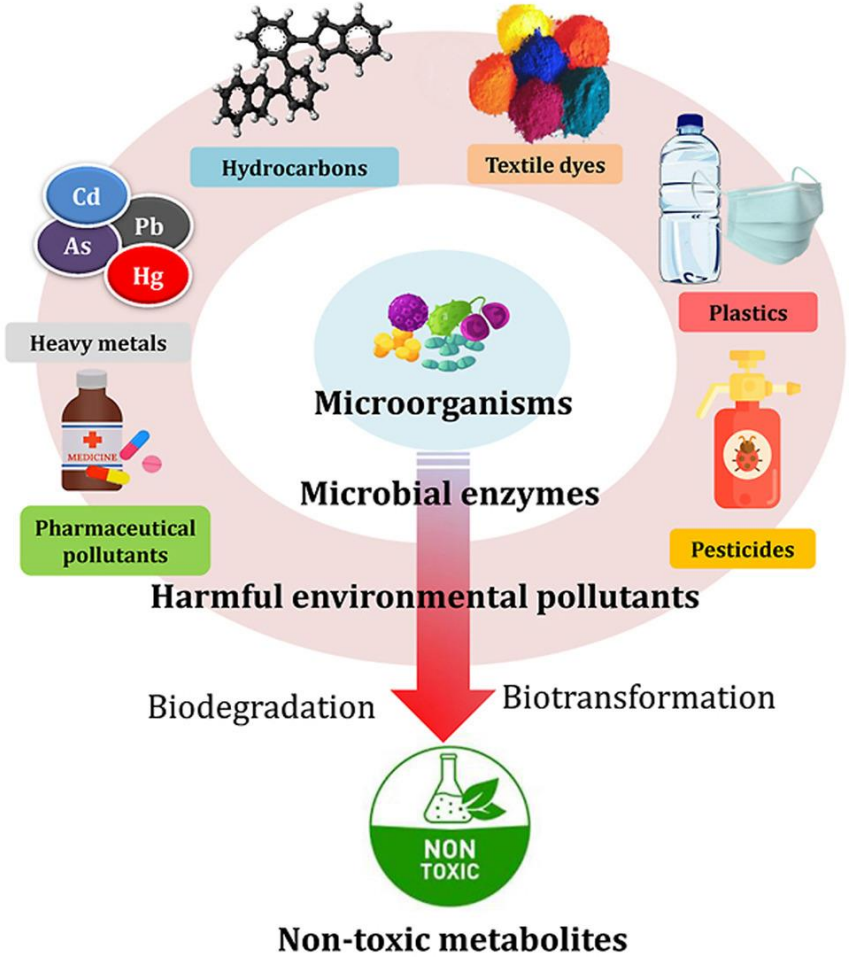
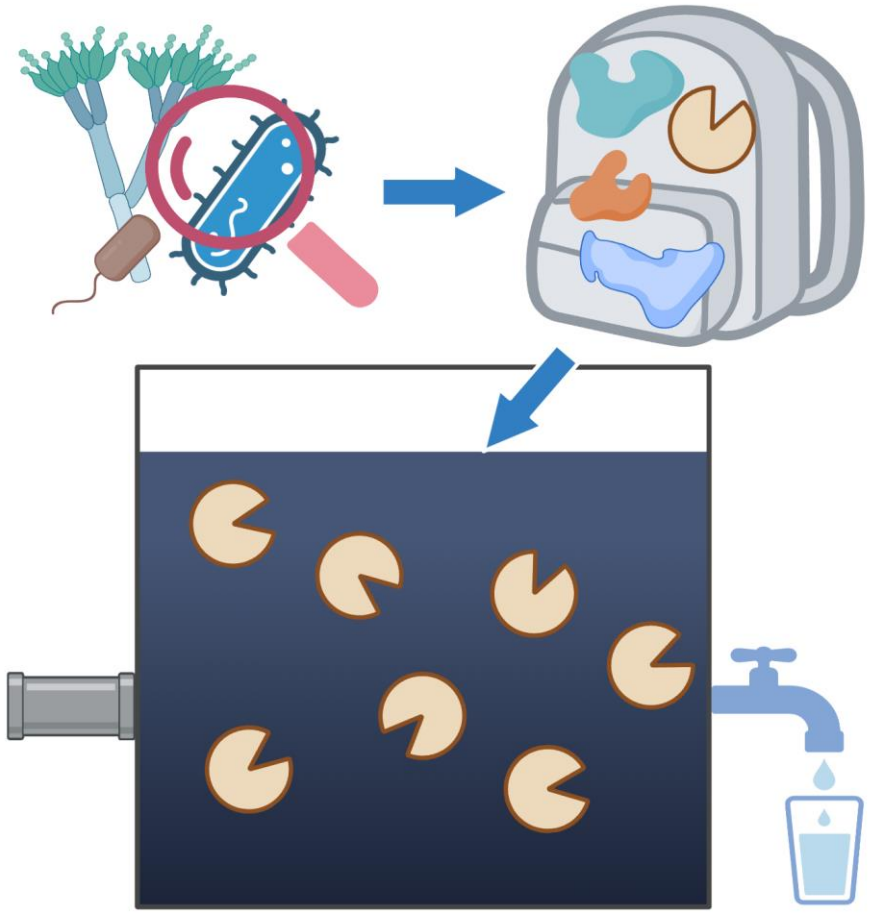
Microbial Enzymes Biotransform Contaminants

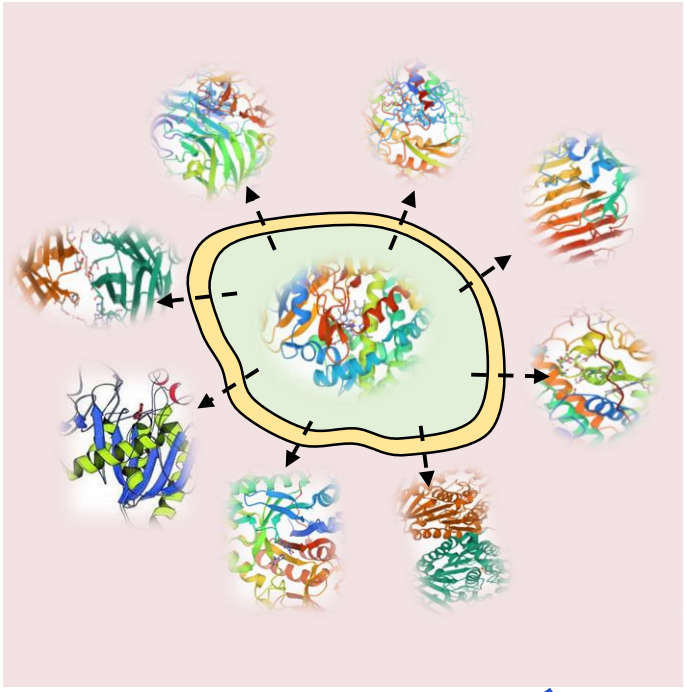


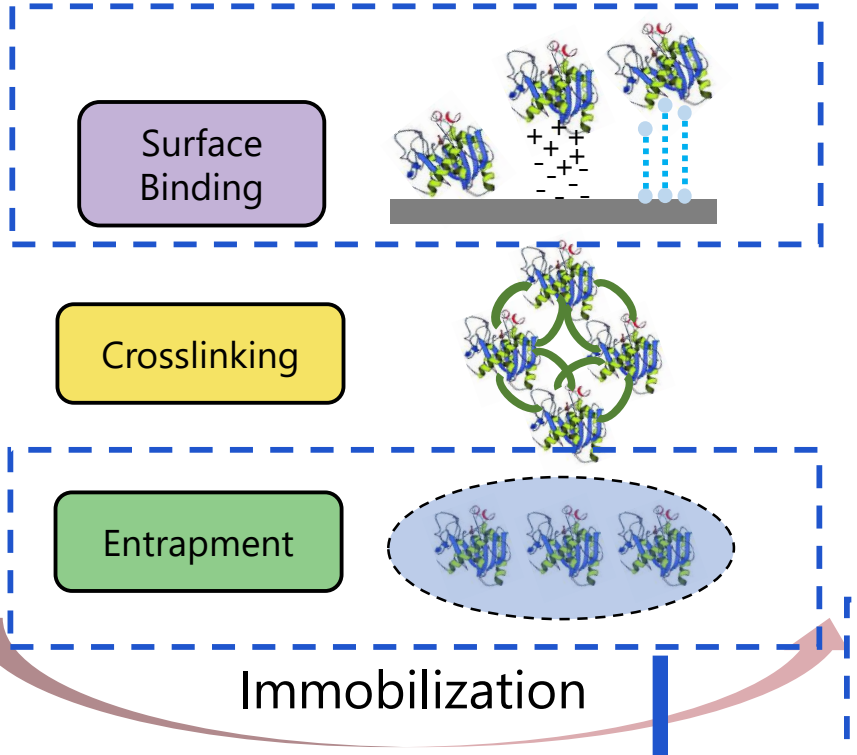


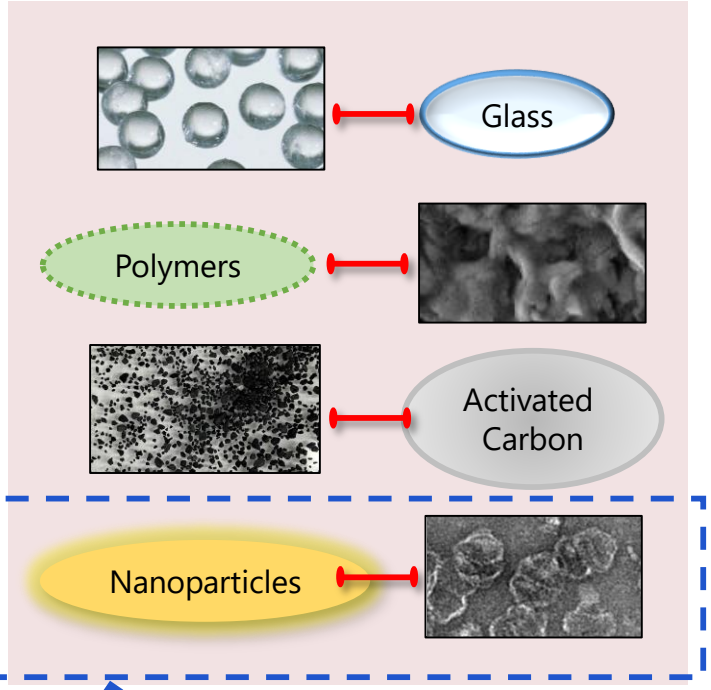
Created by BioRender.com

Narayanan et al., JEM, 2023

Enzyme Immobilization





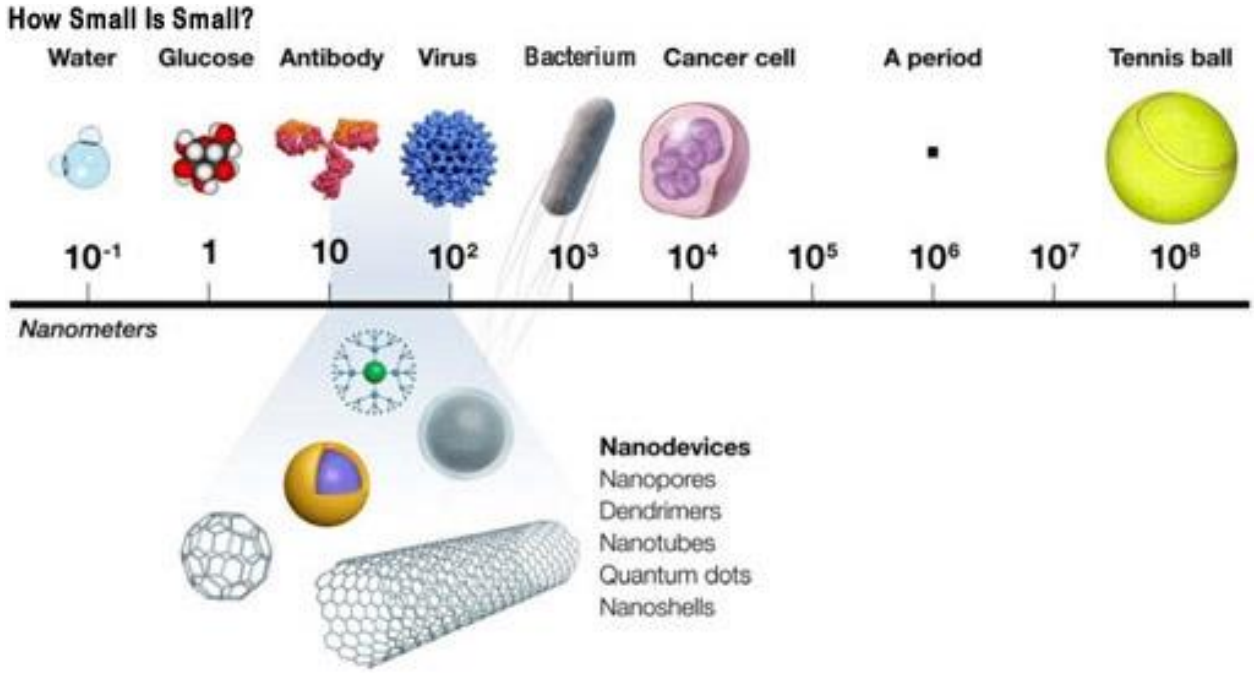


Enzymes immobilized into/onto nanomaterials for stability and longevity

Yifan Gao et al., Biotech. Adv., 2022

Nanoscale: Size Matters

- Nanoscale = 1-100 nm in at least 1 dimension
- Increased surface area = increase reactivity
- Wave/Particle duality = unique nanoscale properties

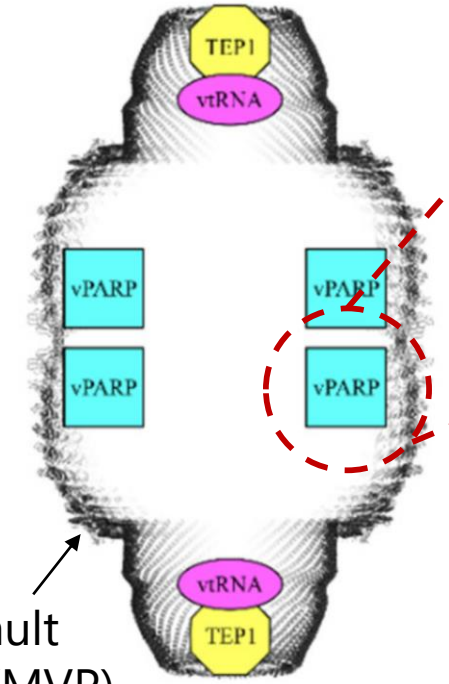


Klaine et al. 2011

The ratio of a nanomaterial to a tennis ball is about the same as the ratio of tennis ball to the moon!

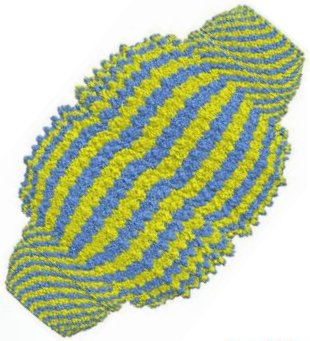
Human hair grows ~6 inches/year = 5 nm/sec!

Vault Nanoparticles



Major vault protein (MVP)

INT serves as a "**Zip code**" to help deliver proteins into vaults



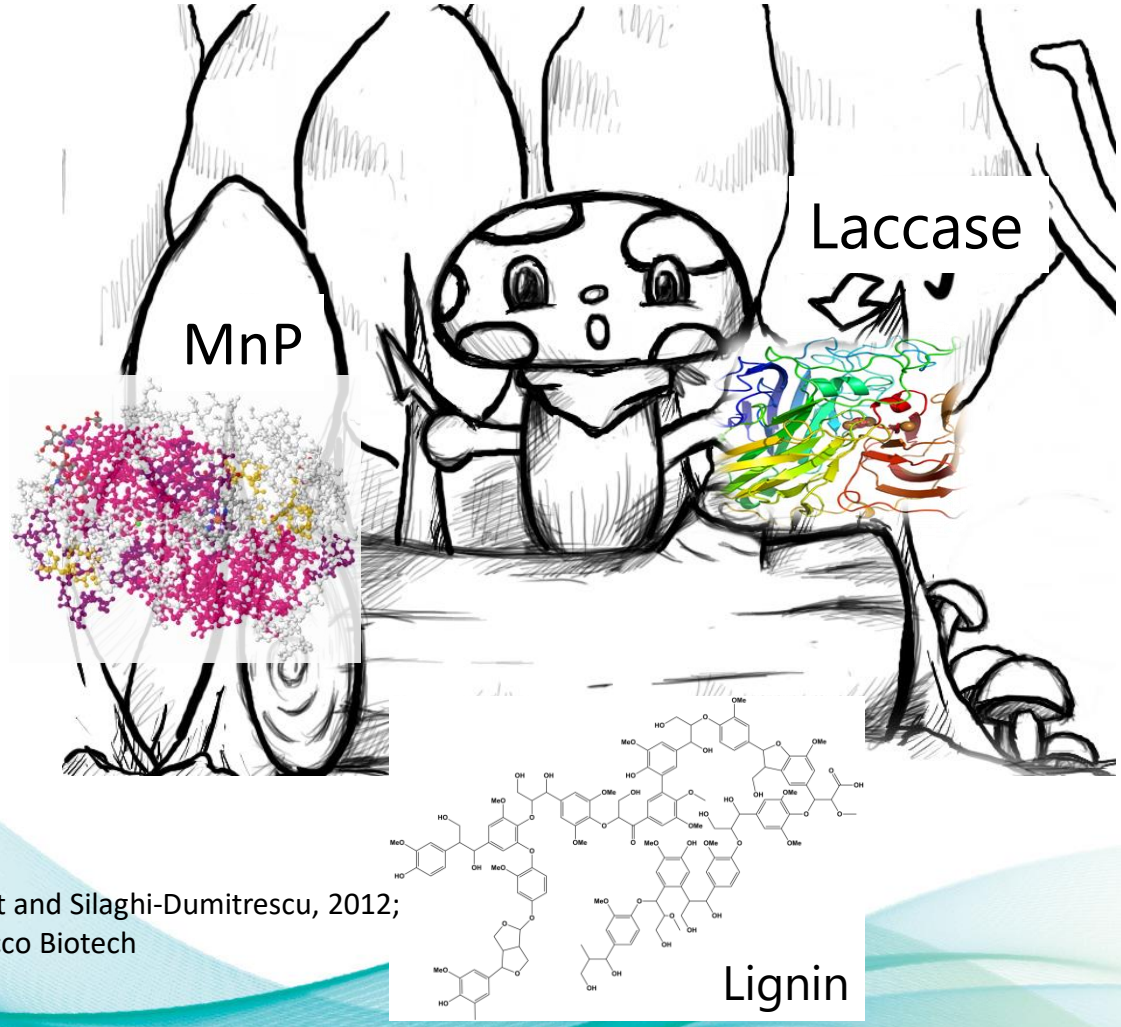
Engineered empty vaults

INT

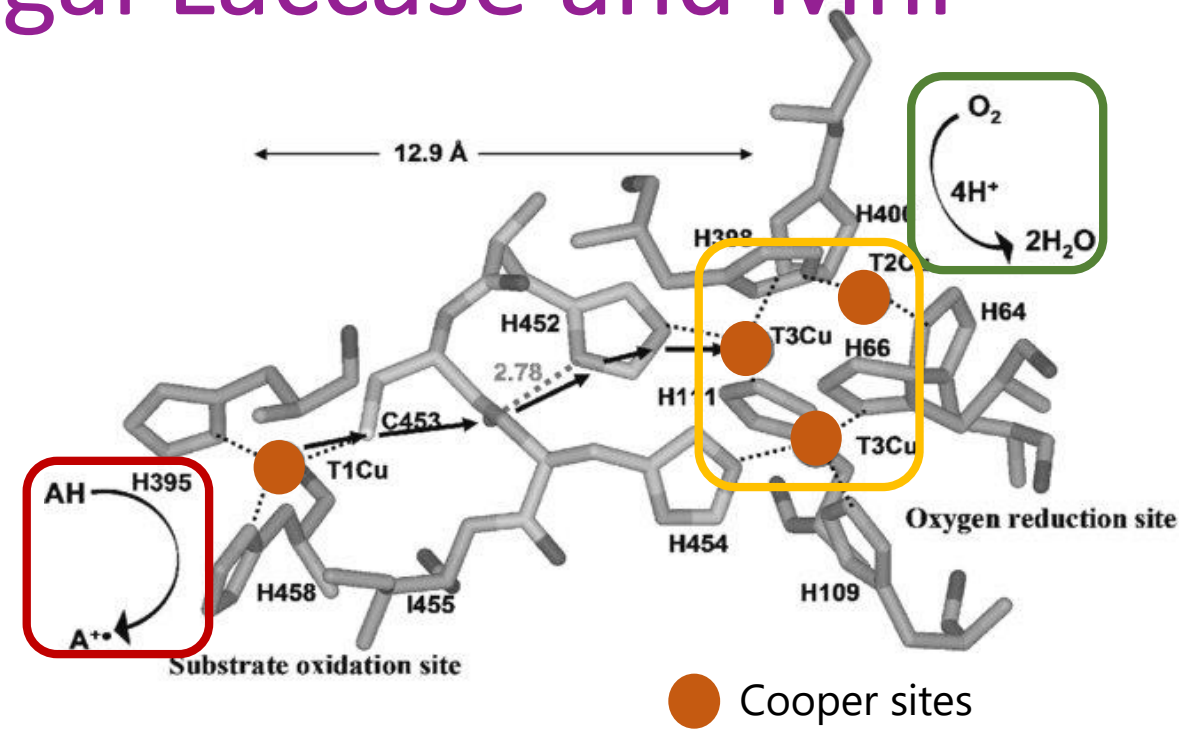
Protein of interest

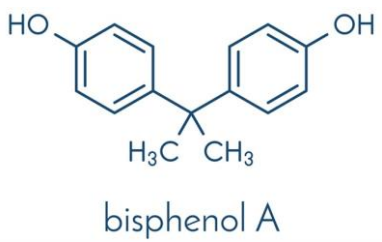
Frascotti et al., Cancers, 2021; Kickhoefer et al., PNAS, 2005

Proteins of Interest: Fungal Laccase and MnP

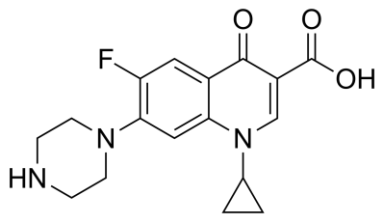


Mot and Silaghi-Dumitrescu, 2012; Gecco Biotech

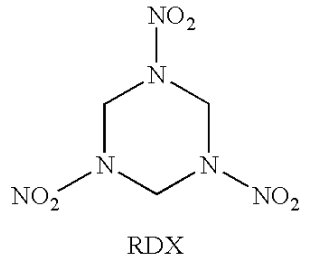




bisphenol A

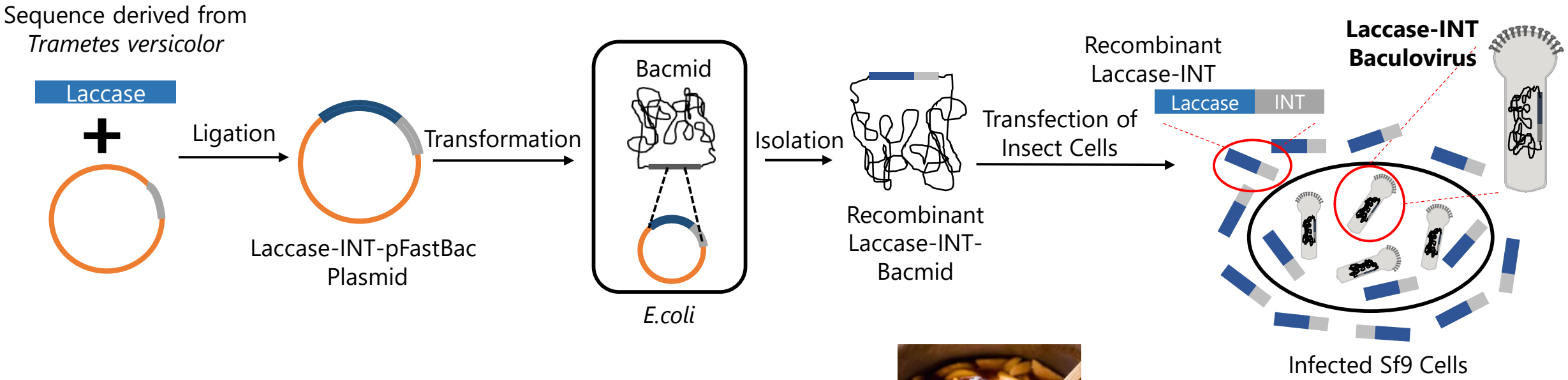


Ciprofloxacin



RDX

Let’s make recombinant proteins

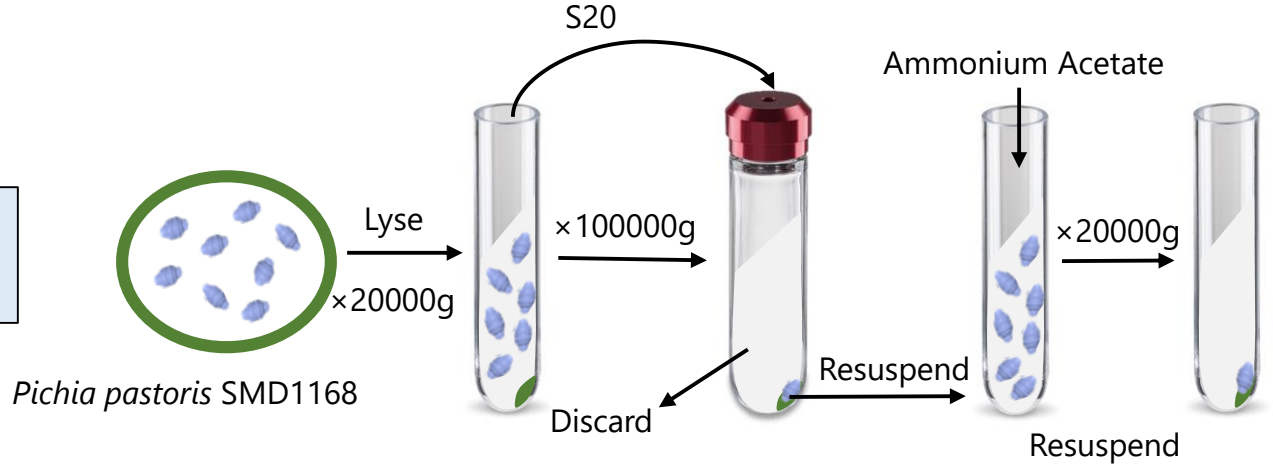


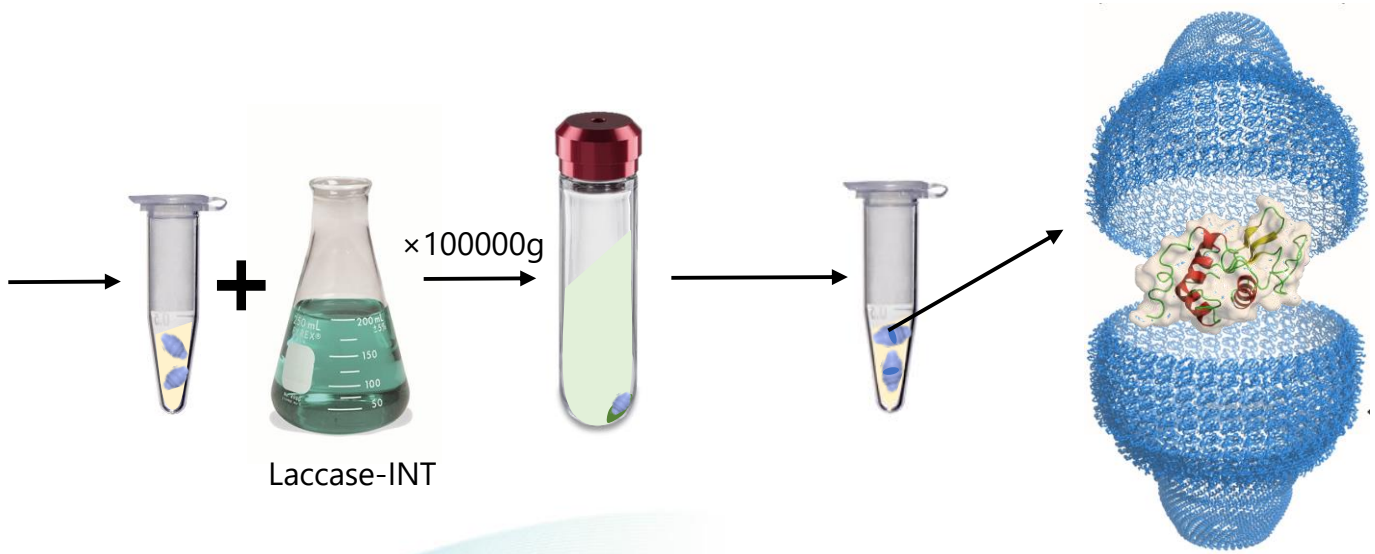
1 Produce recombinant laccase-INT in Sf9 cells

Yifan Gao et al., Bioresour. Technol., 2022

Encapsulate laccases into vaults

2 Produce empty vaults in yeast cells



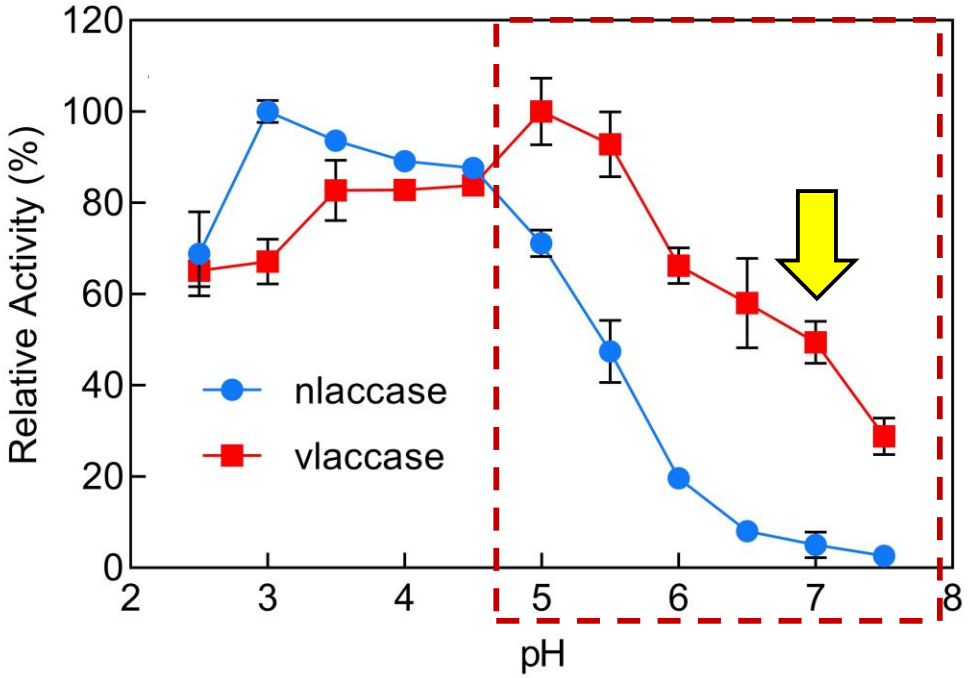


3 Encapsulation by mixing recombinant laccase with empty vaults

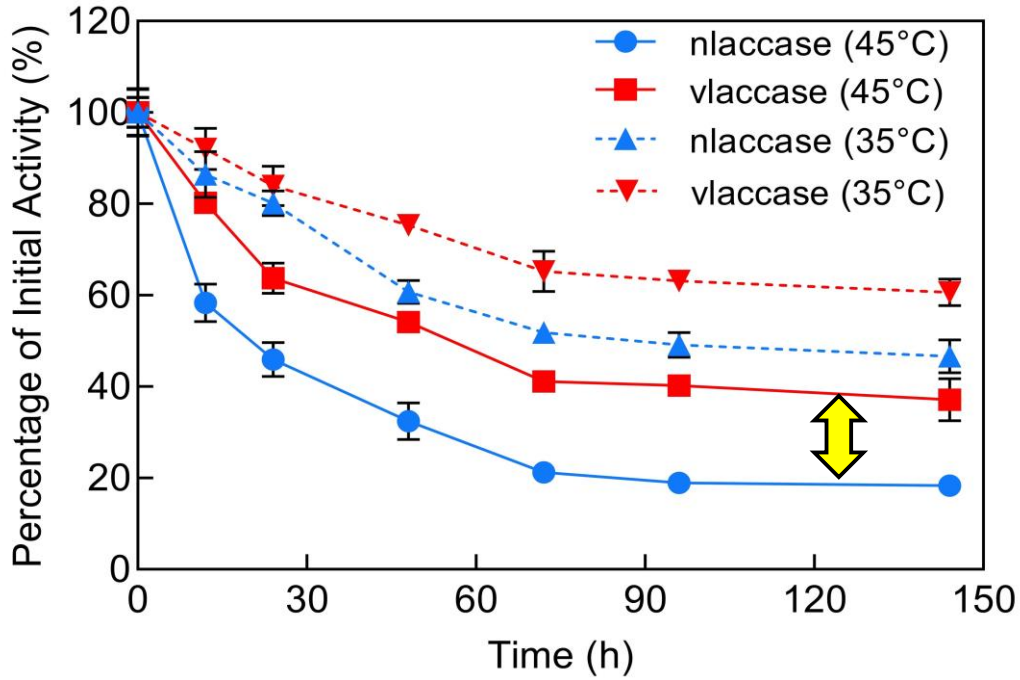
Yifan Gao et al., Bioresour. Technol., 2022

Laccase activity improved after packaging in vaults

nLaccase: natural laccase
vLaccase: vault-packaged laccase



vLaccase had **higher activity** at neutral pH range

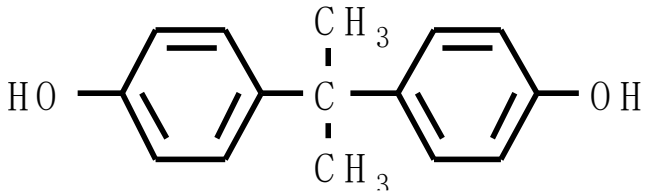


vLaccase had **enhanced thermal stability**

Yifan Gao et al., Bioresour. Technol., 2022

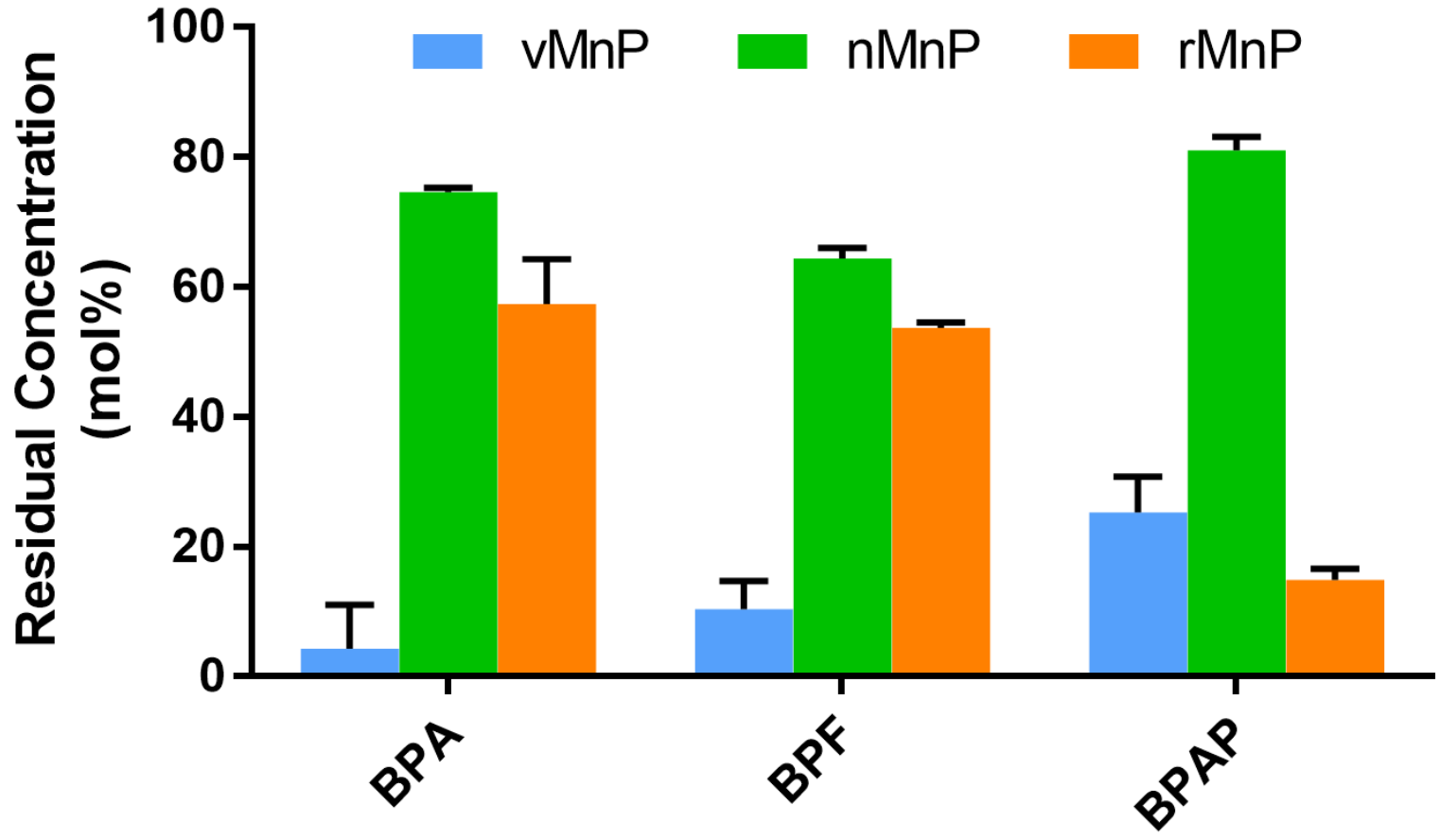
Bisphenol A and its Substitutes

- Weak estrogenic activity → reproductive toxicity
  - Causes chromosomal abnormalities and DNA damage
  - Induces germline apoptosis
  - Reduces brood size
  - Increases embryonic lethality



- Used in production of plastics, epoxy resins, copy paper, PVC, dental sealants, flame retardants, food packaging containers

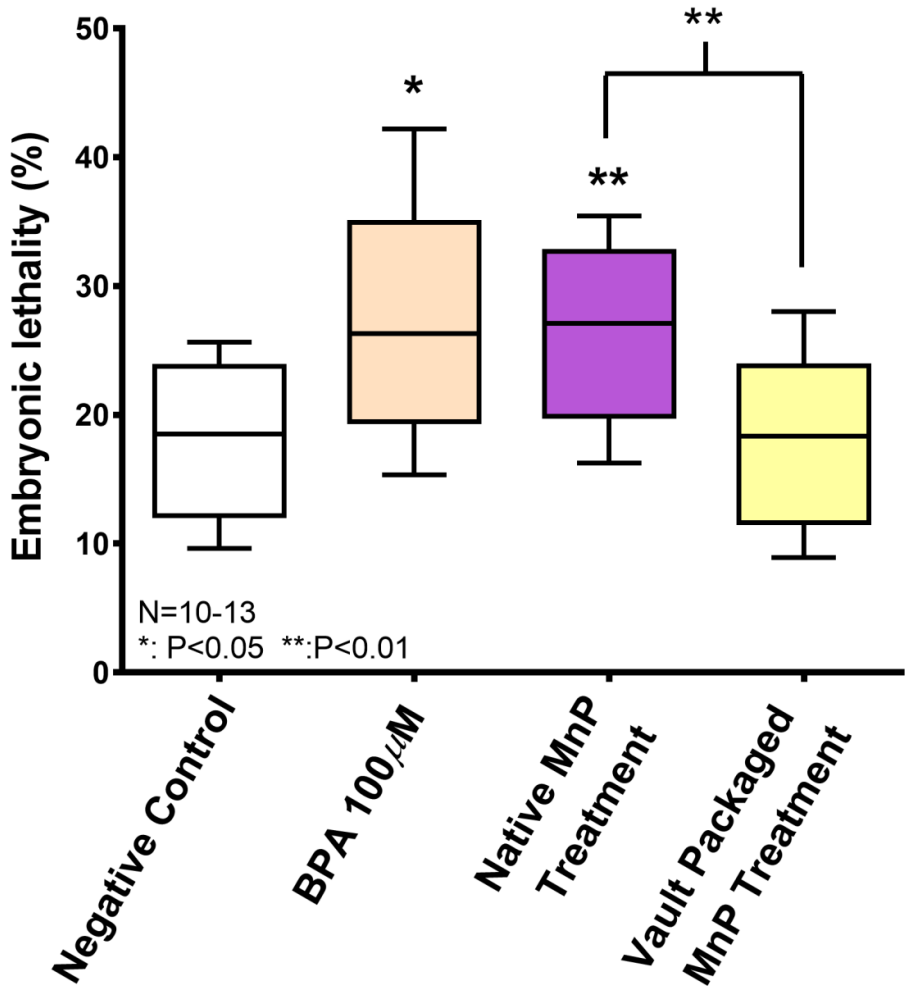
Improved Biodegradation Rates

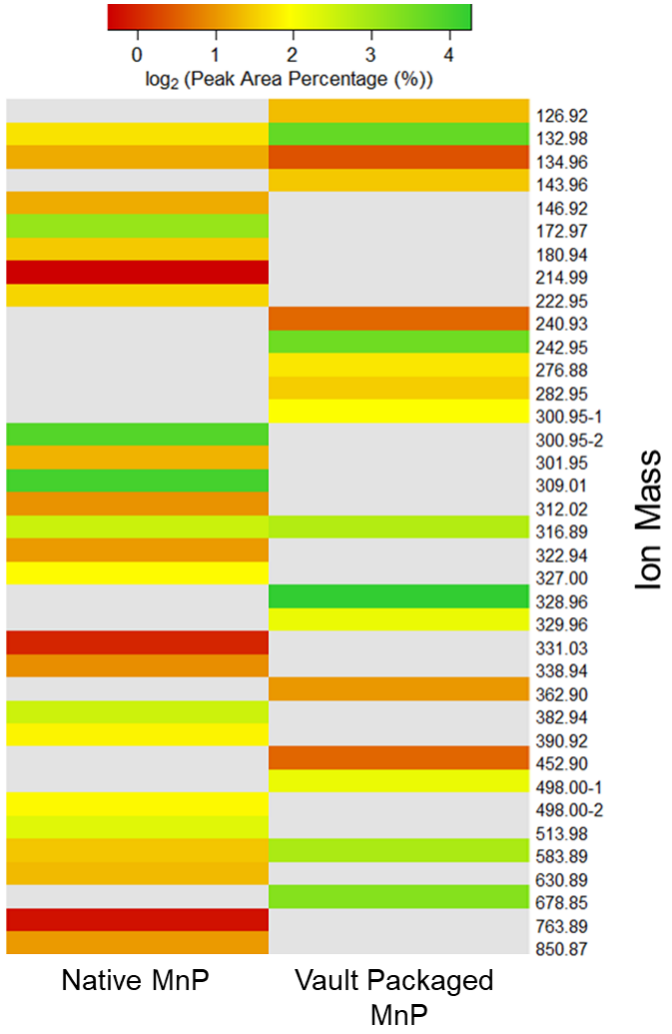


Vault-packaged enzymes showed better thermal stability, wider pH adaptation, and biodegraded bisphenolic compounds at higher rates than unpackaged enzymes or live microbes.

Wang *et al.*, ACS Sus Chem Eng, 2019

Vault MnP Lowers BPA Product Toxicity





Wang *et al.*, ACS Sus Chem Eng, 2019

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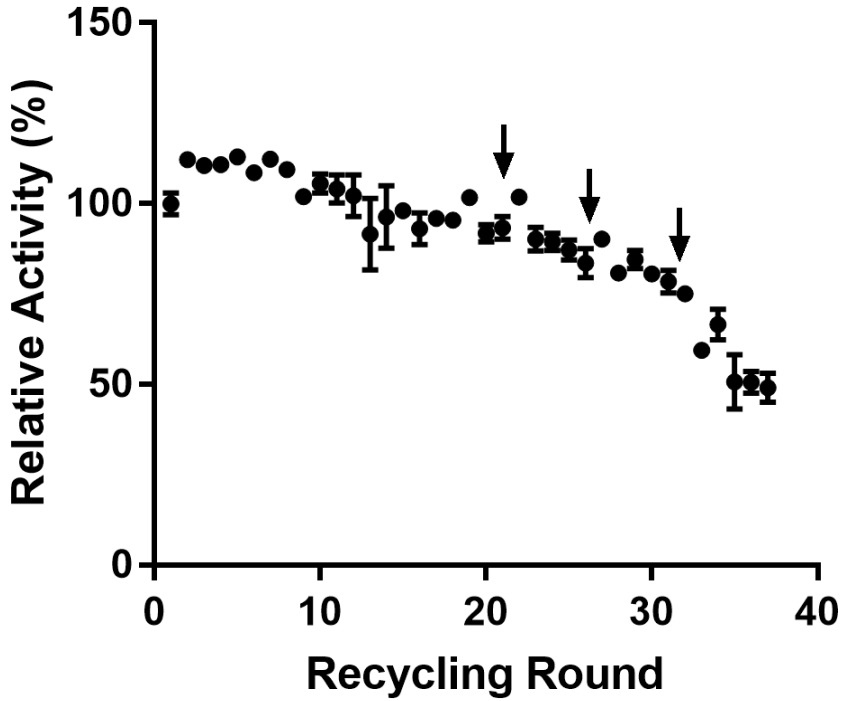
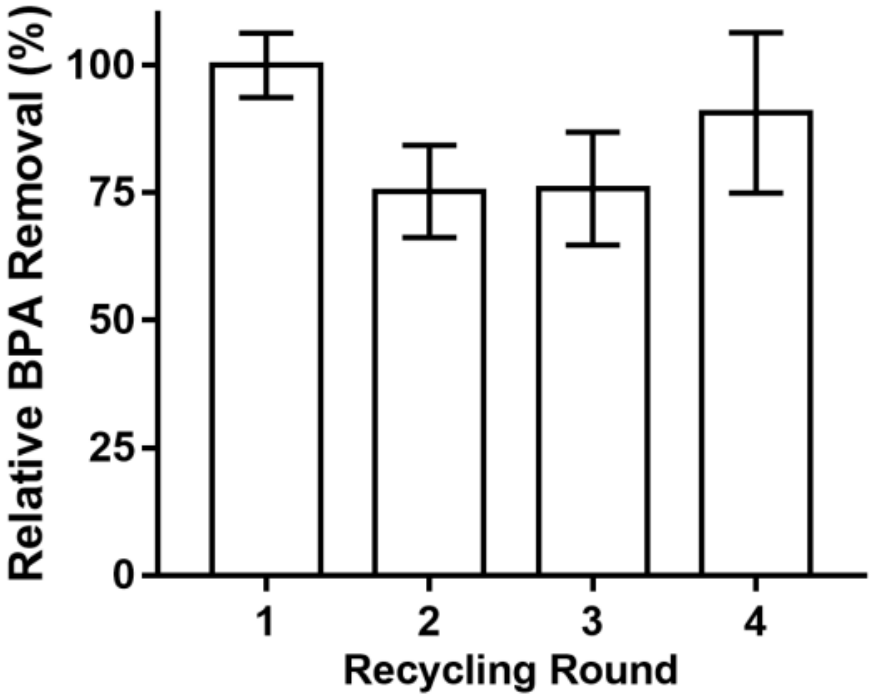
Reusability of MnP-INT/vault/silica

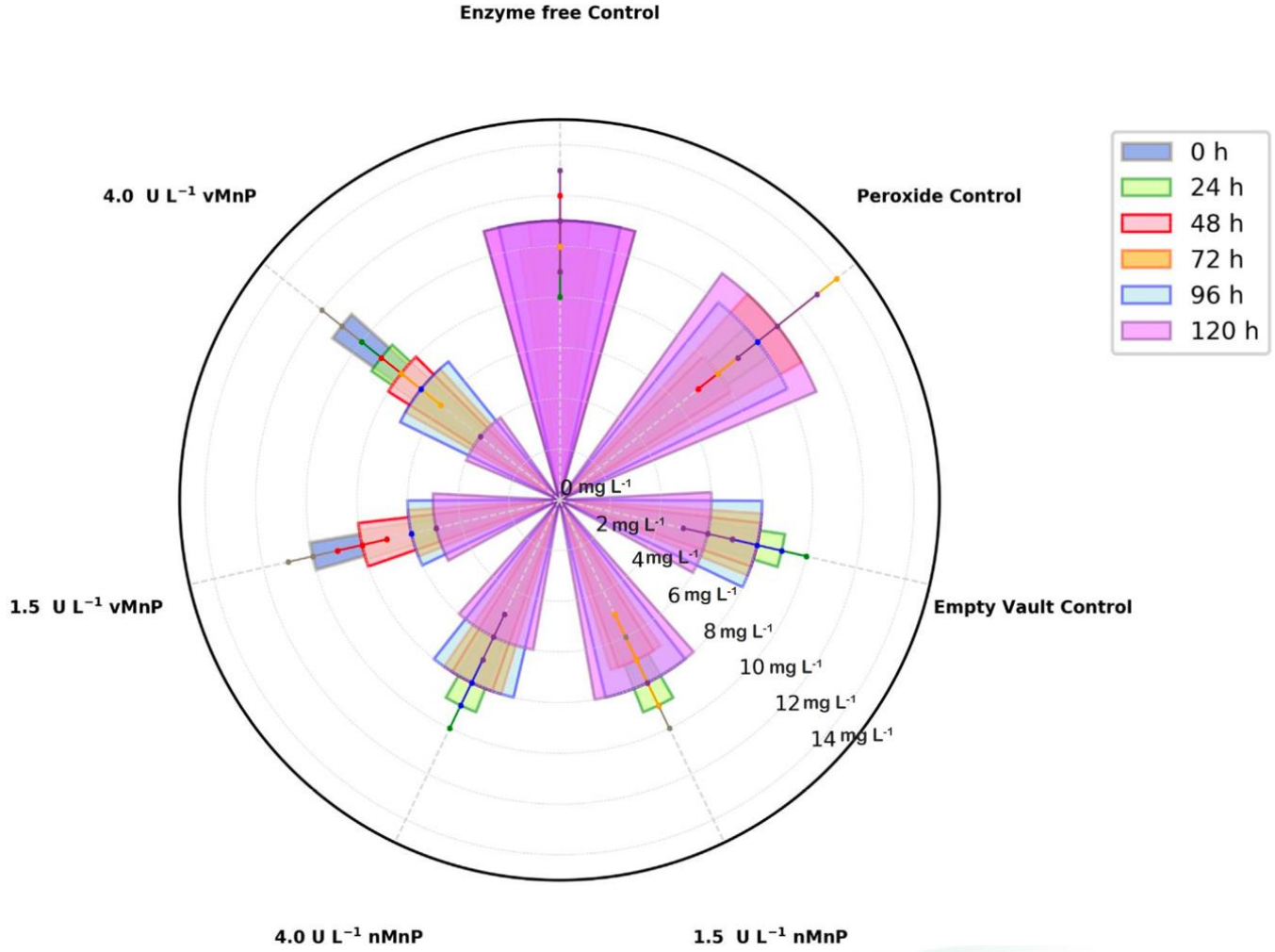
- MnP maintained ~100% activity after 12 recycles, ~90% activity after 25 recycles
- No significant decrease in BPA removal in four repeated uses of the same MnP-INT/vault/silica





Wang *et al.*, Chem Eng J, 2021

Degradation of Nitro-Amino-Aromatics



Vault-packaged enzymes were required in lower amounts than free enzymes to achieve the same degradation rates of dinitrotoluenes (DNT), aminonitrotoluenes (ANT), and diaminotoluene (DAT).

(Lothe et al., *Chemosphere*, 2020)

Synthetic Dyes as Contaminants of Concern

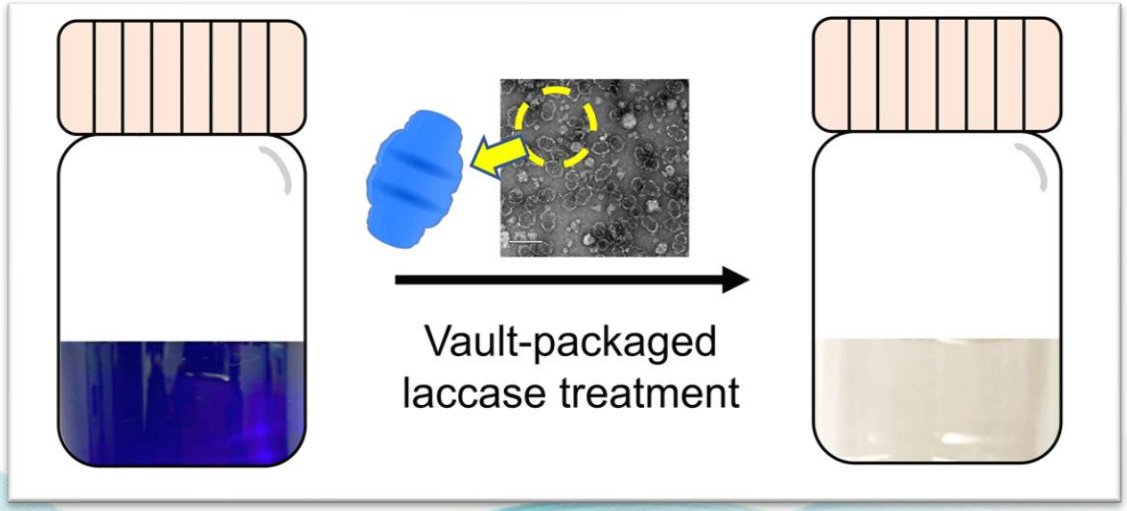
Ecological impact

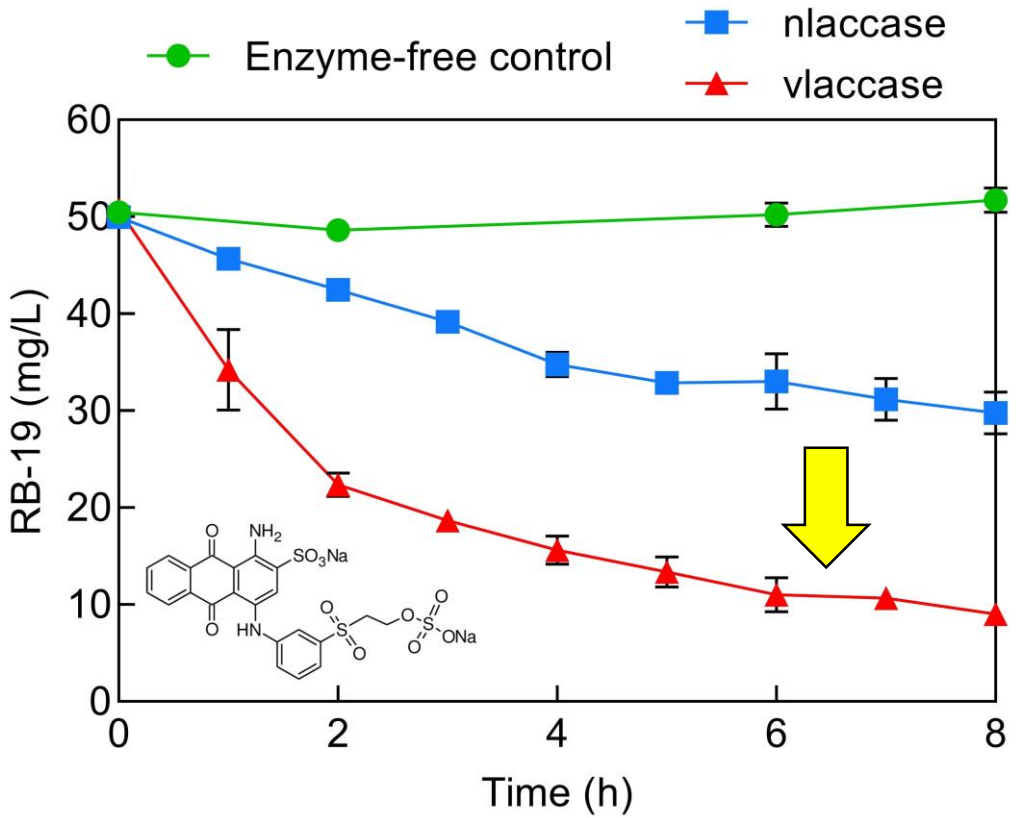
Hurt animals!

Toxic to humans

Decolorization of Synthetic Dyes

Reactive blue 19

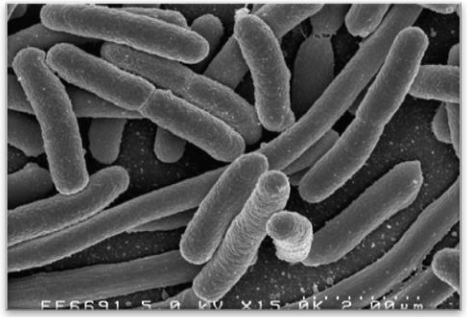




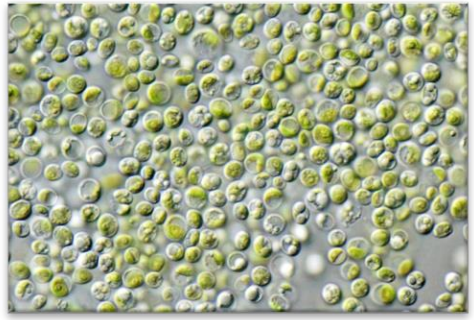
Gao et al., Bioresour. Technol., 2022

Detoxification of Dyes

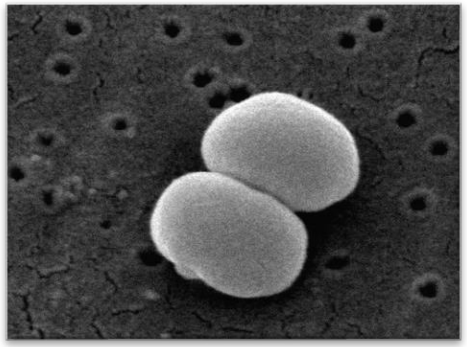
Model organisms



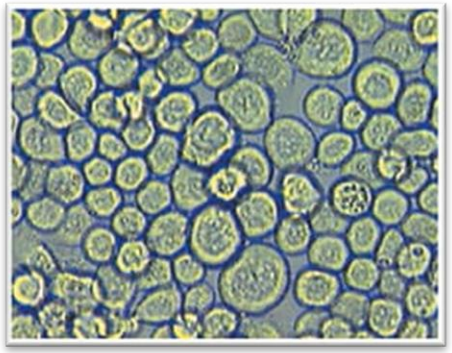
E. coli



C. vulgaris

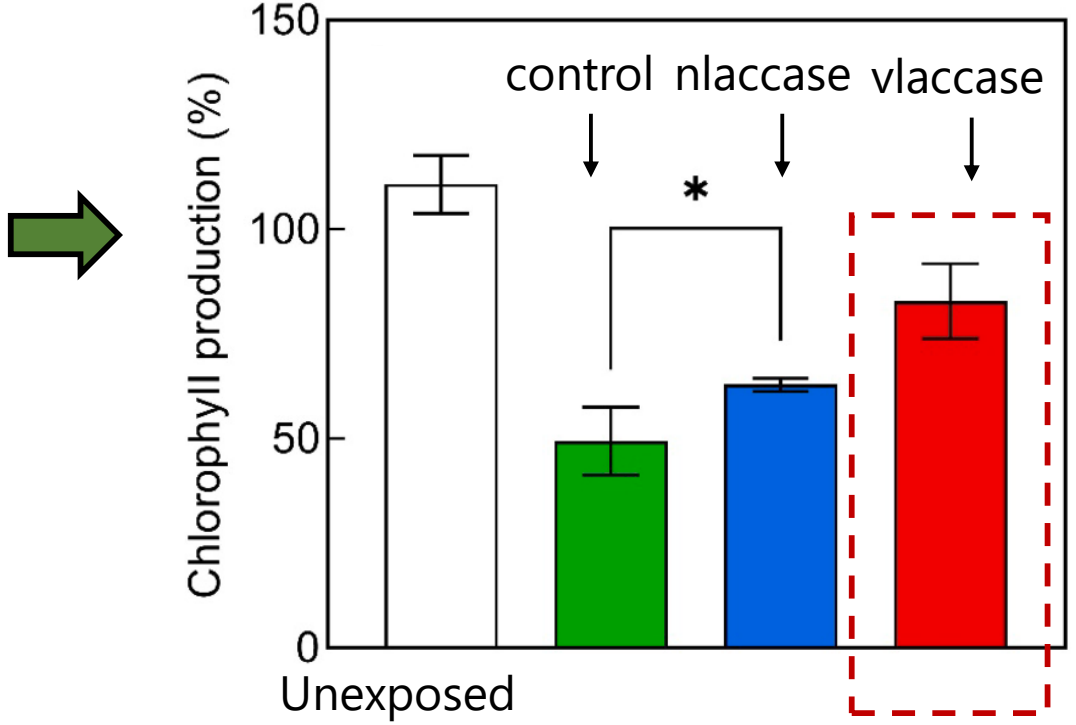


S. epidermidis



Insect cells

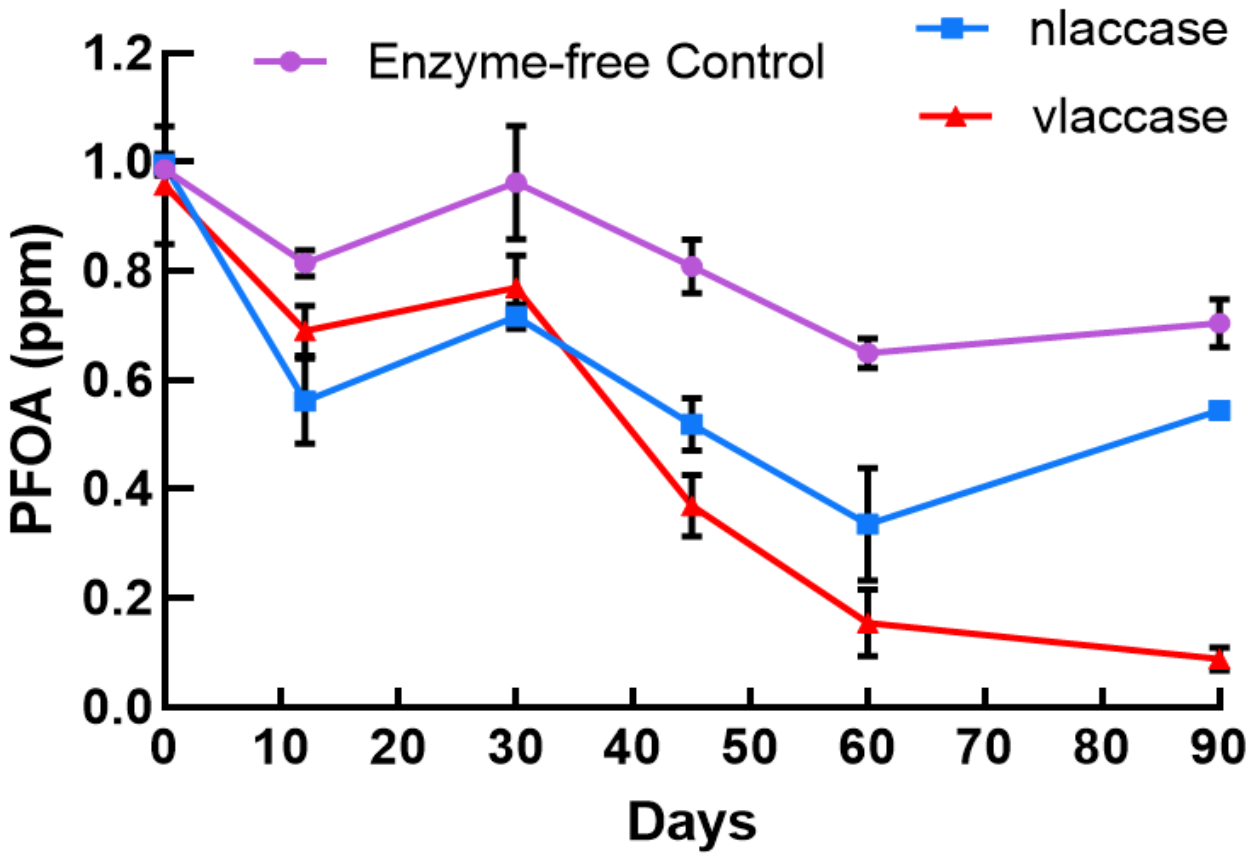
Chlorophyll



High chlorophyll = Low toxicity

Gao et al., Bioresour. Technol., 2022

Removal of PFOA by vLaccase



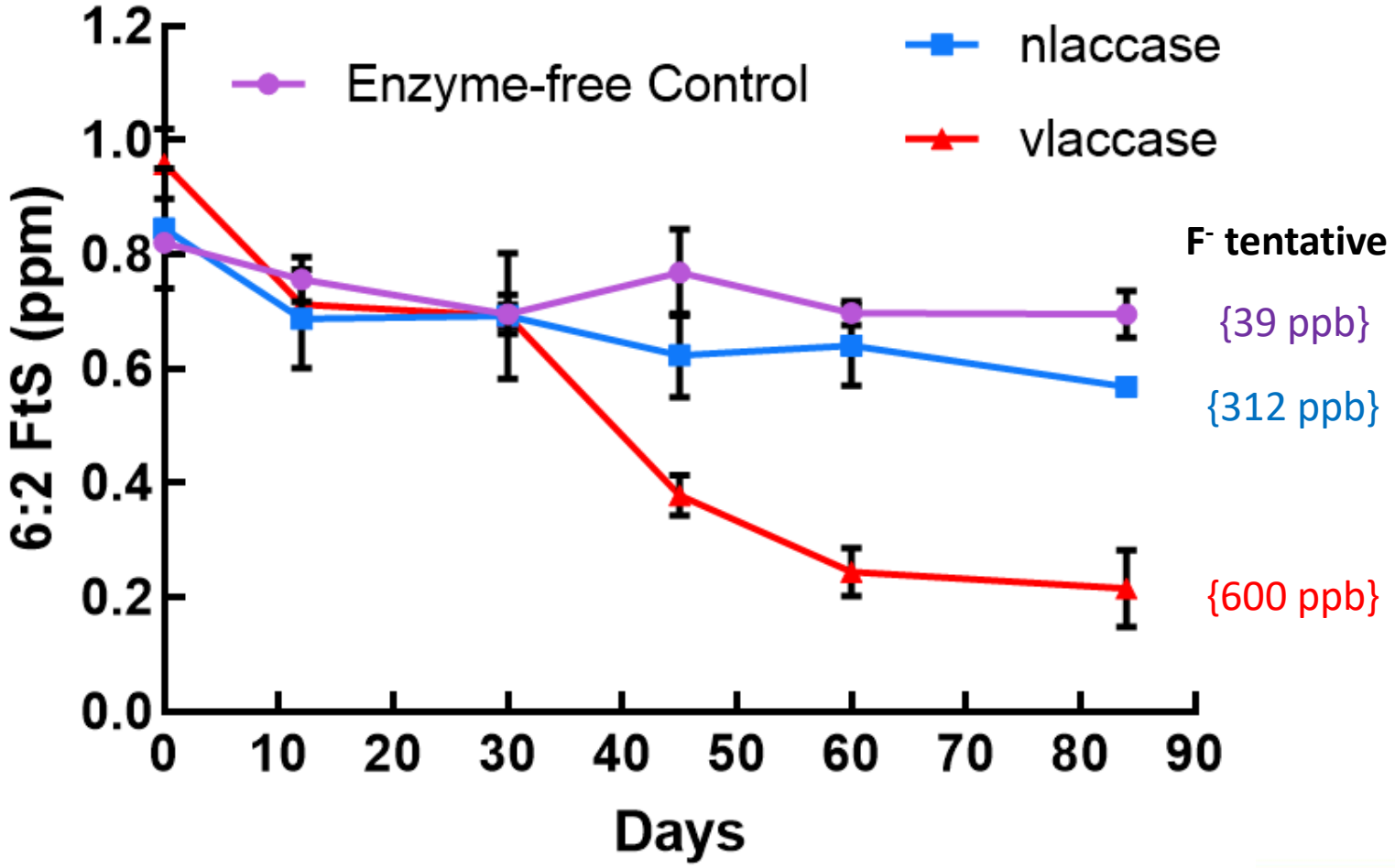
F^- ions

Non-detectable

{290 ppb}

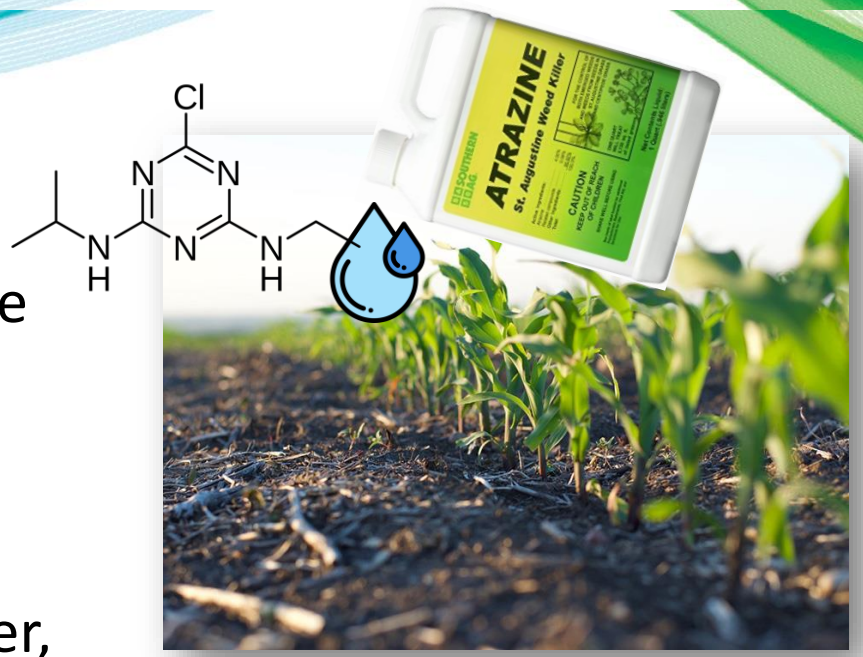
{260 ppb}

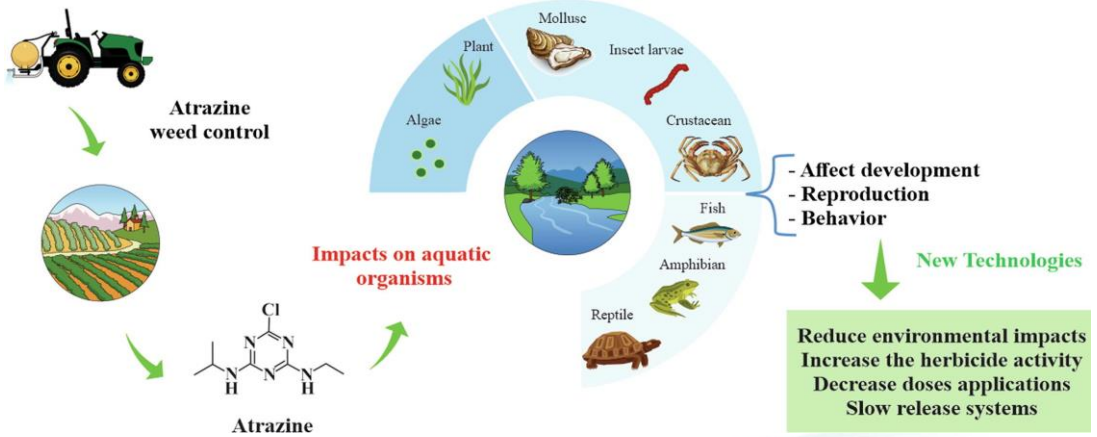
Removal of 6:2 FtS by vLaccase

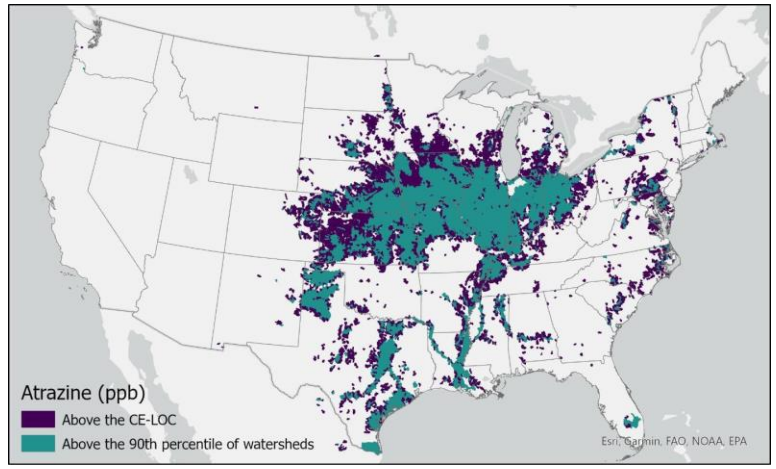


Atrazine – Why do we care?

- Among the most widely used herbicides in agriculture to control annual weeds and grasses
- Endocrine disruption, carcinogenesis, and bioaccumulation in aquatic animals and plants.
- In soil, half-life is more than 100 days; in surface water, half-life is approx. 85 days.



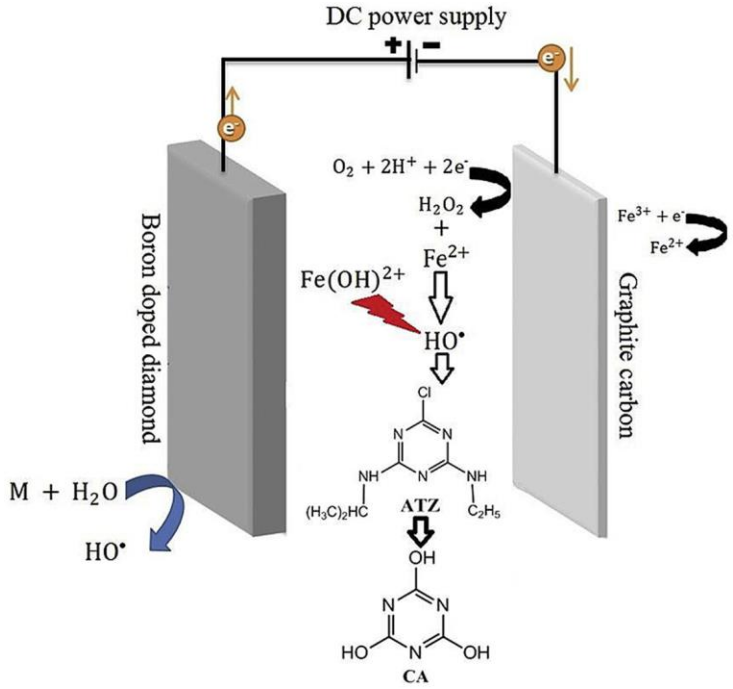




Texas Corn Producers

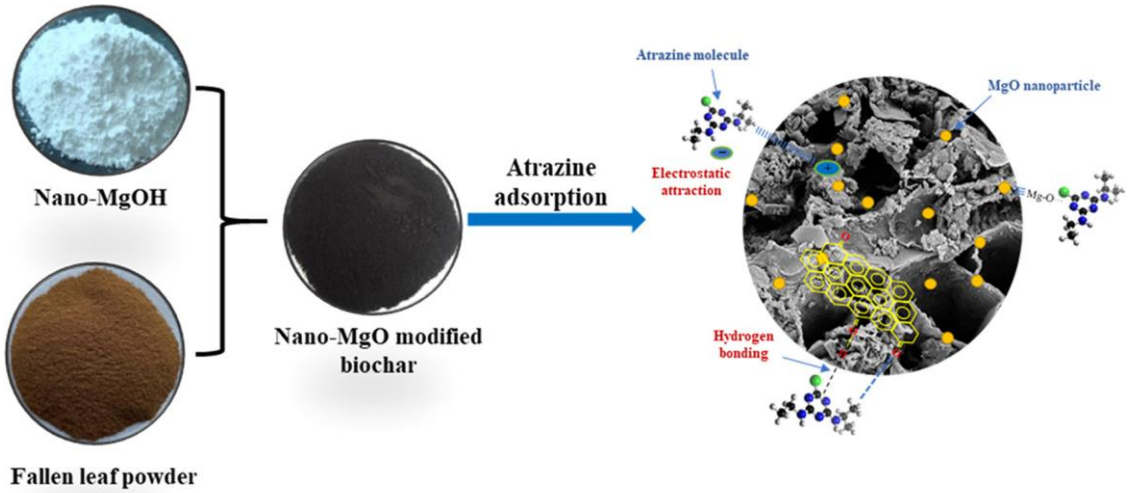
Pereira de Albuquerque et al., STOTEN, 2020; P´erez et al., Chemosphere, 2022

Atrazine Degradation Technologies



Electrochemical advanced oxidative process

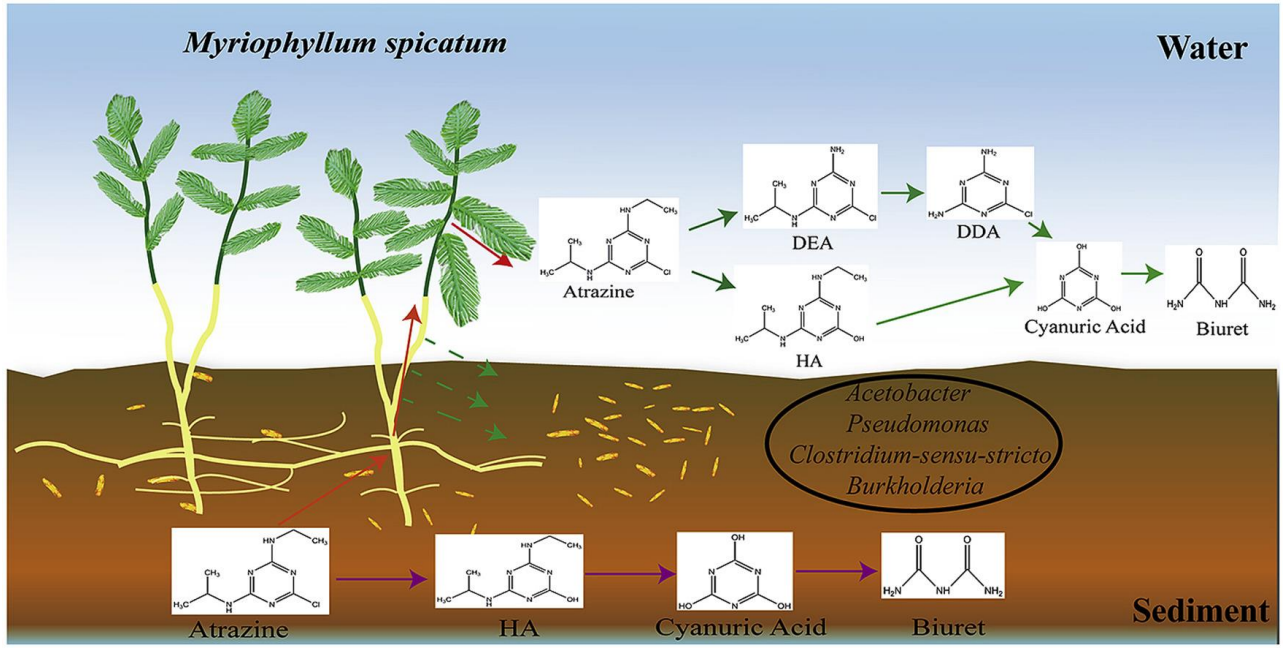
Komtchou et al., Water Research, 2017



Adsorption

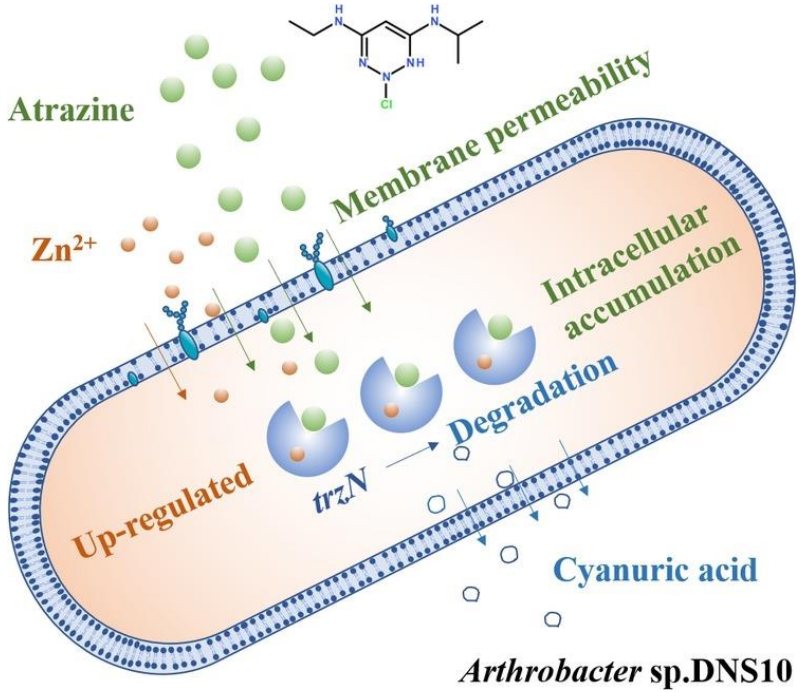
Cao et al., J. Environ. Chem. Eng., 2021

Atrazine Biodegradation



Phytoextraction

Jiang et al., Chemosphere, 2020

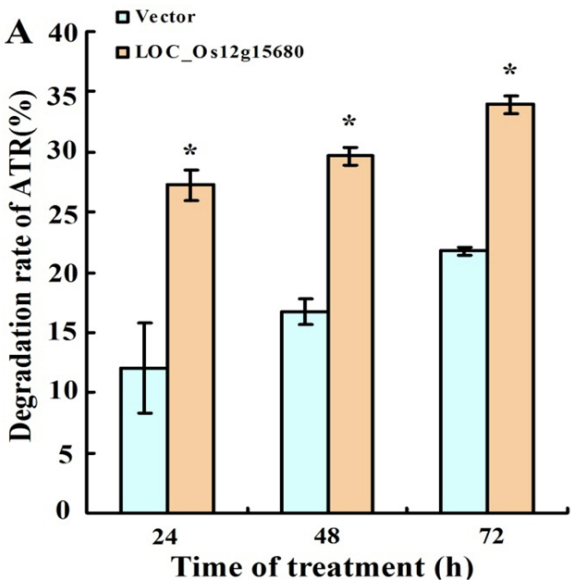


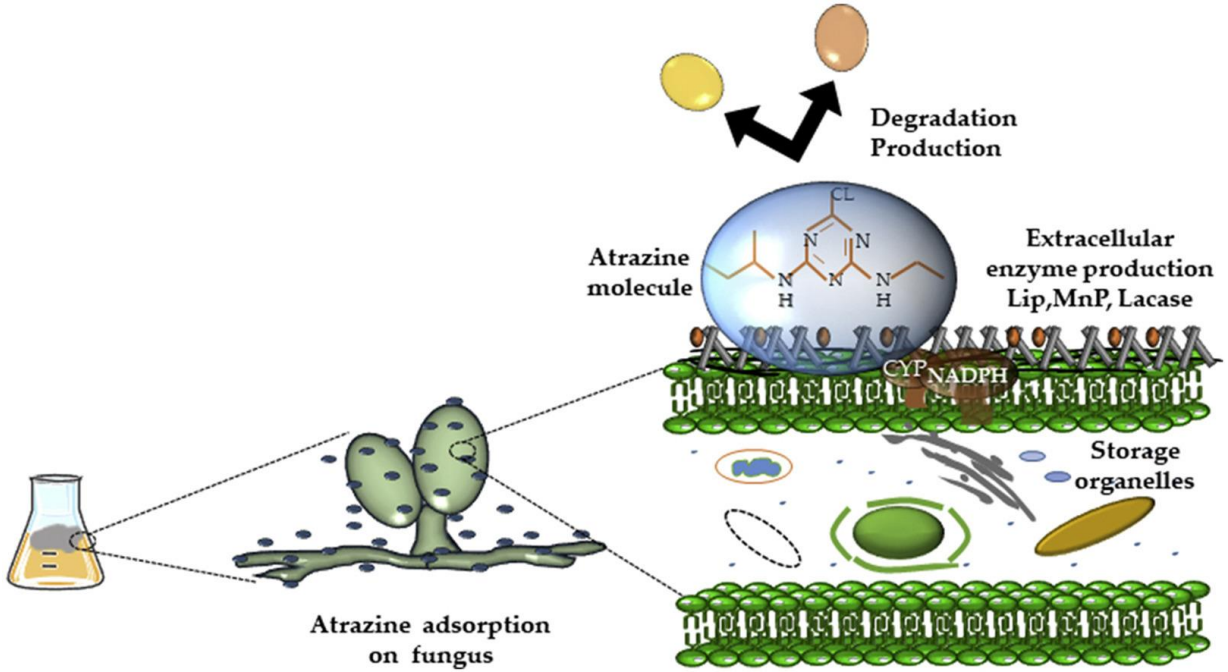
Bacteria

Qu et al., Chemosphere, 2018

Laccase-mediated Atrazine Degradation

- Free plant laccase only achieved 30% of atrazine degradation after 3 days.
- Fungi, *Bjerkandera adusta*, showed atrazine removal (not degradation).
- We used laccase from *Pycnoporus* sp. SYBC-L3, gifted from Huang Lab at University of Georgia.

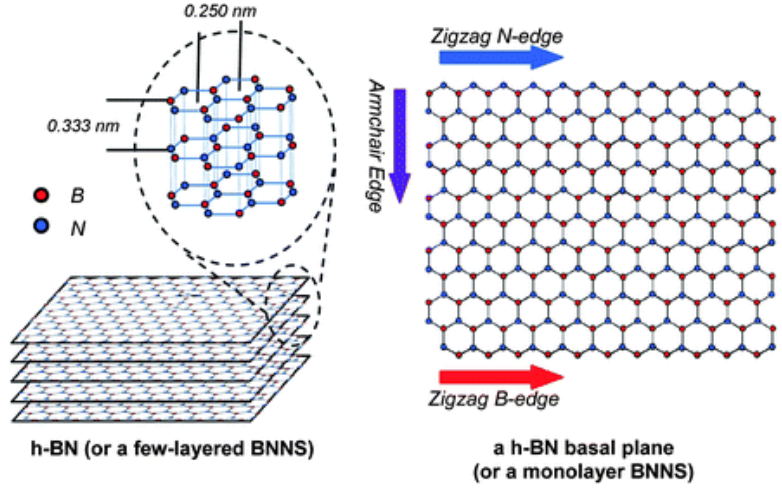


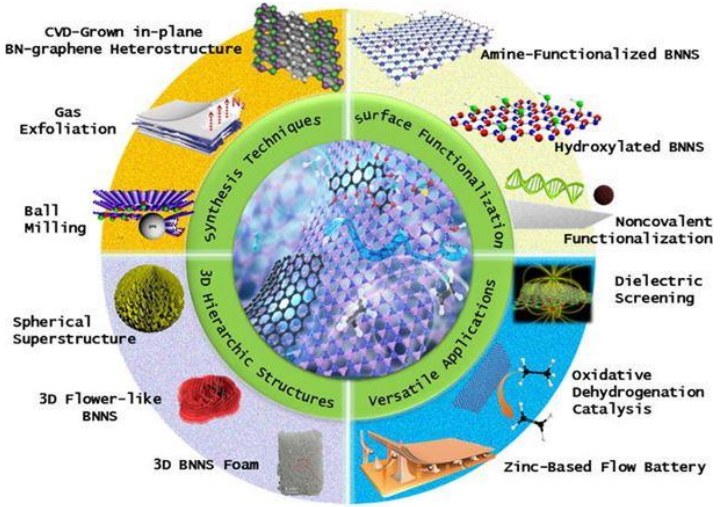


Dhiman et al., Chemosphere, 2020; Huang et al., JAFC, 2016

Boron Nitride as Enzyme Supporting Material

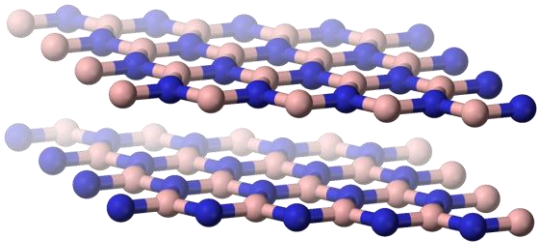
- Bulk hexagonal boron nitride (hBN):
  - Insoluble in water
  - Lower access to enzymes and contaminants
- Boron nitride nanosheet (BNNS):
  - Soluble
  - High surface area: ~2600 $m^2 g^{-1}$; bulk hBN: ~10 $m^2 g^{-1}$ (Rasul et al., 2021)
  - High biocompatibility (Li et al., 2014)
  - Inert (Li et al., Chem. Commun., 2014)
- Boron nitride nanosheets need to be functionalized for enzyme immobilization





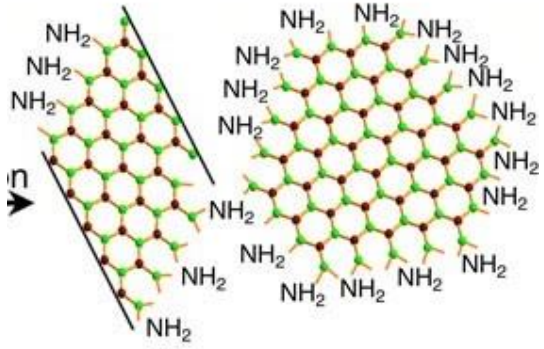
Lin and Connell, Nanoscale, 2012; Dibaji et al., Materials Today, 2020

BNNS for Laccase Immobilization



Boron nitride nanosheets
"White Graphene"

Inert
Biocompatible
Widely used in protein drug delivery



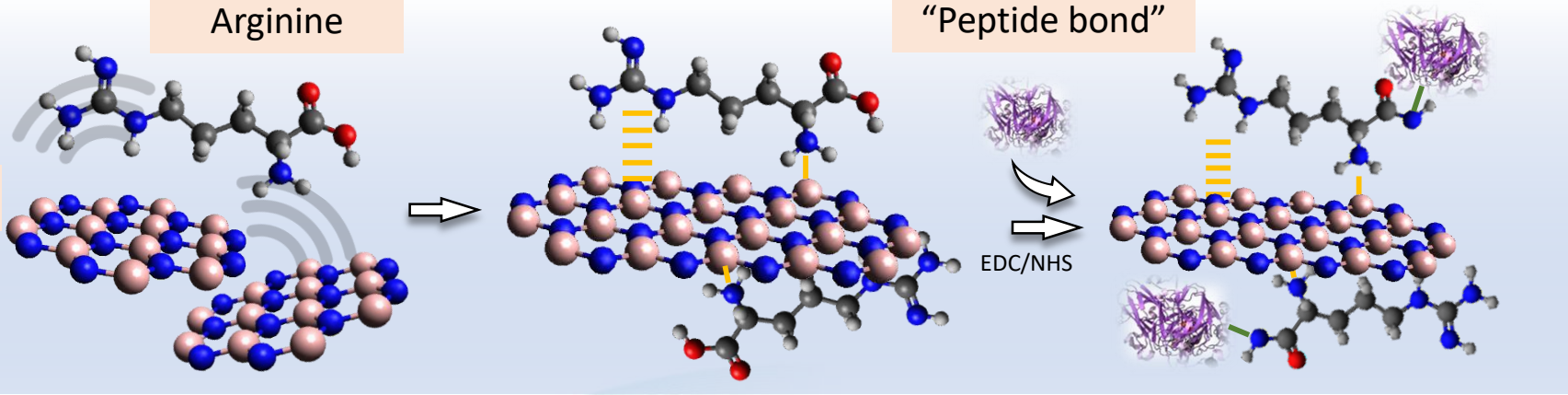
(Lei et al., Nat. Commun, 2015)

But need proper functionalization

Arginine

"Peptide bond"

Ultrasonication

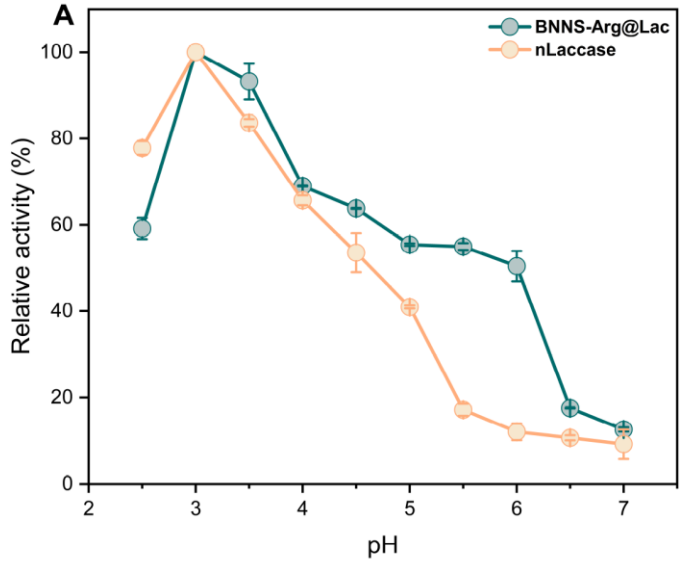


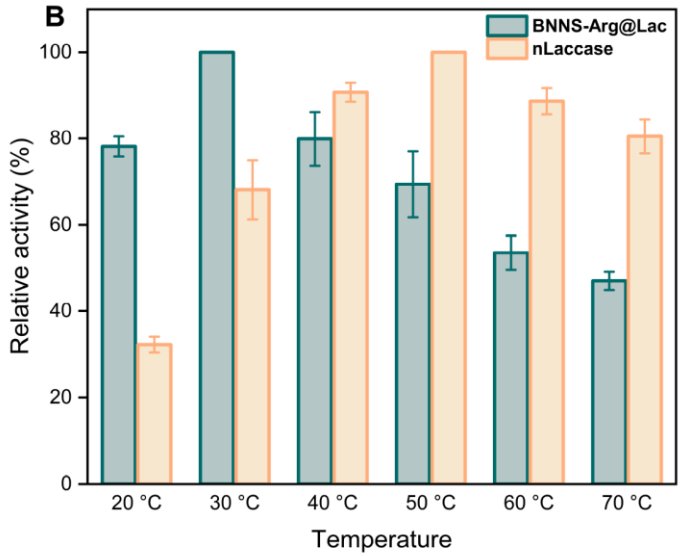
Gao et al., ES&T 2024

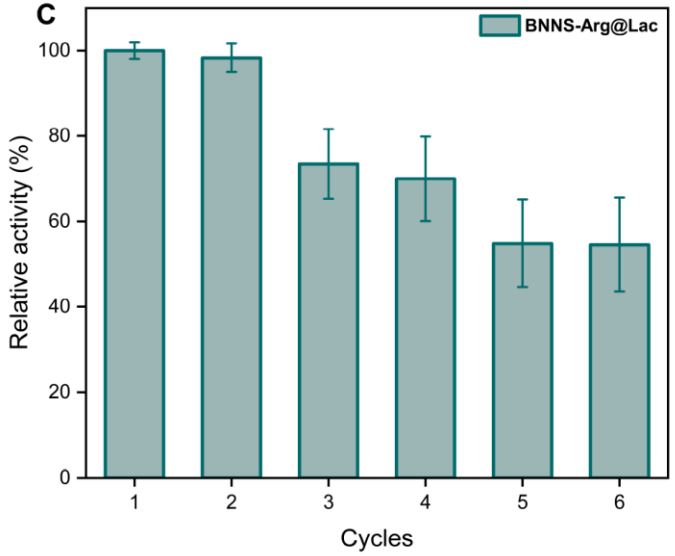
Stability and Reusability of BNNS-Arg@Lac

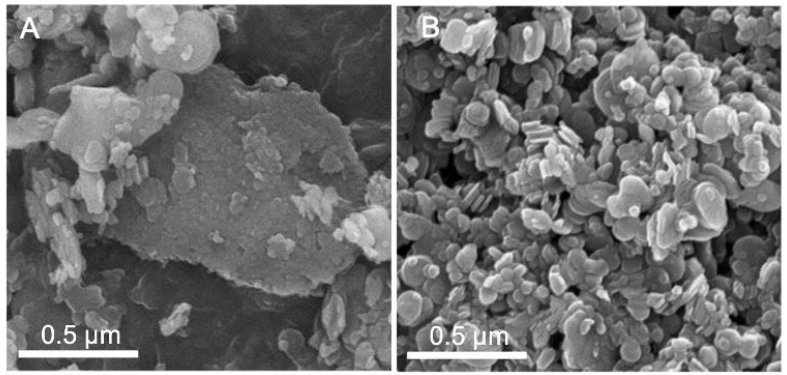
BNNS-Arg@Lac

- retained higher activity at near neutral pH
- exhibited higher activity around 30 °C
- can be used for up to 6 consecutive cycles

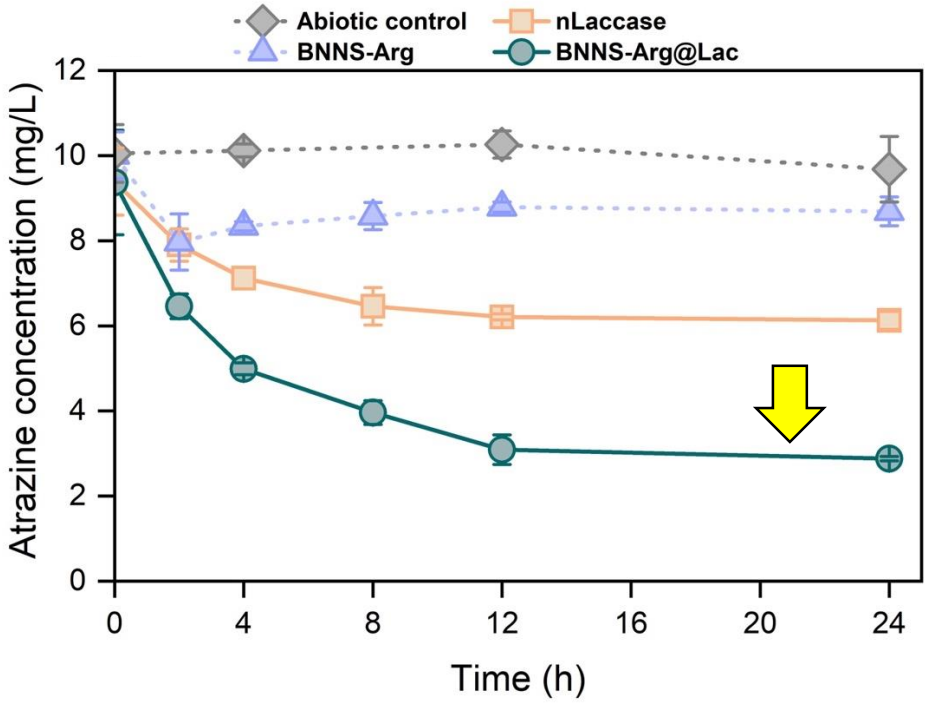




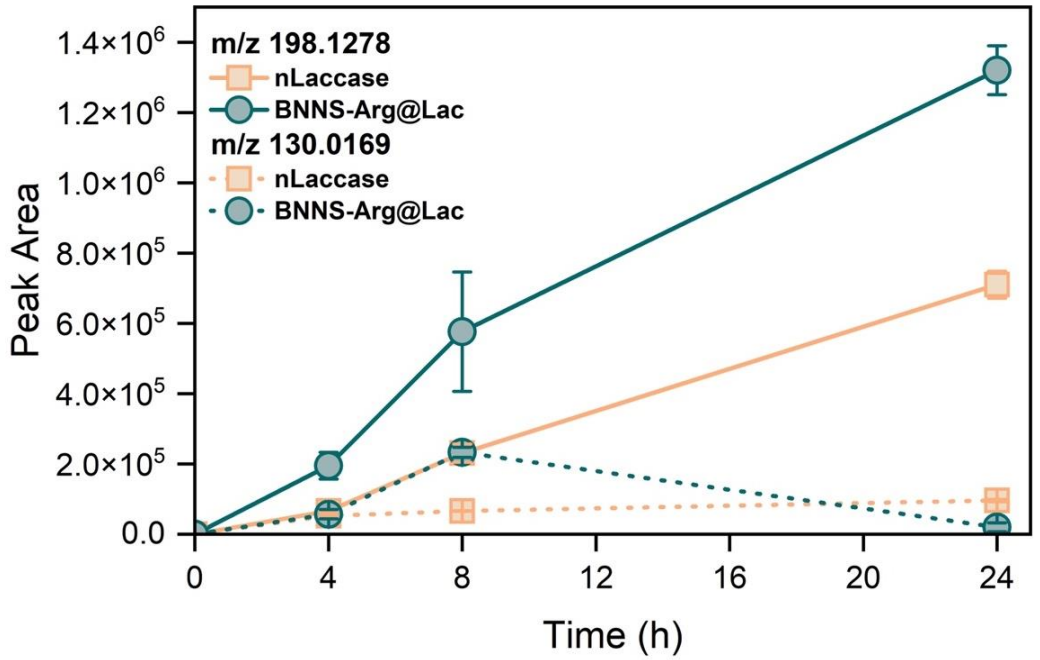




Higher Atrazine Degradation by BNNS-Arg@Lac

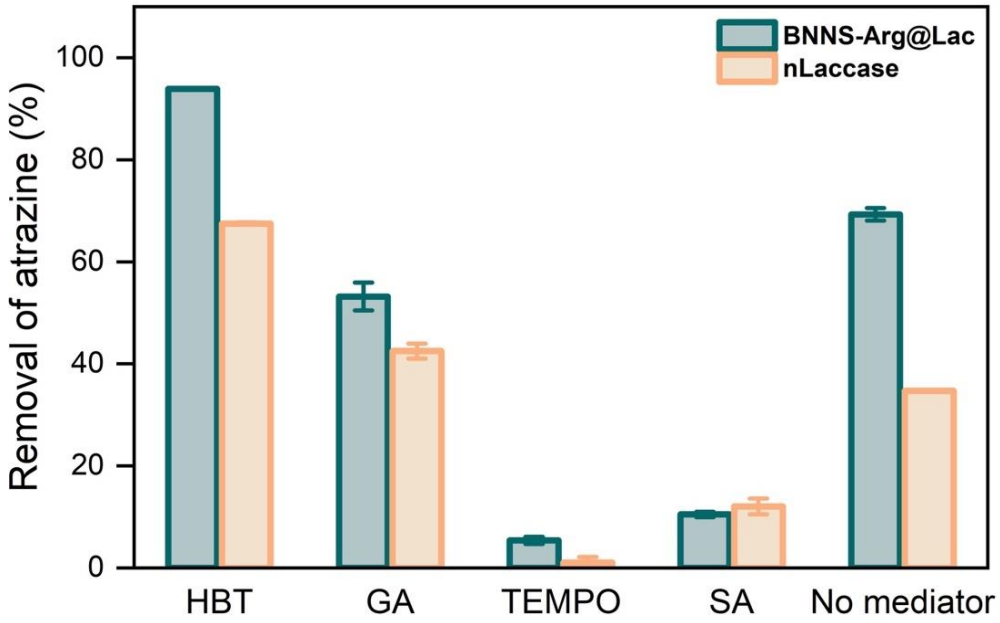


Atrazine degradation rate

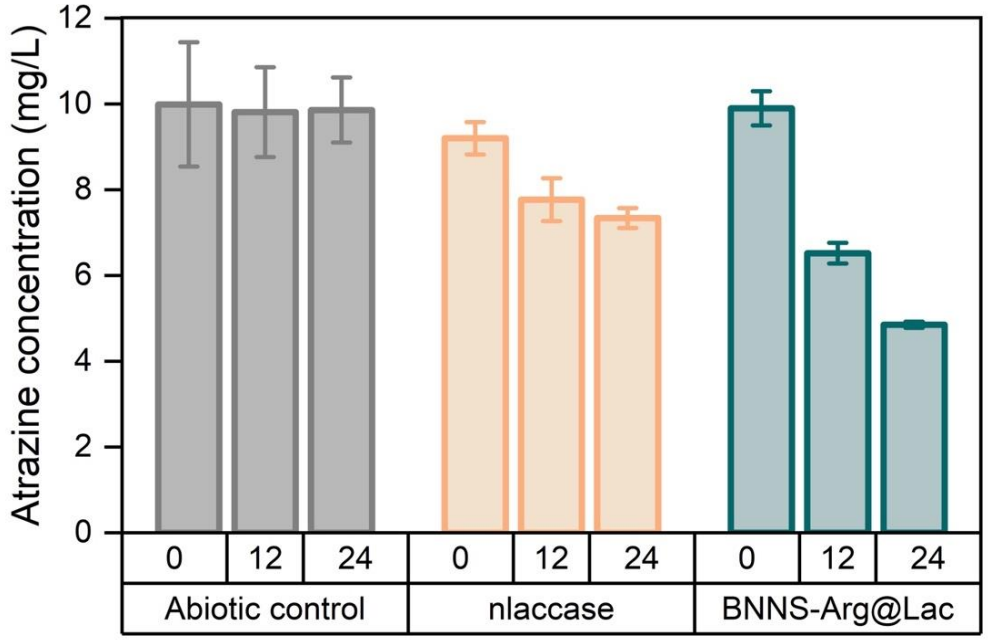


Product yield

Atrazine Degradation in Agricultural Wastewater

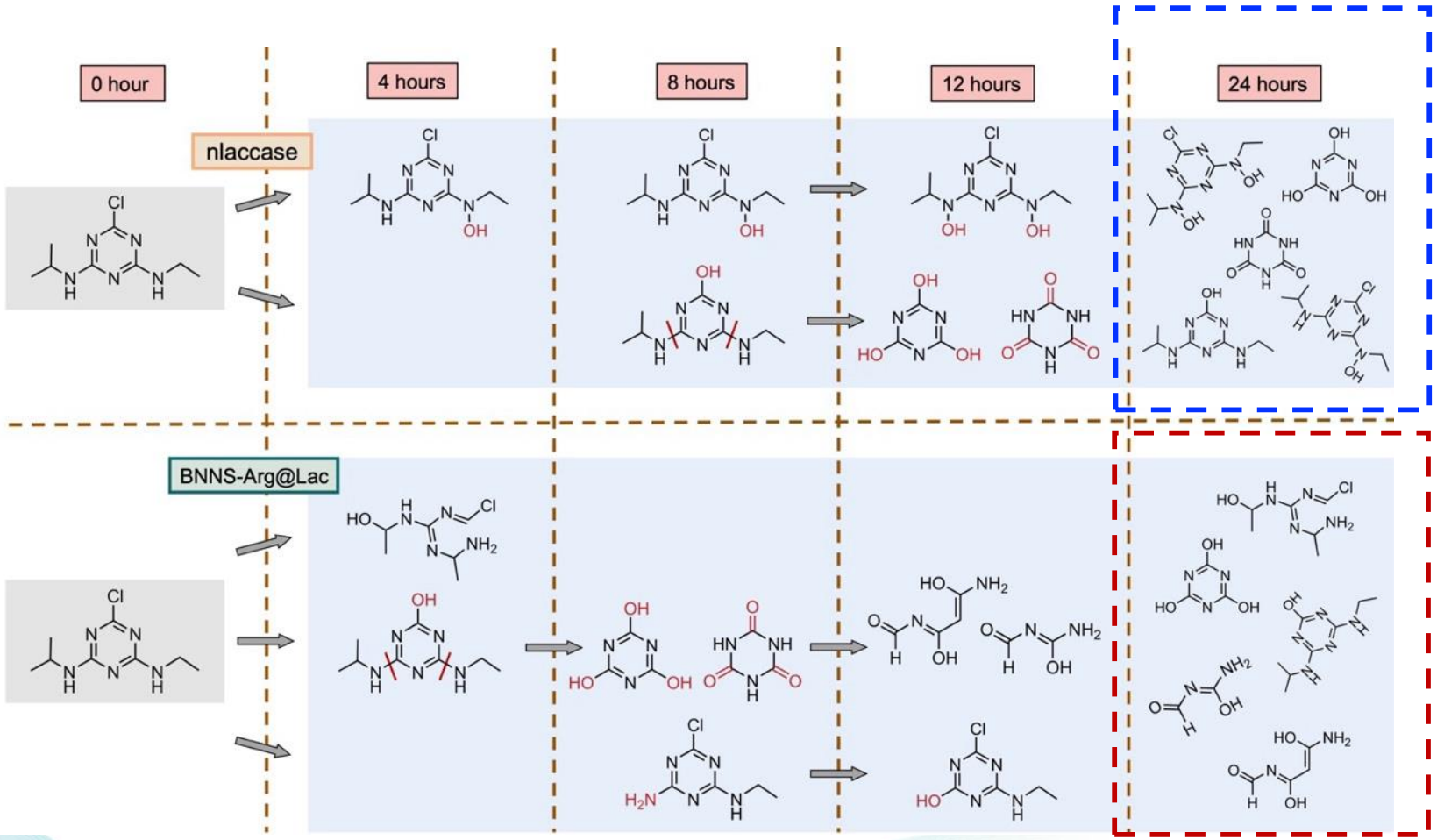


Different mediators

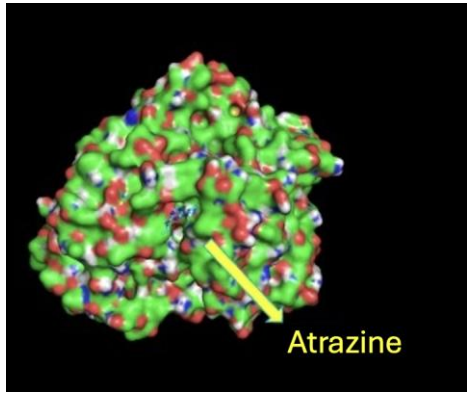


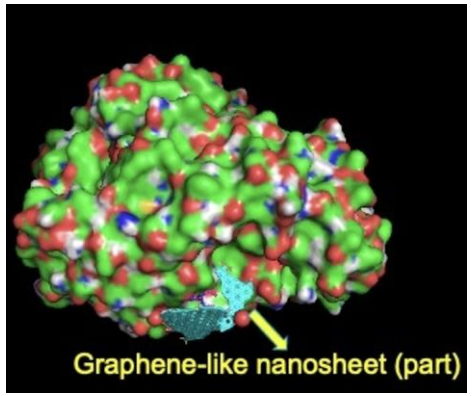
Synthetic agricultural wastewater

Biodegradation Products



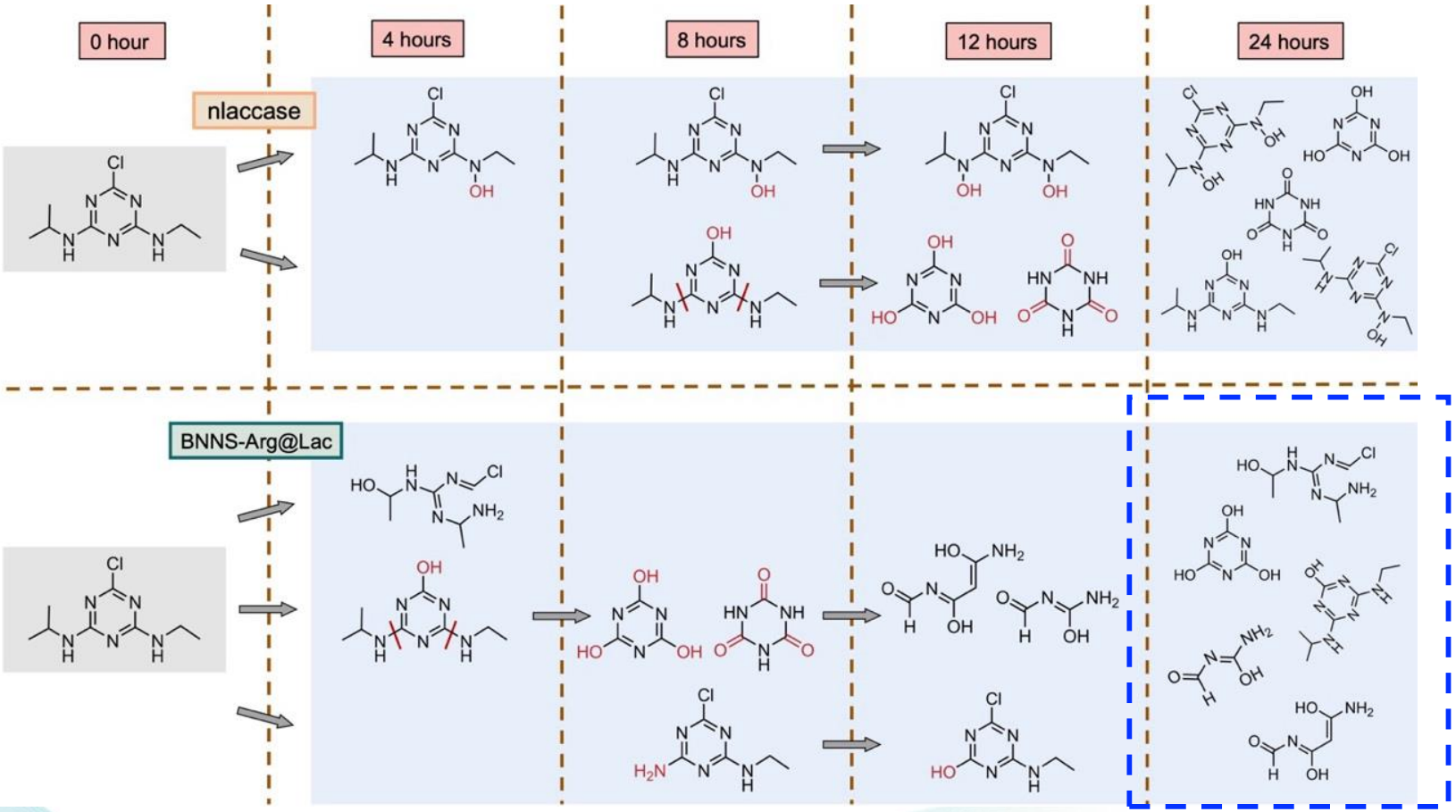
Different end product profile

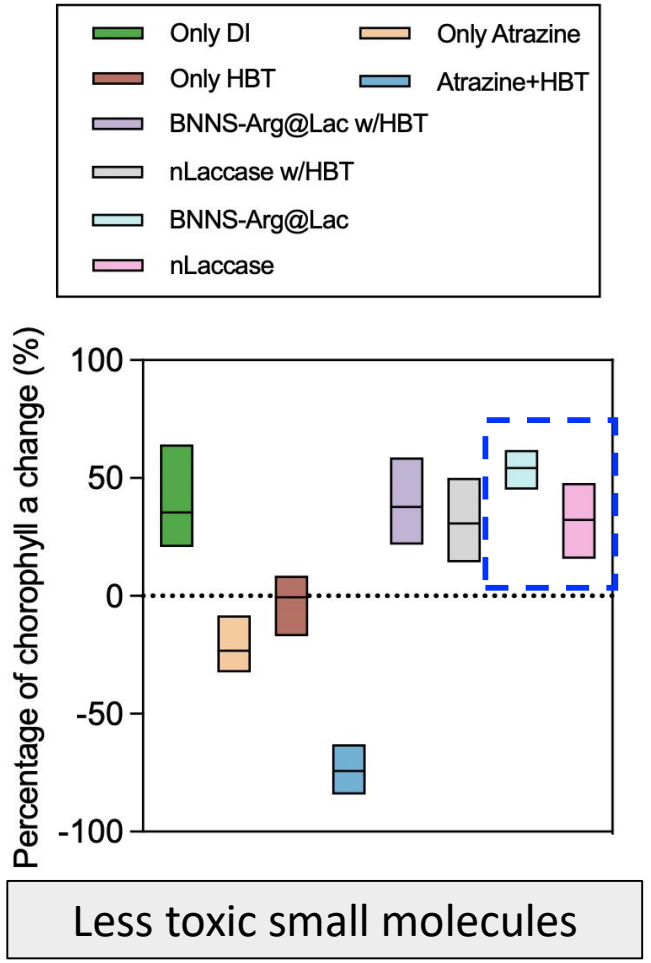




Molecular docking

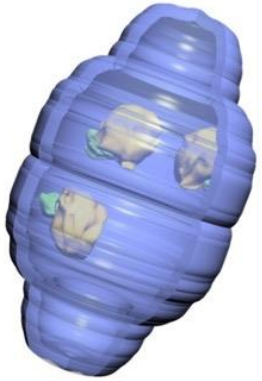
Degradation leading to Detoxification

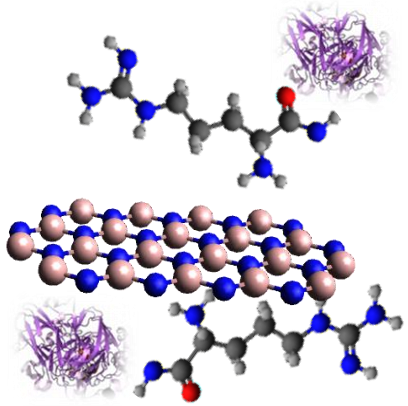




Less toxic small molecules

Summary and Significance





- Innovative enzyme immobilization platforms
  - Vault nanoparticles
  - Functionalized BNNS
- Both demonstrated enhanced stability, catalytic activity, and reusability.
- Can be multiplexed for contaminant mixtures or intermediates in biodegradation pathways
- Generally energy- and cost-efficient for production and scaling up
- Combined with abiotic technologies for industrial processes and water treatment