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BIO MATERIALS INTERFACES

circular bioengineering

Oxidizing Recalcitrant Hydrocarbons with Bacterial Laccase *PIL*

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INTRODUCTION

Environmental samples harbor diverse microbial enzymes that can power a circular economy by selectively transforming and depolymerizing materials, reducing our reliance on fossil-based resources. However, enzymatic depolymerization of recalcitrant, hydrocarbon-rich materials remains a major bottleneck for circular polymer systems. We addressed this gap by studying a novel bacterial laccase *PIL* from *Parvibaculum lavamentivorans*, discovered in contaminated field soil, that exhibits oxidative activity toward long-chain hydrocarbons relevant to polyolefin-derived materials [1].

LACCASES

- **Multicopper oxidoreductase**
- Function: One-electron oxidation of substrates coupled with the reduction of O_2 to H_2O
- Exhibit a broad substrate range, usually phenols and aromatic amines from lignin and humic acids
- Laccase-Mediator system (LMS) allows for oxidation of substrates that are incapable of fitting into the active site or have a high redox potential

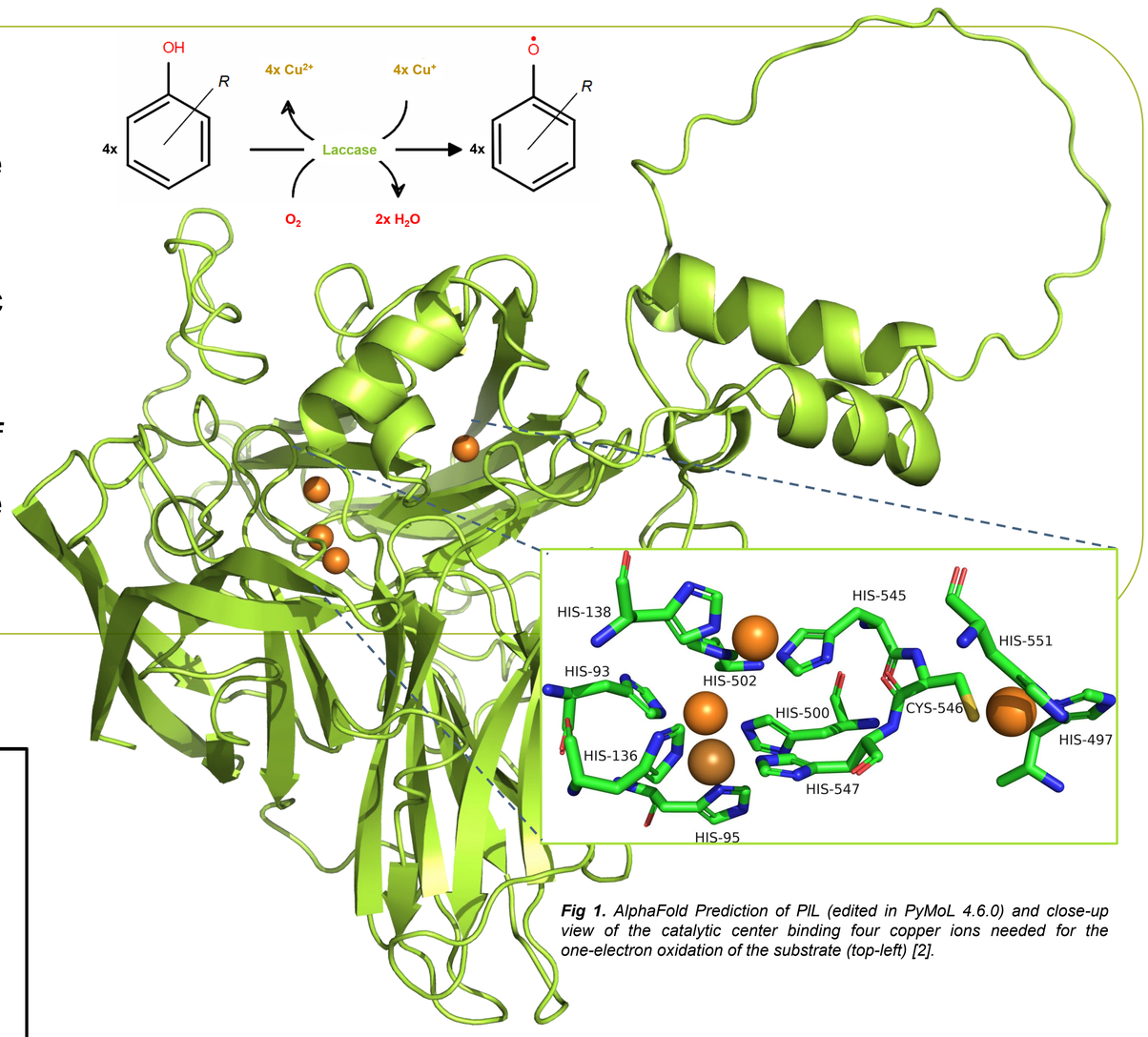

Fig 1. AlphaFold Prediction of PIL (edited in PyMoL 4.6.0) and close-up view of the catalytic center binding four copper ions needed for the one-electron oxidation of the substrate (top-left) [2].

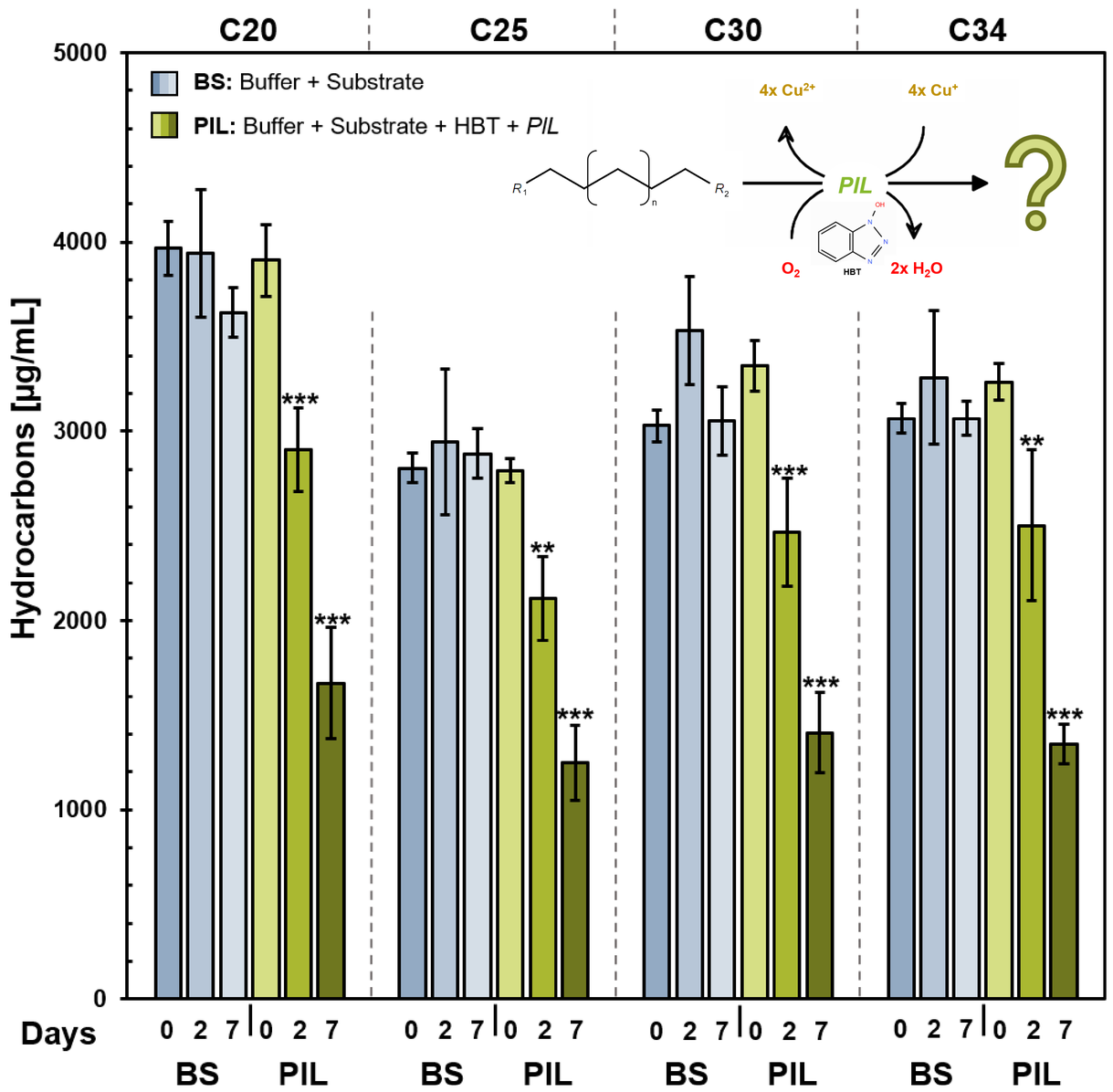

*Fig 2. Weight Loss of different long-chain alkanes (eicosane, pentacosane, triacontane, tetratriacontane) treated with PIL (0.33 U/mL) and mediator HBT (1-hydroxybenzotriazole) after 7 days. Reaction was performed in 0.1M Sodium Phosphate Buffer (pH 6) and 2mM HBT at ambient temperature. Samples were taken after 0, 2 and 7 days and measured via GC-FID. Measurements were conducted in triplicates and data are presented as mean ± standard deviation (n=3). Asterisks indicate statistically significant difference between PIL and BS within a given day (***p < 0.001, **p < 0.01, *p < 0.05, test: two-way ANOVA).*

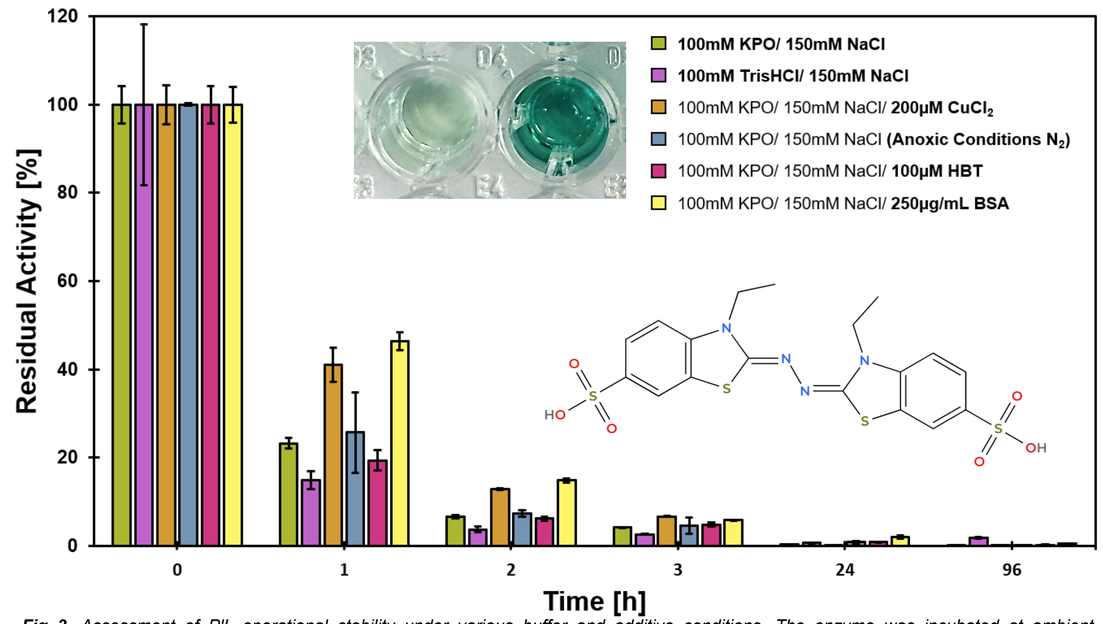

Fig 3. Assessment of PIL operational stability under various buffer and additive conditions. The enzyme was incubated at ambient temperatures and the residual catalytic activity was quantified by monitoring the initial rate of ABTS oxidation to its radical cation (blue-shift, 420nm) spectrophotometrically. All data are normalized to the initial activity at t=0 and presented as mean ± standard deviation (n=3).

CONCLUSION & OUTLOOK

- *PIL* treatment of long-chain alkanes resulted in up to **50% weight loss**
- GC-MS and HPLC could not identify discrete reaction intermediates, further analysis needed to **elucidate the enzymatic pathway**, e.g. LC/MS-ToF
- *PIL* exhibits considerable instability ➔ focus on enhancing enzyme stability and activity through fusion with substrate-binding modules and immobilization [3]

References:

[1] T. Diefenbach, Laccase-mediated degradation of petroleum hydrocarbons in historically contaminated soil. Chemosphere, 348 (2024) 140733.

[2] A. C. Sousa, Laccases: Versatile Biocatalysts for the Synthesis of Heterocyclic Cores. Molecules 2021, Vol. 26, 26(12).

[3] D. Ribitsch, Fusion of binding domains to Thermobifida cellulosilytica cutinase to tune sorption characteristics and enhancing PET hydrolysis. Biomacromolecules, 14(6), (2013), 1769–1776.

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