Breaking the Yield Barrier in Bioplastic Degradation:
Doubling CLE Enzyme Production for Industrial Hydrolysis

Summary

In the push toward a more sustainable future, the ability to efficiently degrade bioplastics like polylactic acid (PLA) is essential. However, translating laboratory discoveries into industrial-scale solutions is often limited by the high volume of enzymes required for effective degradation. This white paper details how MNDL Bio's proprietary AI-driven optimization enabled a two-fold increase in the expression of Cutinase Like Enzyme (CLE), overcoming significant biophysical hurdles that had previously restricted production.

The Challenge: Scaling Sustainable Bioplastic Recycling

The enzyme CLE, derived from the yeast Cryptococcus sp. S-2, is a powerful tool for degrading PLA. To be commercially viable, these enzymes must be produced at high concentrations and very low costs, as PLA degradation requires significant enzymatic loads.

The production of CLE presents specific technical difficulties:

- Structural Complexity: CLE contains two critical disulfide bonds, necessitating an oxidizing environment for proper folding and activity.
- Cellular Location: Efficient production requires directing the protein to the periplasm of E. coli using signal sequences like pelB.
- Existing Plateaus: Previous optimization efforts, including utilizing specialized E. coli SHuffle cells and periplasmic targeting, had already achieved a 100-fold improvement over standard systems, leaving little room for further gains using traditional methods.

The MNDL Bio Approach: Predictive Biophysical Modeling

Standard codon optimization tools often fail at high-titer requirements because they rely on simple Codon Adaptation Index (CAI) frequencies. This approach ignores the physical reality of the cell, such as the speed of the ribosome and the stability of the mRNA transcript.

MNDL Bio’s platform, built on 15 years of academic research, utilizes deep biophysical simulations to resolve expression bottlenecks. For the CLE project, our engine focused on:

- Ribosomal Trafficking: Synchronizing translation speed with the protein’s complex folding needs to ensure functional disulfide bond formation.
- mRNA Structure: Optimizing the transcript to prevent inhibitory secondary structures near the start codon.
- Signal Peptide Integrity: Evaluating the impact of synonymous changes within the pelB leader sequence to maintain efficient targeting to the periplasm.

MNDL Bio generated 10 unique variants: five preserved the original pelB sequence, while five included optimized modifications to the signal sequence.

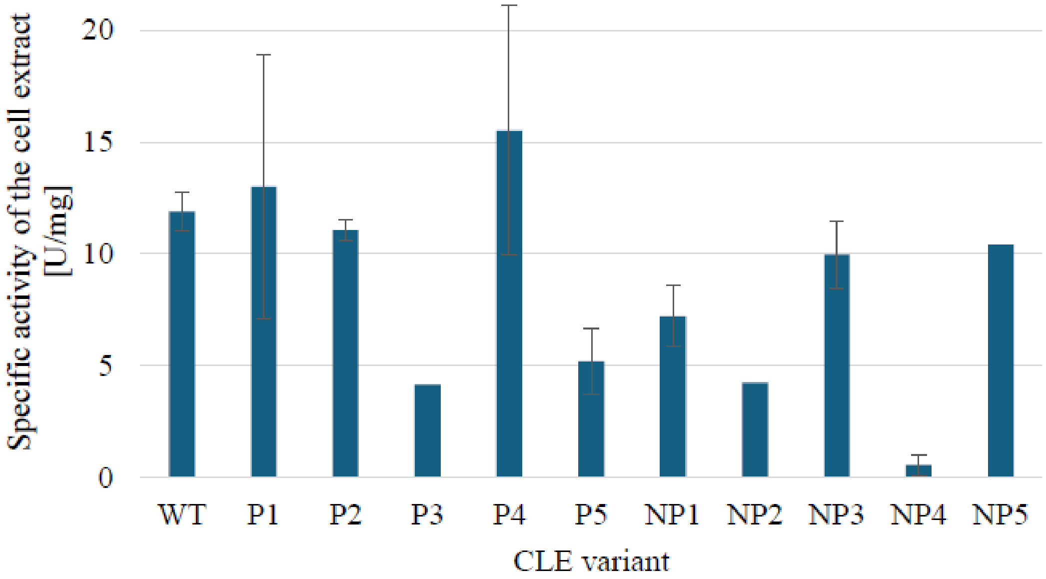
Results: Quantitative Validation of Yield Increases

The optimization process followed a structured protocol to move from initial screening to precise quantification.

Phase 1: Initial Activity Screen

All 10 variants were expressed in E. coli SHuffle cells and assessed for specific activity.

Findings: Variants that kept the original pelB sequence (P variants) outperformed the wild-type (WT).



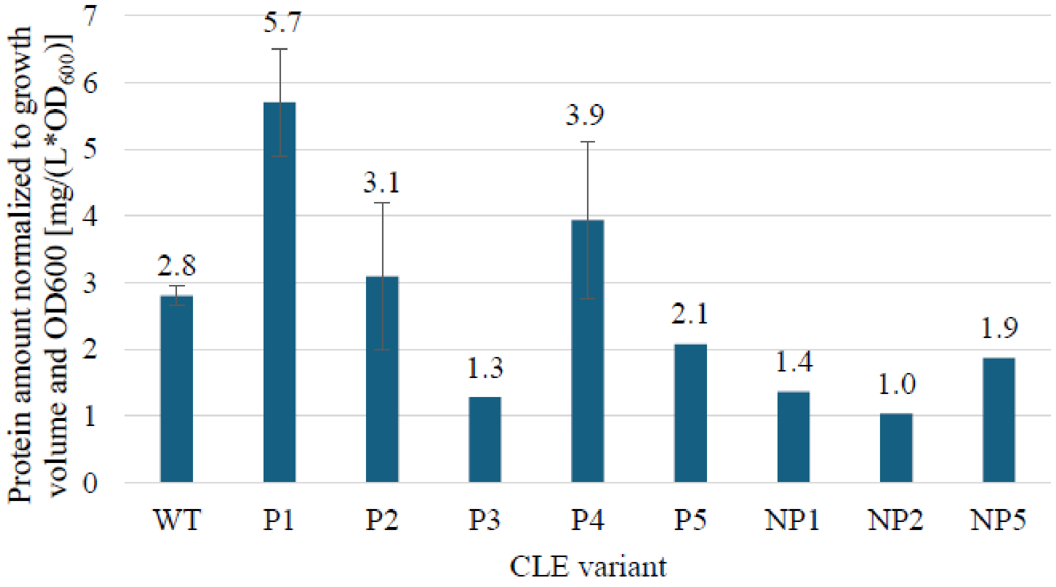
Graph 1.0: Specific activity of CLE variants in soluble cell extracts

Phase 2: Purified Protein Measurement

The best-performing variants were purified using Ni+2 affinity chromatography for exact quantification. To ensure accuracy, protein amounts were normalized to growth volume and cell density (OD600).

Key Quantitative Findings:

- Baseline (WT) Yield: 2.8 mg/(L*OD600).
- MNDL Bio (P1) Yield: 5.7 mg/(L*OD600).
- Total Improvement: +100% (2-fold) increase in normalized enzyme yield.



Graph 2.0: Purified CLE levels normalized to volume and cell density

Future Perspectives: Deeper Optimization for Global Scale

Achieving a 2-fold increase in a single iteration is a significant milestone, especially given the existing high-performing baseline. This initial project utilized MNDL Bio's online platform to focus on coding sequence optimization.

In expanded partnerships, MNDL Bio pushes titers even further by targeting the full genomic context:

- Non-Coding Region Engineering: Optimizing promoters and UTRs to fine-tune translation initiation.
- System Calibration: Training AI models on specific industrial strains to identify unique cellular constraints.
- Genomic Integration: Pinpointing the most stable chromosomal locations for long-term production.

Conclusion

The results of this collaboration demonstrate that even when traditional optimization methods reach their limit, biophysical modeling can unlock additional potential. By doubling the production yield of CLE, MNDL Bio has provided a clear pathway for the cost-effective, industrial-scale degradation of bioplastics.

We have replaced trial-and-error with predictive design, moving sustainable biotechnology from the research lab to commercial reality.