# Engineering of a Microbial Host for the Secretion of Biomass-Degrading Enzymes

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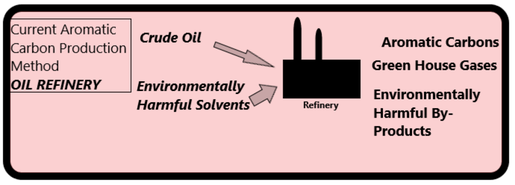
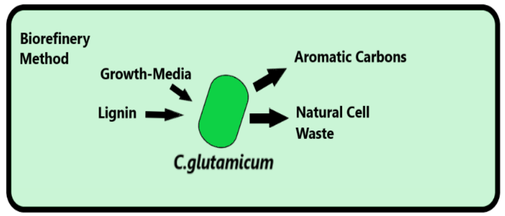
## Purpose

- Lignin, a natural biopolymer found in plants is composed of many aromatics
- This project aims to tap into lignin as a sustainable source of aromatic carbons in order to reduce reliance on petroleum-sourced aromatics in industries



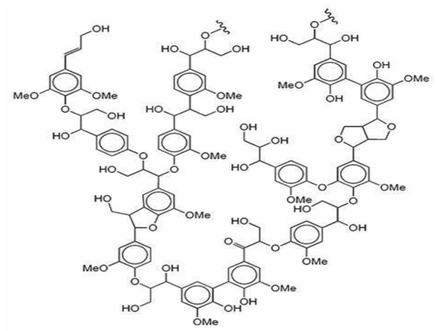



*Figure 1: Diagram of Lignin*

## Why *C. glutamicum*

- natural resistance to aromatic carbons,
- Industry proven
- Utilizes aromatics in its metabolism

## Theory

- Plasmids contain genes that code for peroxidases and laccases
- Exports peroxidase and laccase out of the cell
- Signal peptides coded in plasmid to allow for exportation of enzymes

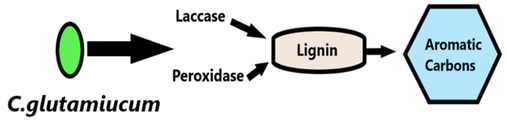



Figure 2: *C.glutamicum producing laccases and peroxidases for the depolymerization of lignin into aromatic carbons*

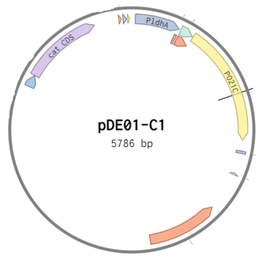



*Figure 3: Example of plasmid containing enzyme coding genes and signal peptides*

## Procedure

- Plasmids Sequencing Verified
- Goal is to optimize enzyme expression
- Growth Media analysis
- pH analysis
- Quantification by OD absorbance reader

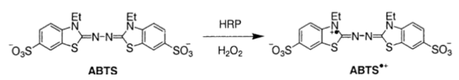



*Figure 4: Reaction of ABTS with Horseradish Peroxidase and hydrogen peroxide. The product of this reactant is dark blue in color*

## Results

- No enzyme activity present
- Proteins may not be folding
- Unwanted reactions occurring in cell supernatant
- Certain medias inhibiting enzyme activity
- pH test inconclusive
- Future testing of components required

## ABTS Assay and Graphs

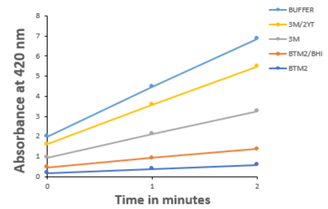



*Figure 5: ABTS assay absorbance scans of Horseradish Peroxidase activity in different growth medias.*

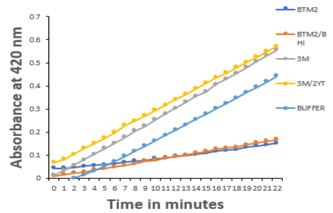



*Figure 6: ABTS assay absorbance scans of Aspergillus' Laccase in different growth medias.*

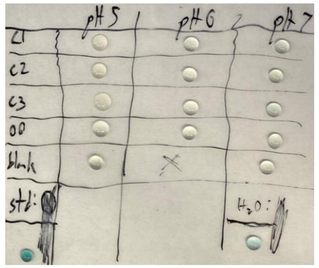



*Figure 7: ABTS drop assay of C. glutamicum strains in BTM growth media at different pH levels*

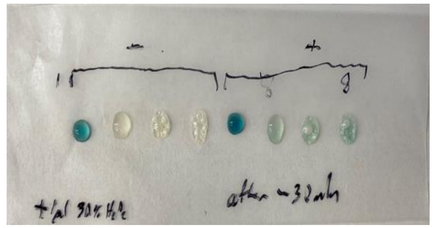


*Figure 8: ABTS drop assay of spent growth media, used by C. glutamicum strains, waster, and new growth media*

## Future Testing

- More testing of the growth media components
- Used growth media will be characterized by GCMS and HPLC
- Growth Assays

## Acknowledges

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FURI

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