Production of rMnP from Yeast Strains for Biofuels Processing

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Big Picture

Why produce manganese peroxidase?

- Manganese peroxidase can be used to create biofuels from waste biomass.
- Small scale production of rMnP enzyme for analysis (our project).
- Produce enzyme in larger, more concentrated quantities for industrial processing.

Introduction

Manganese peroxidase (MnP) (Figure 1) facilitates the lignin degradation by oxidizing the manganese ion which acts on bonds in the lignin molecule through radical formation. The active degradation of lignin requires H_2O_2 and a heme molecule properly folded within the enzyme.

Figure 1: Manganese peroxidase structure

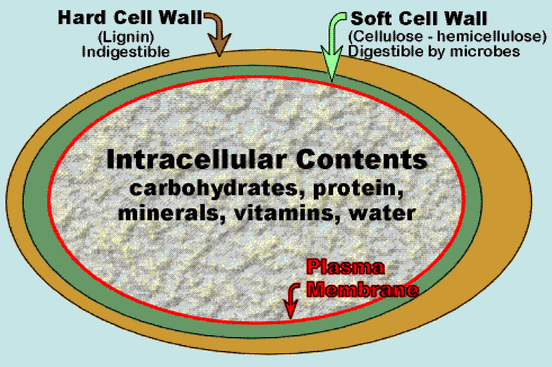

Figure 2: Biomass cell schematic (University of Guelph, Ontario Canada)

Soft Cell Wall: cellulose and hemicellulose can be fermented and refined to produce biofuels.

Hard Cell Wall: Lignin gives structure to the biomass but is indigestible and must be degraded by MnP.

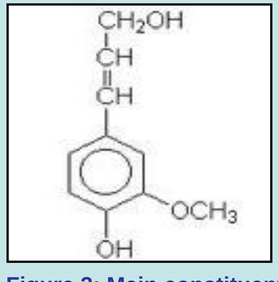

Figure 3: Main constituent in lignin structure.

Objectives

- Produce rMnP in the bioreactor with selected yeast strain (secreting and peroxisome targeted) of 1000 U/L of activity.
- Design a protocol to lyse and investigate newly engineered peroxisome targeted strain.
- Ensure enzyme does not degraded during lysis (sonicator shown in figure 4).
- Produce rMnP for industry.

Figure 4: Sonicator used for lysis of yeast cells

Methods

The manganese peroxidase (MnP) gene was cloned into the yeast, *Pichia pastoris,* with a signal that directs the enzyme to be secreted into the culture broth (***secreting strain***) This strain was grown in the bioreactor (figure 5) at specific conditions to produce rMnP.

Figure 5: Bio-reactor with controller.

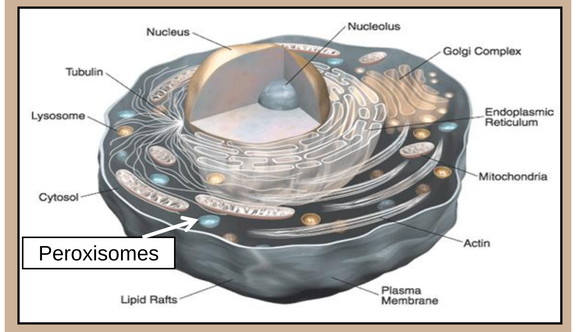

Figure 6: Cell schematic showing organelles (Life Technologies Department of Fluorescent Imaging)

Newly engineered strain contains an alcohol oxidase promoter and uses a methanol media to promote the produced rMnP to be targeted to the peroxisomes (***peroxisome targeted***). The peroxisomes are expected to be a more protected location for the enzyme until harvesting. To harvest rMnP the cells must be lysed.

Sampling and Testing

A sample was collected from the bio-reactor every 4-6 hours and the cell density and enzyme activity was tested by taking the absorbance (A) as shown in Equation 1 and 2 . During fed batch growth, heme was added to allow for enzyme production.

Equation 1:

$$Cell\ Density\ \left(\frac{g}{L}\right) = 0.3 * (A@600\ nm) * (Dilution\ Factor)$$

Equation 2:

$$Activity\ \left(\frac{U}{L}\right) = \left[\frac{A@469\ nm * Assay\ Vol. * 10^6}{49600 * Sample\ Vol.}\right]$$

Secreting Strain Cultivation

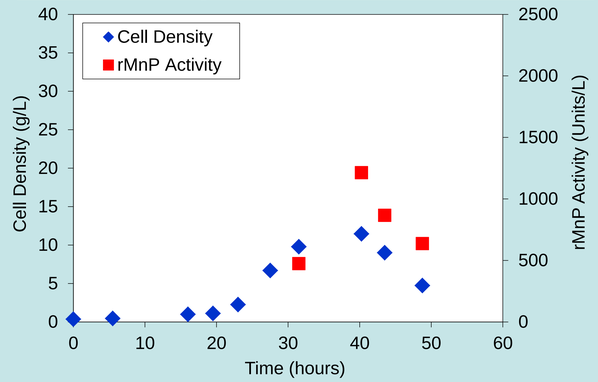

Figure 7: Bio-reactor run with secreting strain on glucose. Maximum enzyme activity was1200 U/L.

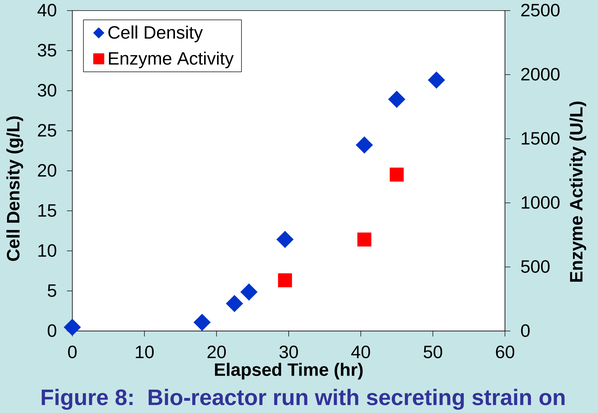

Figure 8: Bio-reactor run with secreting strain on glycerol and methanol. Maximum activity was 1200 U/L.

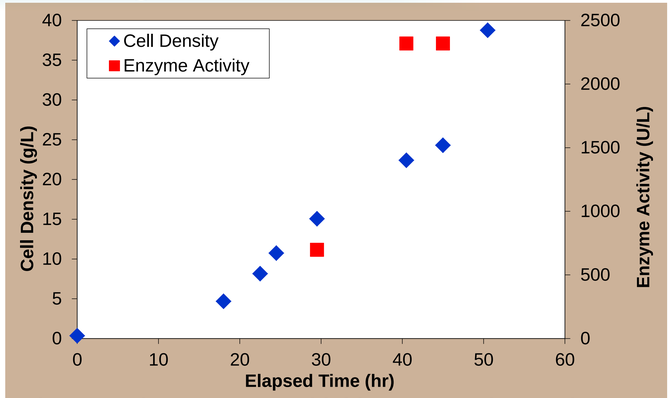

Figure 9: Bio-reactor run with his-tag secreting strain grown on glycerol and methanol. Maximum activity was 2300 U/L

Targeted Strain Investigation

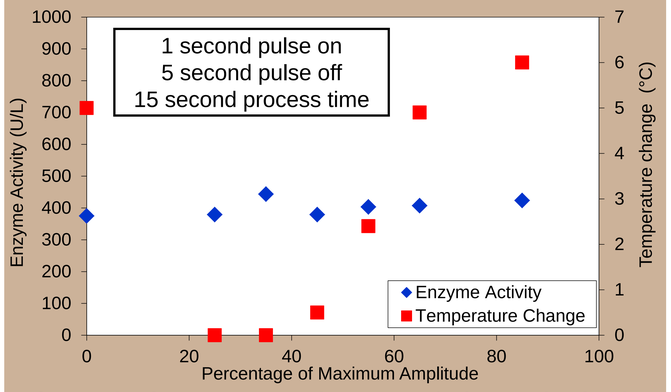

Figure 10: The temperature increases during sonication but the change does not degrade the rMnP at the range of sonication amplitudes tested.

Future Work

- Grow, purify and submit enzyme produced to Novozyme for characterization.

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Acknowledgements

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