Production of the Lignin Degrading Enzyme Recombinant Manganese Peroxidase in Bioreactors.

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Introduction

Lignocellulosic materials (e.g., wood, crop residues) will likely play an increasingly important role as raw materials for manufacturing and energy production in a sustainable environment. One of the major impediments to using these feedstocks is the presence of lignin. Sustainable biomanufacturing will require suitable methods for the removal of lignin in the process of separation, modification and conversion of lignocellulosic materials into manufacturing feedstocks and biofuels. Lignin is also a major source of carbon in soils. Understanding enzymatic degradation of lignin can facilitate the use of biomass for fuels and chemicals and help more clearly elucidate the role of soil carbon storage and release in the carbon cycle.

Many white-rot fungi (basidiomycetes) produce the native manganese peroxidase (nMnP) (Figure 1) enzyme to oxidize and depolymerize lignin in wood and soil. The breakdown of lignin allows the fungi to access the energetically and chemically rich cellulose and hemicelluloses needed to grow and reproduce. The catalytic cycle of the enzyme begins with the binding of H_2O_2 to form an iron-peroxide complex. Reduction of H_2O_2 oxidizes the iron-heme complex to a Fe^{4+} -oxo-porphyrin-radical, which in turn oxidizes Mn^{2+} available in wood and soil to chelated Mn^{3+} (Figure 2).

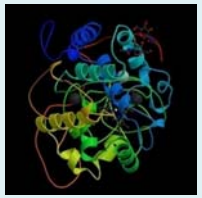
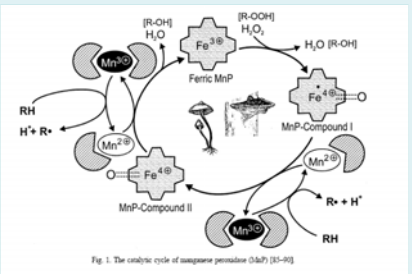


Figure 1. Crystal structure of MnP



Hofrichter, M., Review: Lignin conversion by manganese peroxidase (MnP), *Enzyme and Microbial Technology* 30 (2002), pp. 454-466

Figure 2. Lignin degradation catalyzed by MnP

The Mn^{3+}, chelated with organic acids such as oxalate or malonate, acts as a low molecular mass electron transfer mediator that can diffuse into the cell walls of host material and oxidize phenolic molecules by hydrogen and one-electron abstraction. Chelated Mn^{3+} cannot directly oxidize non-phenolic structures that constitute the majority of lignin, so other mechanisms involving lipid peroxidation have been proposed. In these mechanisms, the chelated Mn^{3+} oxidizes saturated or unsaturated carboxylic acids thereby forming peroxyl radicals or super oxide, which are oxidants strong enough to degrade C_α-C_β and β-aryl ether bonds in non-phenolic lignin.

Methodology

White rot fungi are the native producers of MnP however they produce low levels of MnP under stressed conditions which include nitrogen limitation and low nutrient levels.

As a result a gene encoding manganese peroxidase (mnp1) from the white-rot fungi *Phanerochaete chrysosporium* was cloned using a constitutive promoter into a protease deficient (pep4$^-$) strain of the methyltrophic yeast *P. pastoris*.

Figure 3. rMnP Bioreactor

P. pastoris was chosen because it grows to high cell density in industrial relevant bioreactors (figure 3 and figure 4), many cloning vectors are commercially available, and the recombinant protein is secreted into the culture broth and these traits are yield a promising method to produce commercially relevant quantities of rMnP.

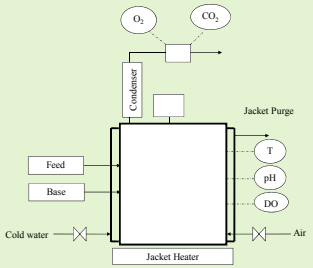


Figure 4. Schematic of Bioreactor

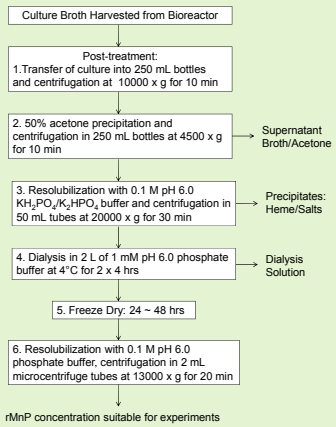


Figure 5. Flow Chart of Concentration Steps

Results

The effect of fed-batch temperature on cell growth and rMnP production was evaluated to determine the optimum temperature.

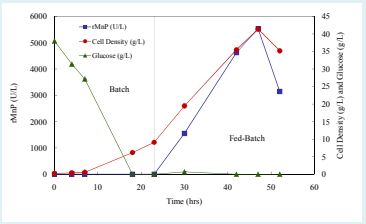


Figure 4. Production of rMnP in the bioreactor at optimum conditions which are batch temperature 30°C and fed–batch temperature 25°C

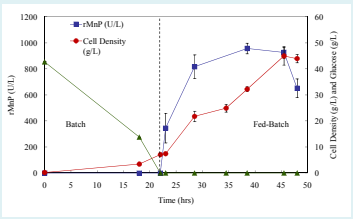


Figure 5. Production of rMnP in the bioreactor at non-optimum conditions with the batch temperature of 30°C and fed–batch temperature 30°C

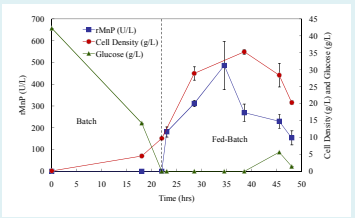


Figure 6. Production of rMnP in the bioreactor at non-optimum conditions with the batch temperature of 30°C and fed–batch temperature 35°C

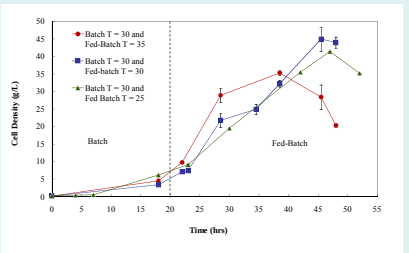


Figure 7. Comparison of *P. pastoris* cell density during bioreactor runs where the batch temperature was held constant at 30°C and the fed-batch temperature was altered to be 25°C, 30°C, or 35°C

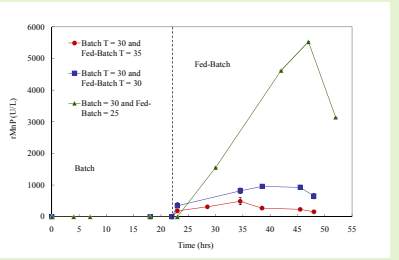


Figure 8. Comparison of *P. pastoris* rMnP production during bioreactor runs where the batch temperature was held constant at 30°C and the fed-batch temperature was altered to be 25°C, 30°C, or 35°C

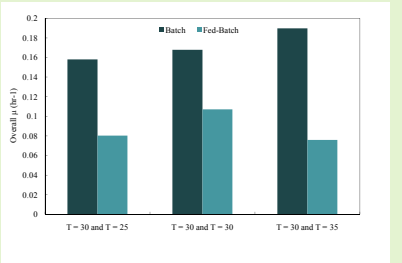


Figure 9. Comparison of the growth rates (μ) of *P. pastoris* in bioreactor runs where the batch temperature was held constant at 30°C and the fed-batch temperature was altered to be 25°C, 30°C, or 35°C

Conclusions

The optimum fed-batch temperature is 25°C because the highest yield of rMnP is achieved. rMnP has previously been shown to be thermally unstable which might be a possible reason for the lower yields at 30°C and 35°C.

Acknowledgements

SBI

This work was supported by Subsurface Biosphere Initiative