Co-synthesis of Bioenergy Proteins To Increase Microbial Biofuel Competitiveness

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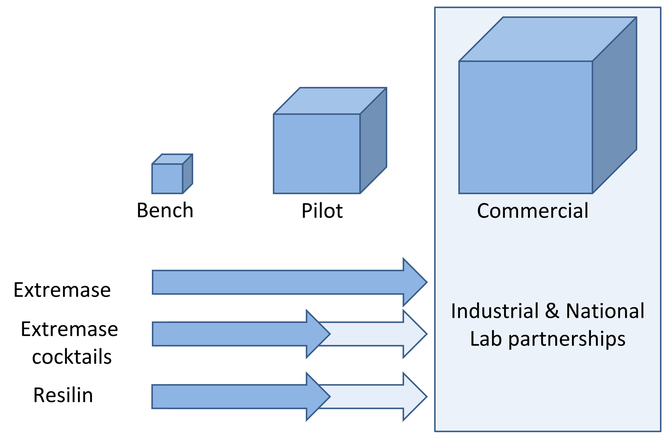
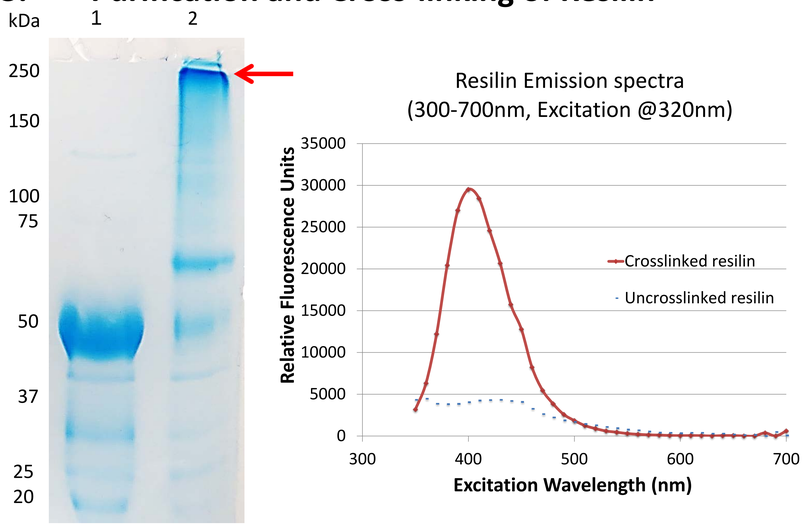
1. Abstract

To achieve sustainability, low value commodity products like biorenewable fuels and chemical feed stocks must be optimized for large scale production. Adding to the value train will facilitate this goal. Here we present initial efforts about the addition of co-product synthetic capacity to bioenergy relevant microbes that leverages previously low value added microbial protein. This project targets conventional algal-biodiesel and yeast-cellulosic ethanol using two proteins; Resilin and Extremase. Resilin is an efficient energy storage insect protein and Extremase is a hot acid-resistant cellulase. We have successfully made Resilin using an *E.coli* host expression system with a production yield of 6 grams per 10 L fermentation. Resilin is under evaluation as a biocomposite material by protein cross-linking to make hydrogels, thin films and 3D structures. Extremase production employs an *S. cerevisiae* secretion system and has been combined with related enzymes to achieve a more potent hydrolytic cocktail for lignocellulose saccharification. Further increases in production scale are underway notably at 80 L fermentations. The resulting materials will provide the basis for studies on protein biomechanics and collaborative interactions with regional industries and US National Laboratories.

2. Stages in Bioprocess Scaling

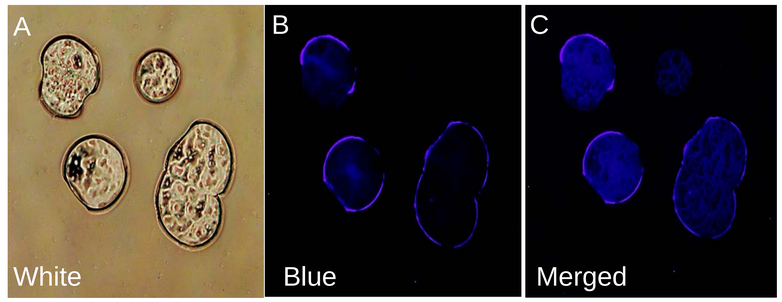


3. Purification and Cross-linking of Resilin



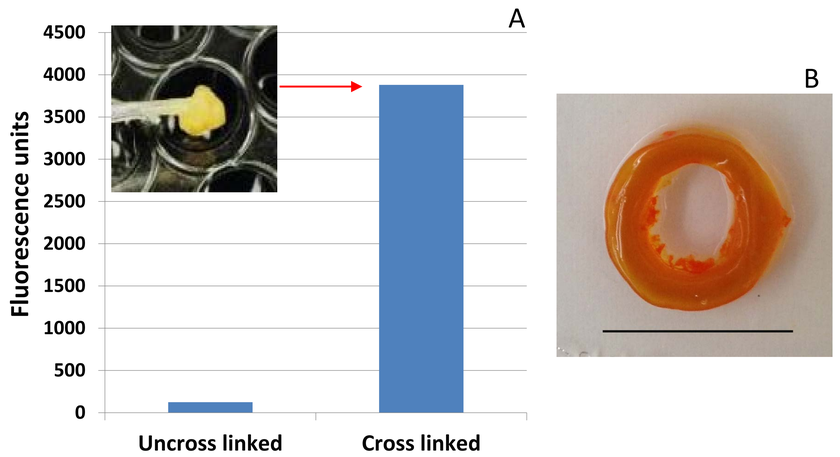
A) HRP-cross-linking: Soluble Resilin, 1 mg/mL HRP and 10 mM hydrogen peroxide, produces polymerized Resilin (arrow); lane 1, Uncross-linked Resilin; lane 2, Cross-linked Resilin (B)Fluorescence of Resilin. The fluorescence detected by scanning 300-700nm, both uncross-linked and cross-linked Resilin protein data is shown.

4. Cross-linked ResChBD bound to Chitin beads



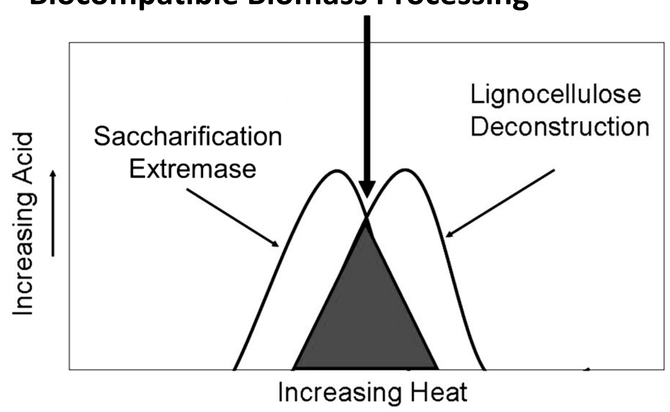
Functionalizing spheres with Resilin protein hybrids: Chitin coated agarose beads were created with recombinant Resilin fused to a protein chitin binding domain. Beads (~1mm diameter) were then treated to form Resilin cross-links by peroxidase activation, mixed with naked-untreated beads and examined by fluorescence microscopy. (A) Bright field image; (B) Blue fluorescence image; (C) Merged image.

5. Resilin Fluorescence



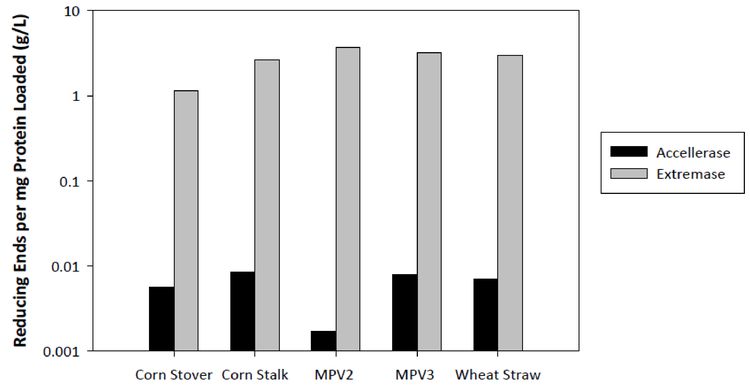
(A) Photo-Fenton (iron initiated) cross-linking of Resilin (inset) and the resulting fluorescence; (B) 3D printing of Resilin using a photo-polymerization process (diameter ~9mm). Size bar represents 1 cm

7. Biocompatible Biomass Processing



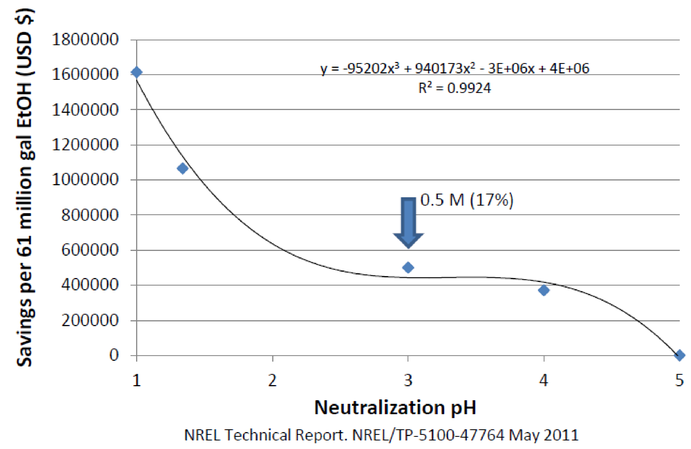
- Pretreatment uses hot acid that must be neutralized before commodity enzyme addition.
- Extremase is a hot acid-compatible enzyme for biomass processing.
- Enzyme cocktails are required for efficient bioconversion

7. Saccharification of Lignocellulose



Comparison of Extremase cocktail to Accellerase 1500 cocktail. Pretreated lignocellulosic substrates were incubated with equal volumes of each cocktail at pH 3.0 and 80 C for 24 hours. Reducing ends were assayed by DNS assay at 540 nm with normalization to substrate-only controls.

8. Reduction in Cost of Industrial Pretreatment



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9. Conclusion

- Coupling biofuel production to protein co-product synthesis would transform unwanted or poorly used protein biomass into bioenergy products with high intrinsic value
- Extremase by itself or in the presence of β-Glycosidase as a cocktail outperforms commercial enzyme in substrate hydrolysis and sugar release.
- Extremase is also capable of hydrolyzing both cellulose and hemicellulose
- Low pH and high temperature stability of Extremase reduces the cost of neutralization and energy input into commercial production of ethanol in a pretreatment-compatible process
- Scaling up for production of recombinant bioenergy products presents a strategy that has been implemented successfully with high product yield and purity

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