Co-synthesis of Bioenergy Proteins To Increase Microbial Biofuel Competitiveness

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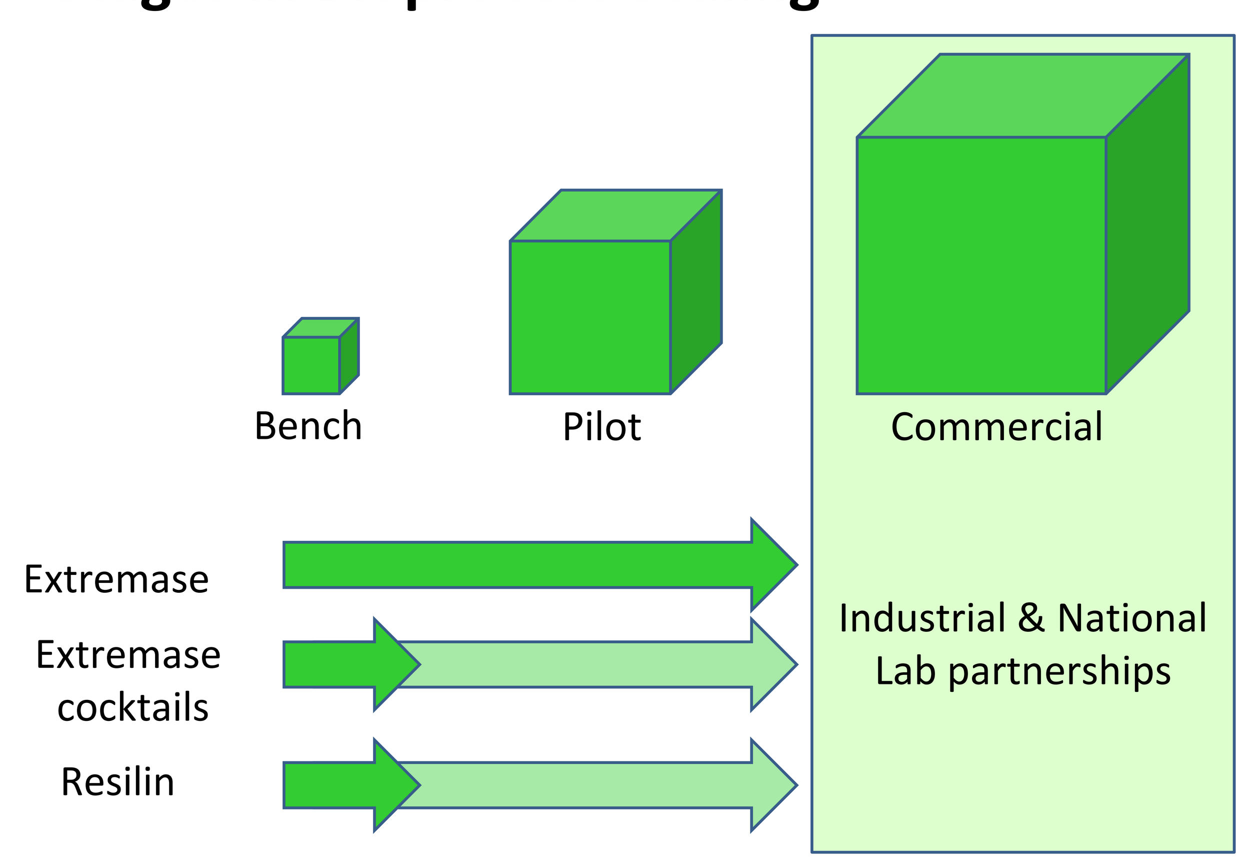
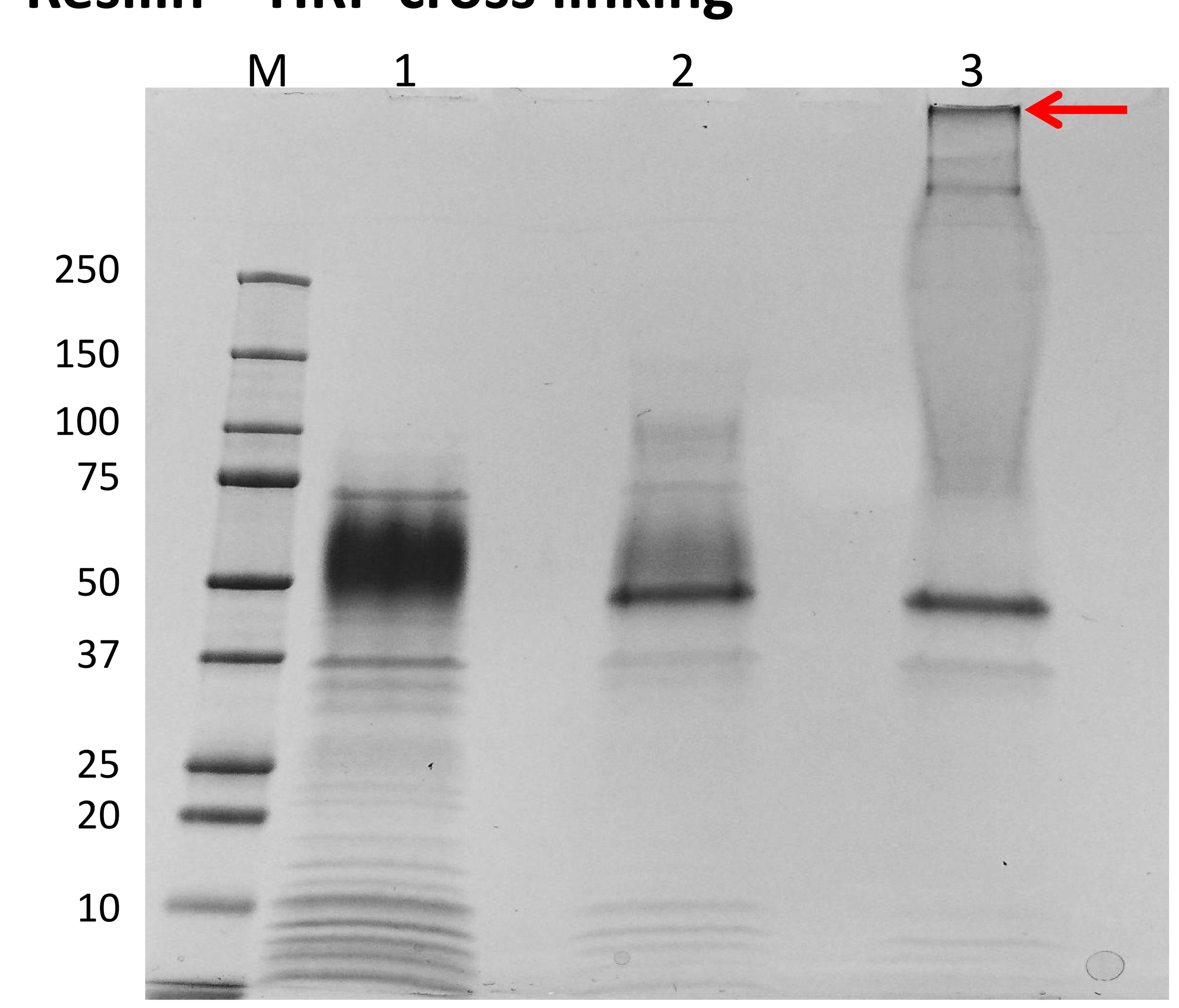
1. Abstract

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

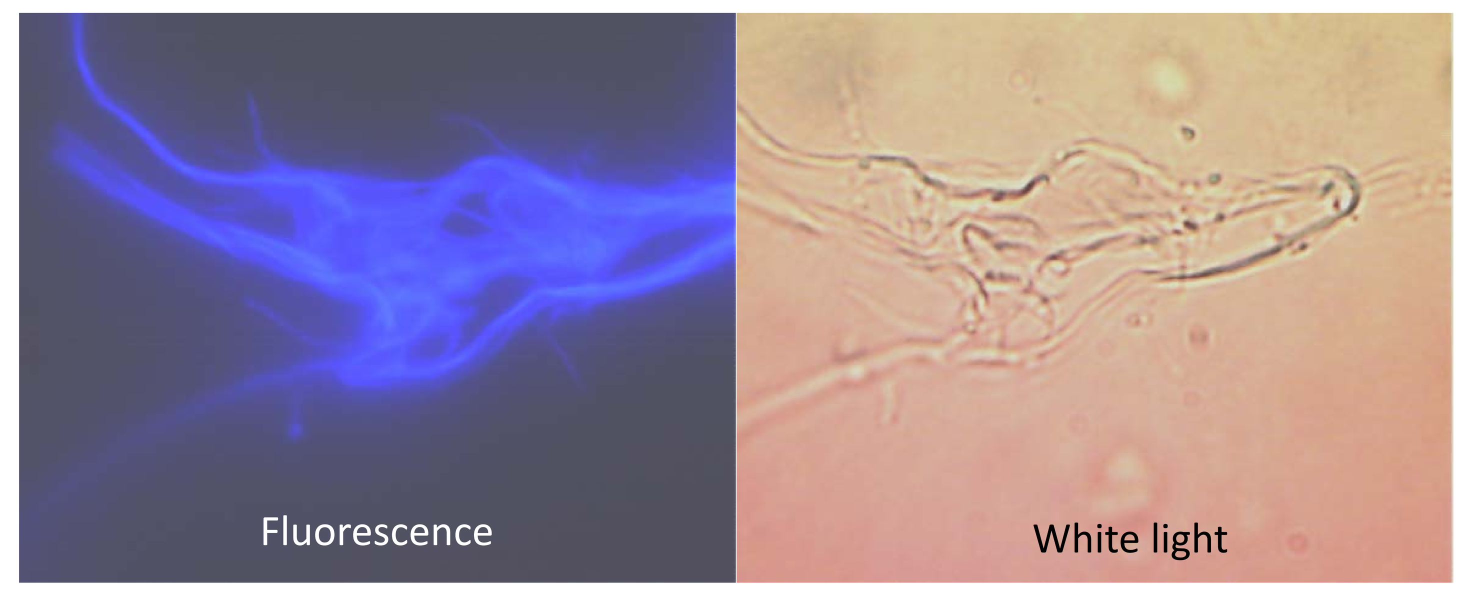


3. Resilin – HRP cross linking



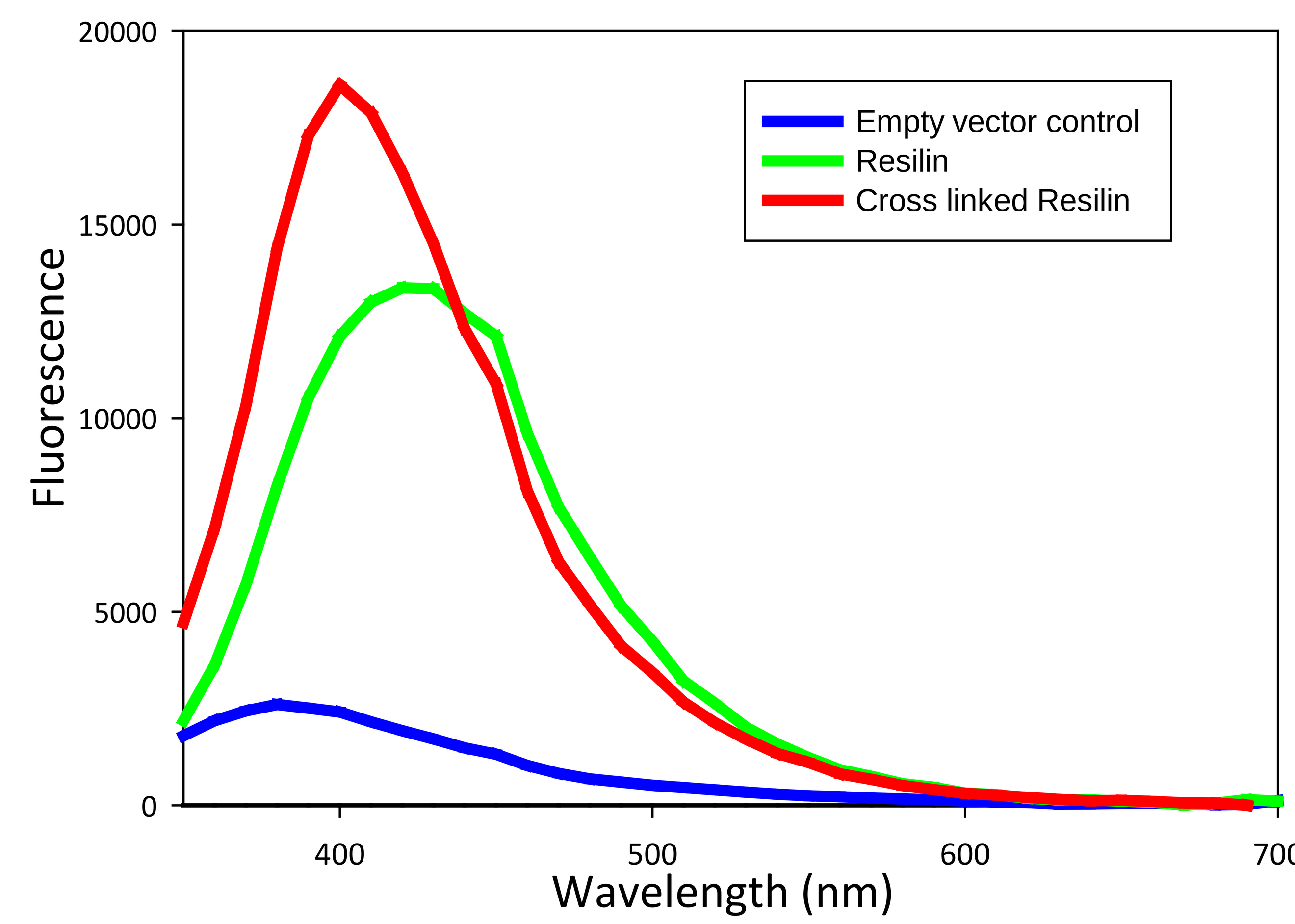
Lane 1, Heat fractionated Resilin protein (1 mg); Lane 2, Resilin HRP mixture without H_2O_2; Lane 3, Resilin HRP mixture with H_2O_2. The solution containing 50 mg/mL heat fractionated Resilin, 1 mg/mL HRP, and 10 mM hydrogen peroxide produces polymerized Resilin after cross linking, barely enters the gel (arrow).

4. Resilin Film : 358 Excitation 461 Emission



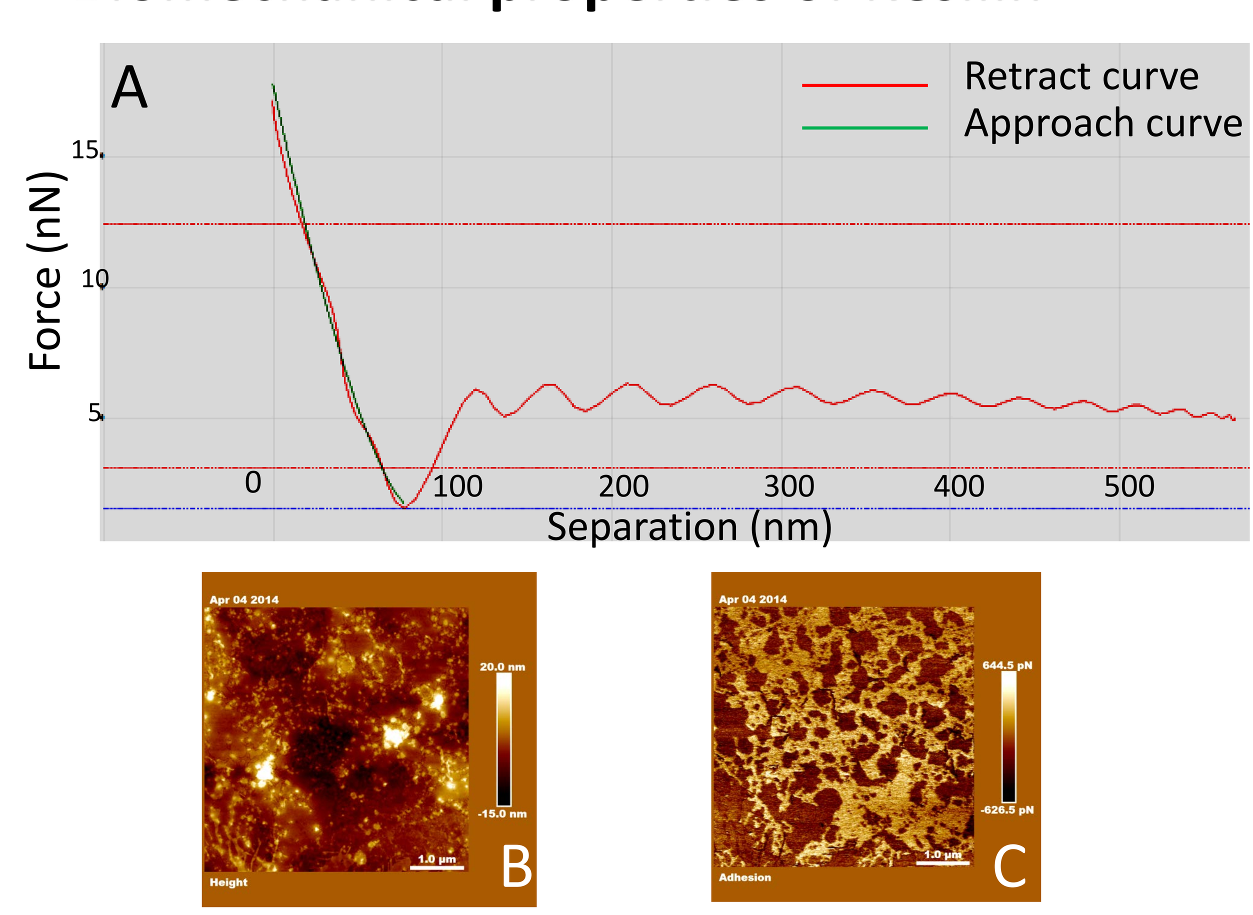
Fluorescence analysis of heat fractionated resilin sample. The pictures were taken by Nikon fluorescence microscope with a DAPI filter cube (Excitation: 358 nm)

5. Resilin Fluorescence



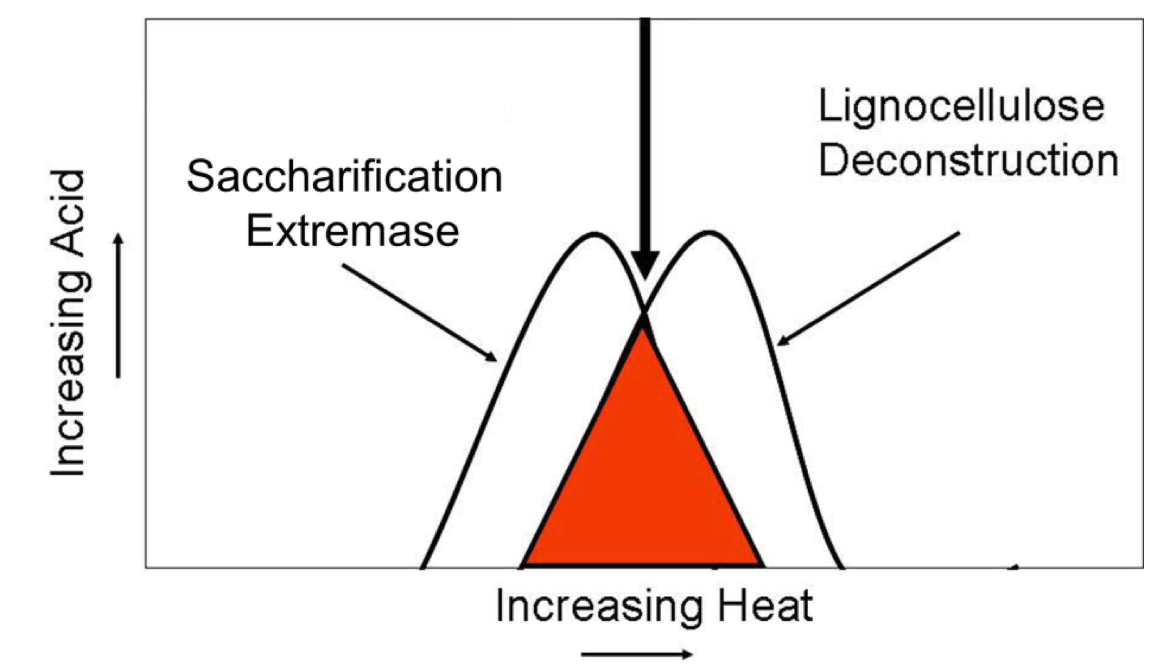
Fluorescence emission spectra of heat fractionated resilin and HRP cross linked resilin. The emission scan was from 300-700nm with excitation @ 320nm

6. Biomechanical properties of Resilin



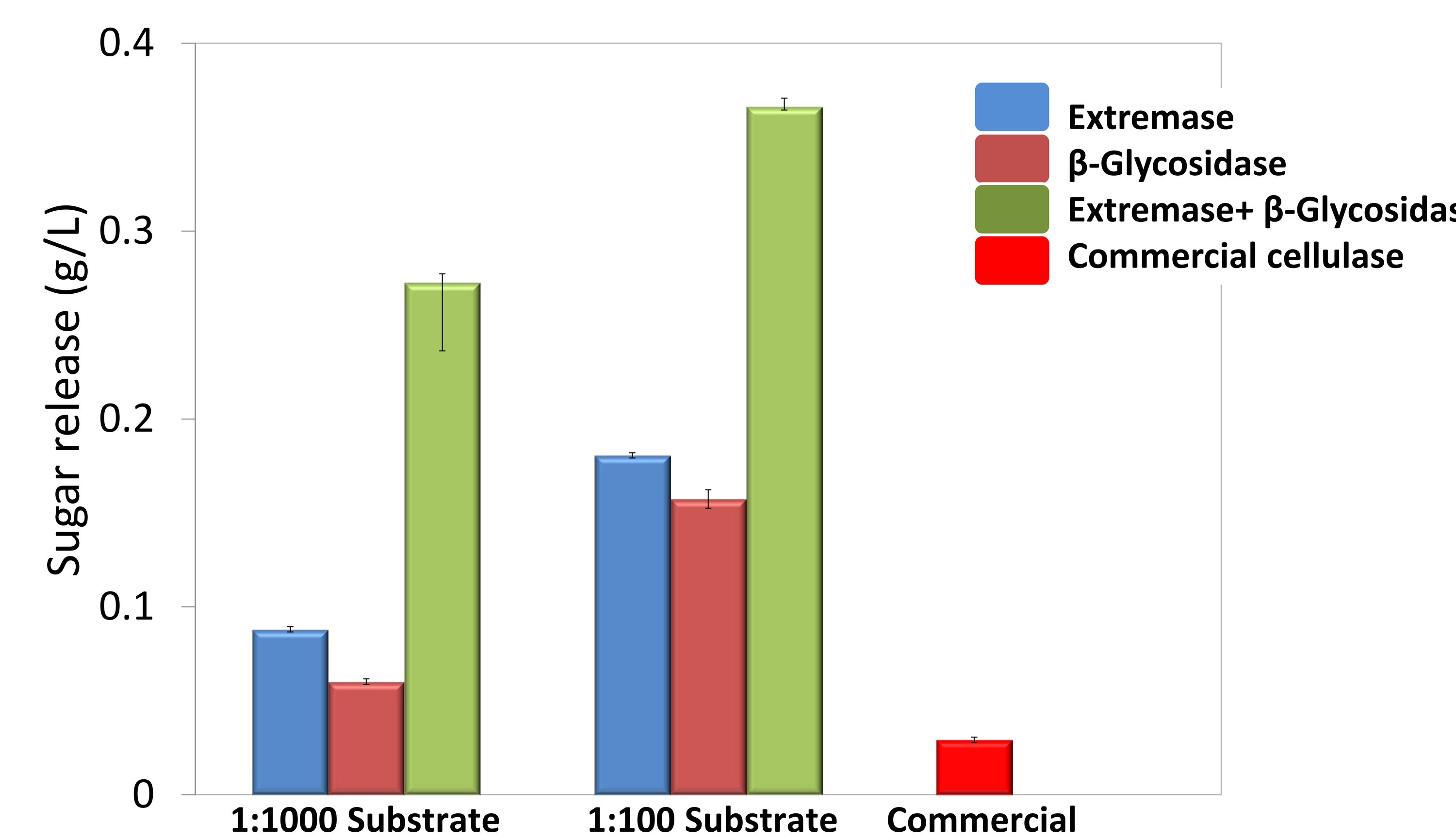
Elasticity of HRP-H_2O_2 cross-linked Resilin by AFM : Force distance curve was recorded for cross linked resilin (A). A squared area of the length of 5 um is scanned to image the resilin surface. Two images , height (B) and adhesion (C), are depicted here. In adhesion image, the lighter color shows the softer sections.

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

8. Extremase Cocktail Stover Hydrolysis



Consolidated bioprocessing with Extremase at 80 ºC and pH 3.0. Utilization of an Extremase cocktail for hydrolysis of cellulose to sugars represents an sustainable strategy for industrial bioethanol production

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
- Cross linking procedure has to optimized for better structural and mechanical assessments of Resilin
- Extremase by itself or in the presence of β-Glycosidase out performs 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
- Scaling up for production of recombinant bioenergy products presents a strategy that has been implemented successfully with high product yield

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