

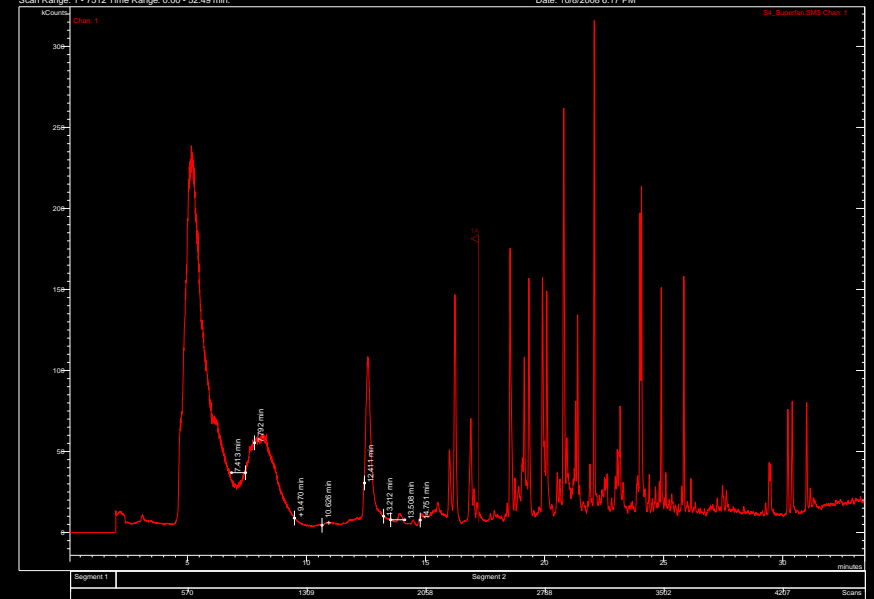
Development of Biocatalyst Systems for Renewable Biofuel Production



Chromatogram Plot

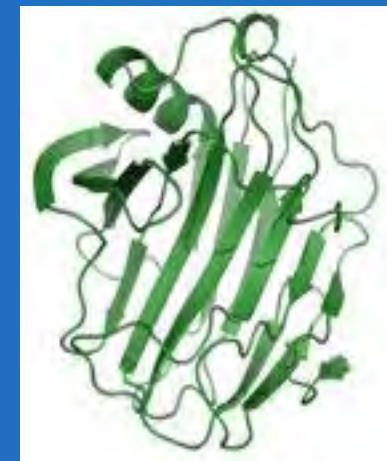
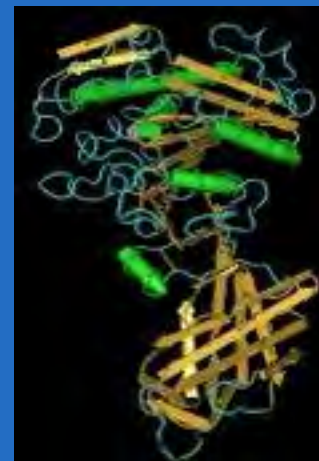
File: ...y documents\work\research\gc_msms\chidata1003080dof4_superfan.msms
Sample: S4_Superfan
Scan Range: 1 - 7512 Time Range: 0.00 - 52.49 min.

Operator:
Date: 10/8/2008 6:17 PM

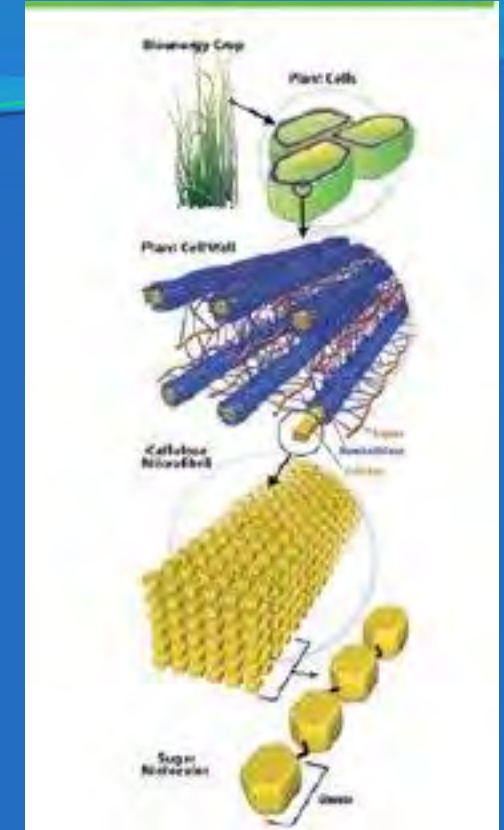
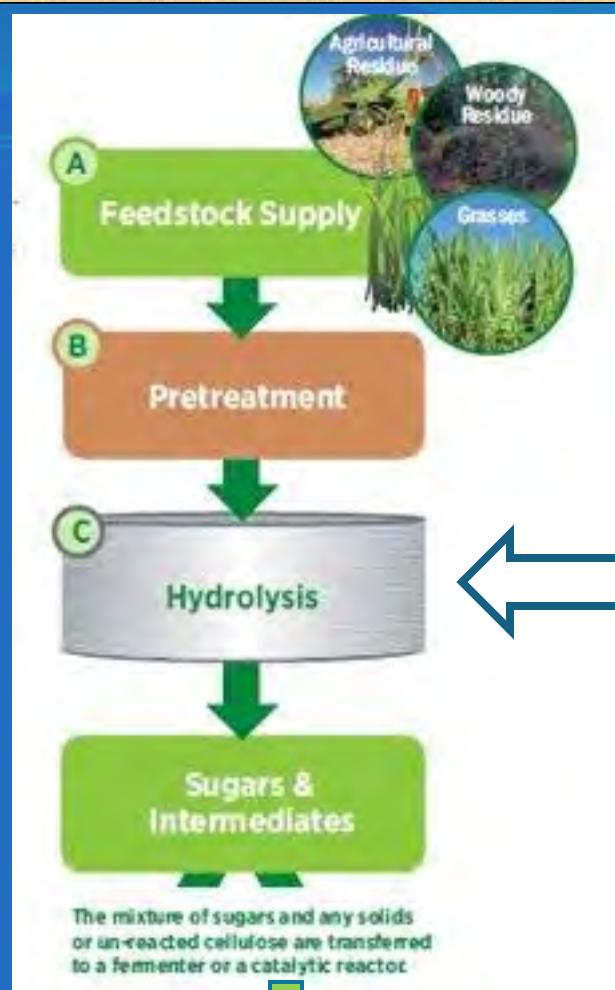


Enzyme?

- Proteins that catalyze the chemical reactions
- Enzymes will not be consumed by the reactions



Advanced Cellulosic Biofuels



High enzyme costs to convert cellulose to sugars (Saccharification) have historically been one of the biggest challenges to a commercially viable cellulosic biofuel process.

Enzymes Needed for Biofuel Production?

- Low cost

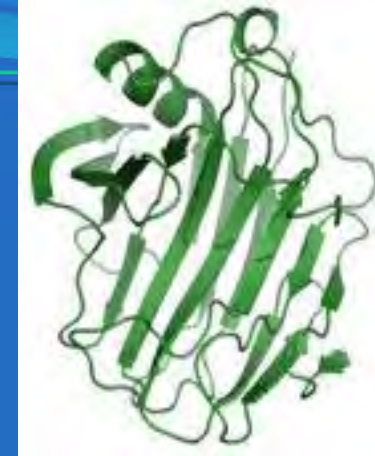
- Stable

thermal stable, long half-life, resistant to high/low pH ,
resistant to phenolic compounds

- Effective

reduce enzyme loading and cost

Enzymes of Interest



1. **Cellulase**-catalyzed hydrolysis of beta-1,4-D-glycosidic linkages in cellulose (cellulose → simple sugars)
2. **Lipase**-catalyzed synthesis of fatty acid methyl esters (fat → biodiesel)

Delivery Platforms

1. Spores
2. Carbon
3. Nano-materials (e.g., carbon, nano gold)

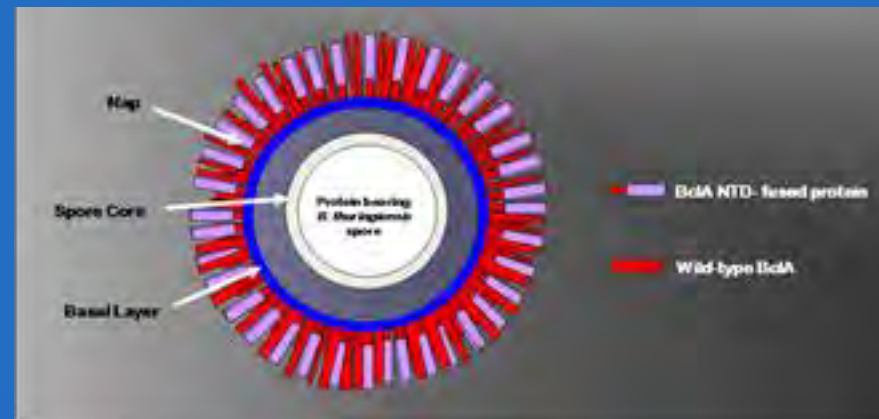
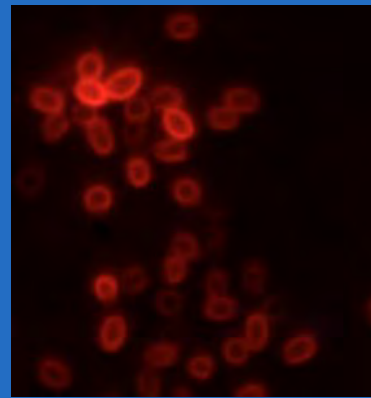
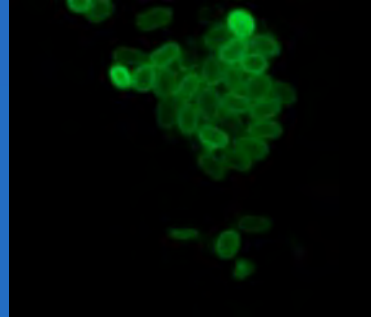
Development of Biocatalyst

Express Enzymes on Surface of Spores by Gene Fusion



B. thuringiensis spore

Exosporium nap (BT-1)
Exosporium basal layer
Spore coat
Cortex



Expressing Fluorescent Proteins on Surface of Spores by Gene Fusion

ORF

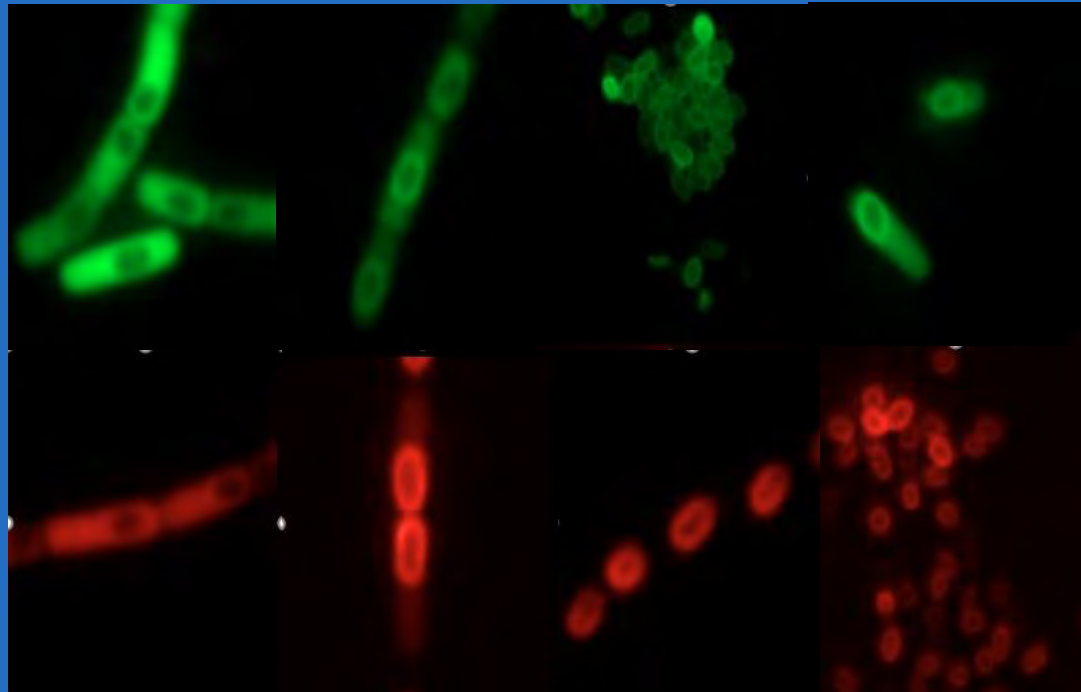
eGFP

(dimer)

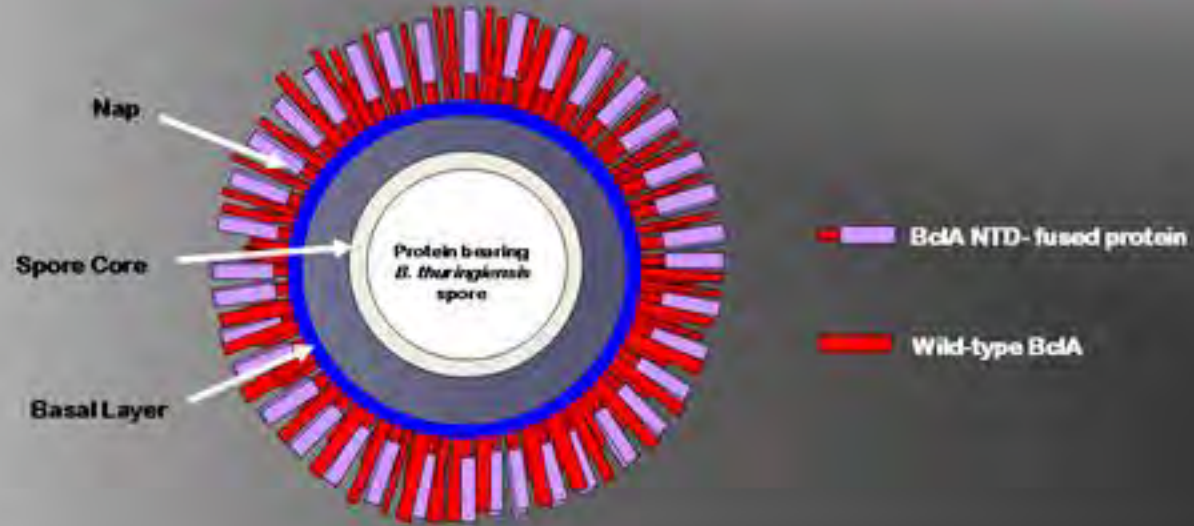
ORF

DsRed

(tetramer)

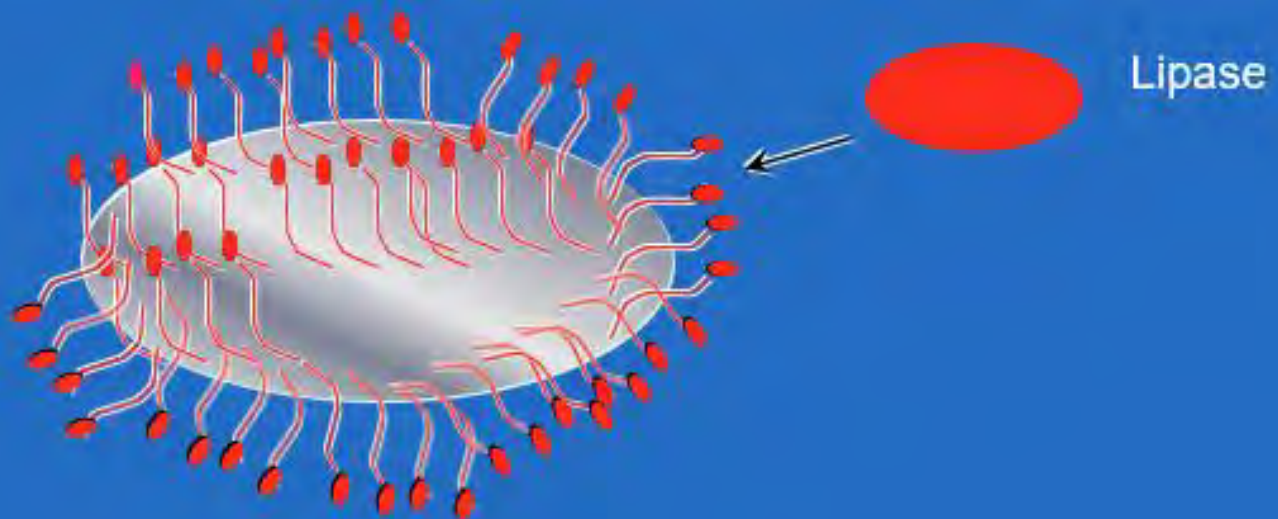
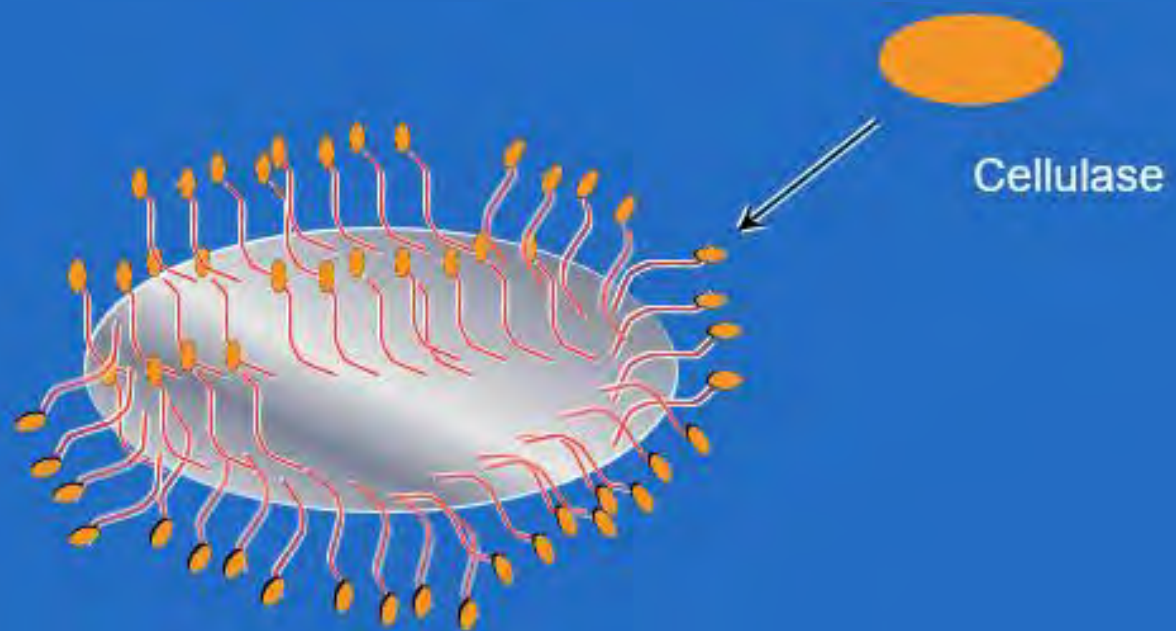


Bacillus thuringiensis Spore-based Display System



Enhanced stability from 3 weeks to > 7 years
Cheap to produce
Can be recovered and reused

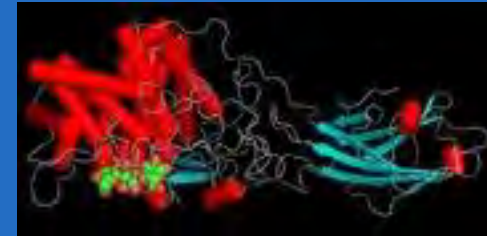
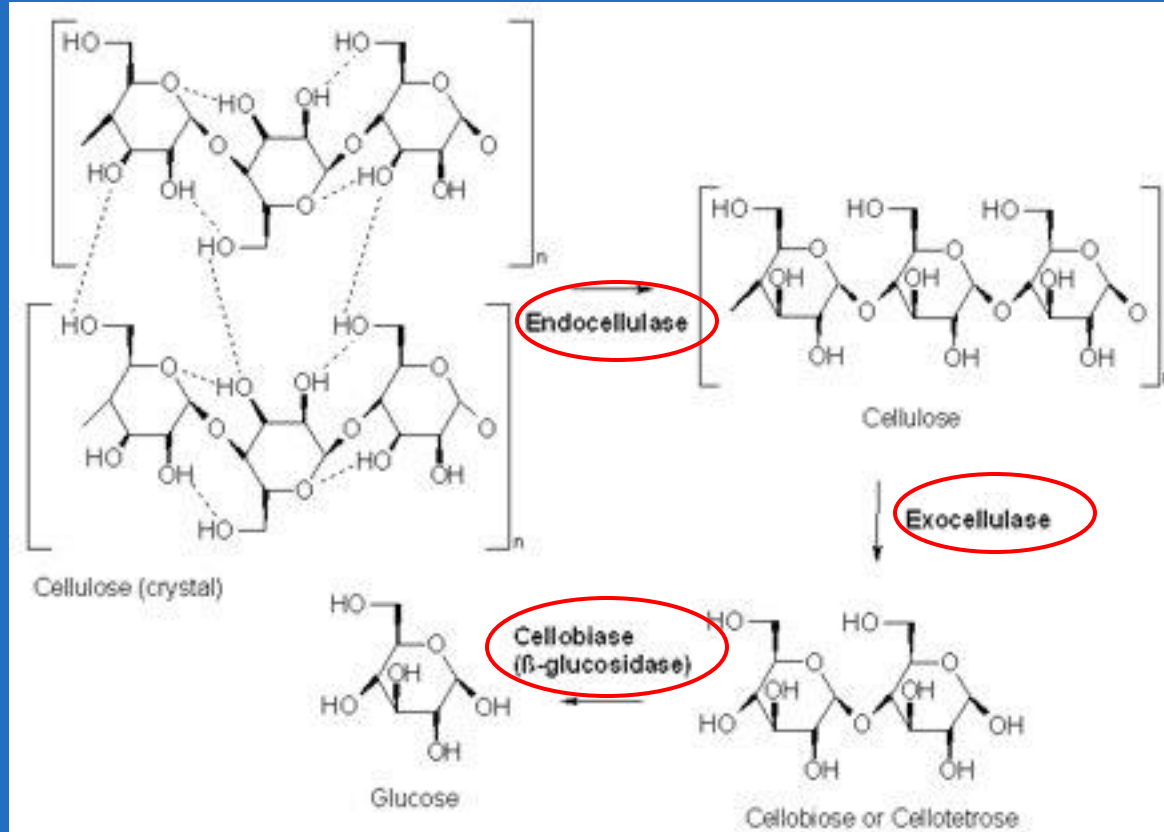
Prototype (dead spores + catalytic enzymes on the surface)



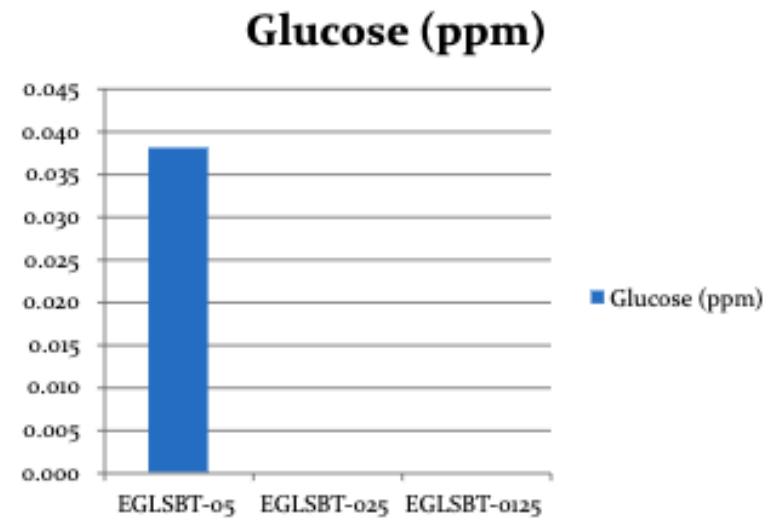
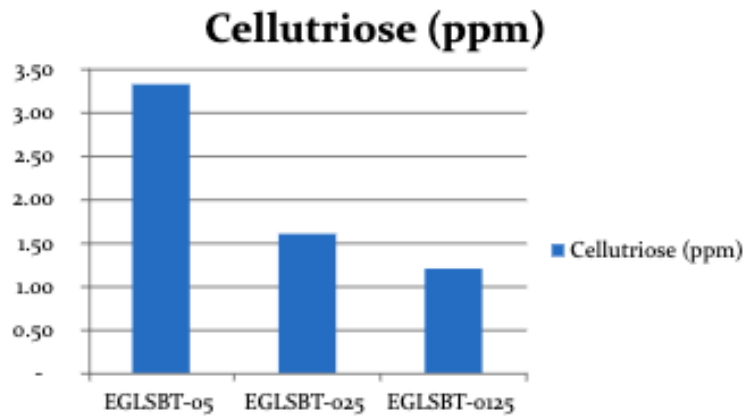
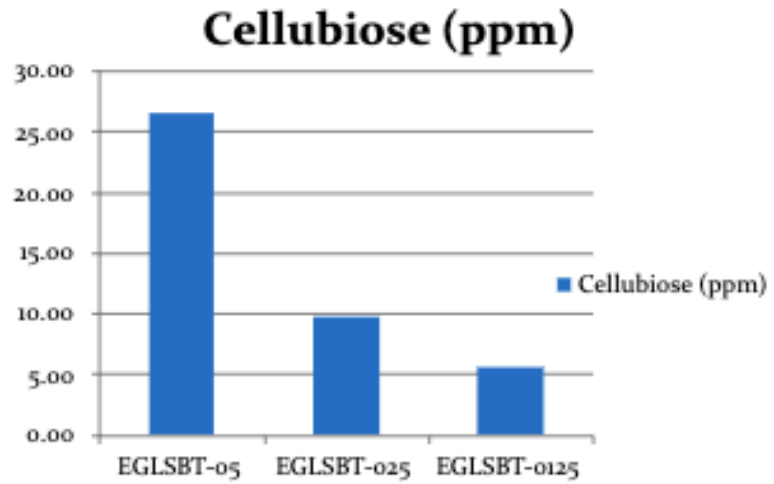
Express Lipase on the Surface of the Biocatalysts for Biodiesel Production



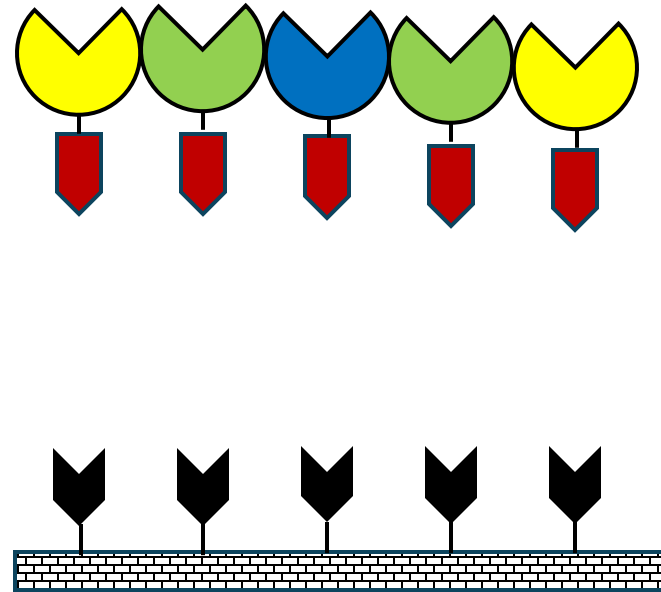
Endocellulase, Exocellulase and β -glucosidase Are the Key Enzymes Required to Convert Cellulose to Glucose



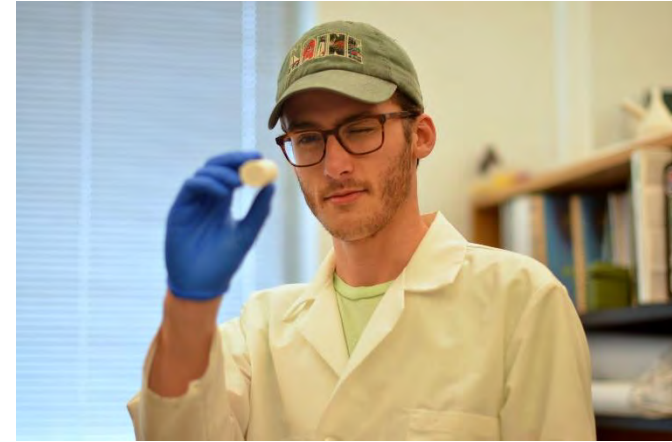
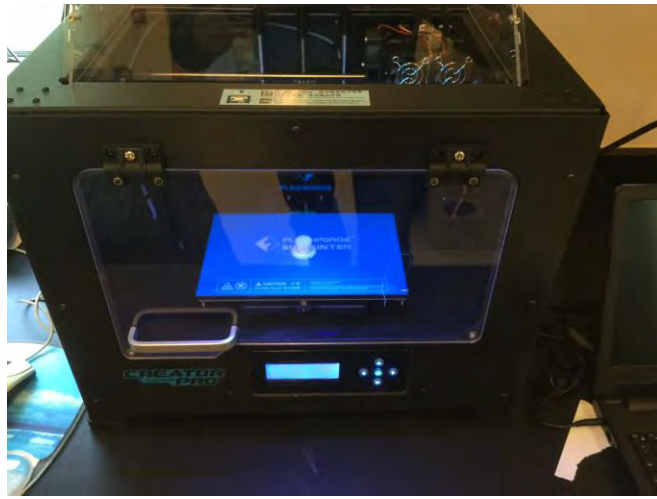
Spore Expressed Endonuclease



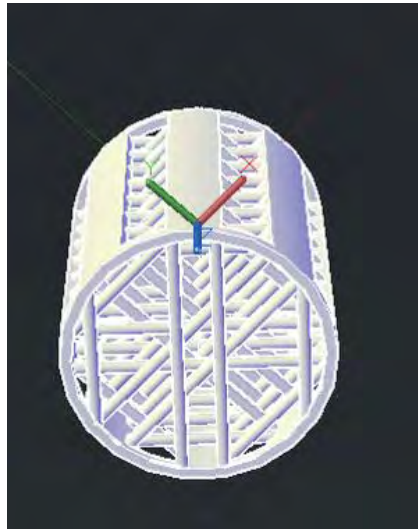
Approach 2 Continuous-Flow Bioreactor System



(carbon fibers, polypropylene, polystyrene, nylon, glass fiber, carbon nanotubes, magnetic particles)



1st Generation



2nd Generation

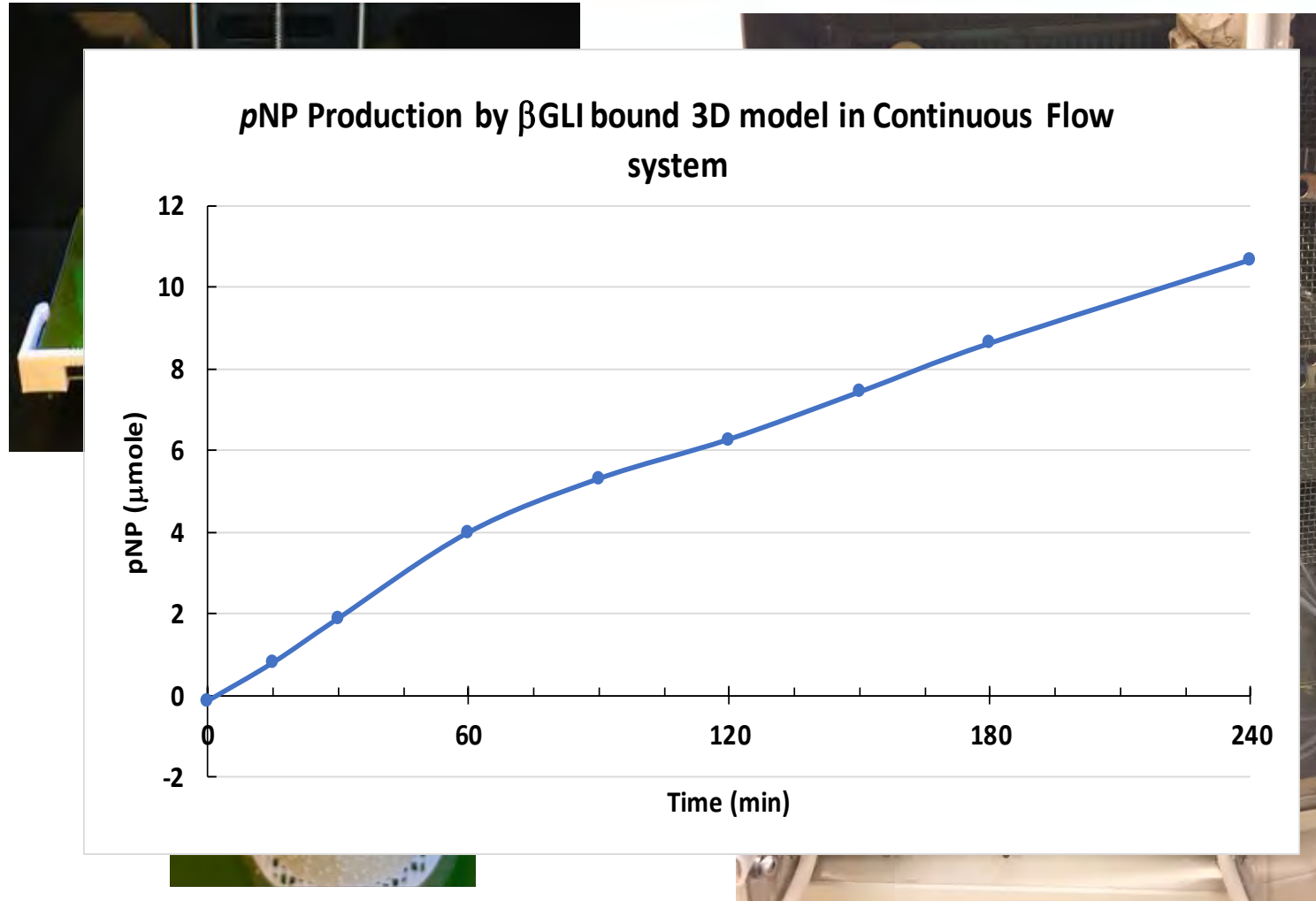


15th Generation

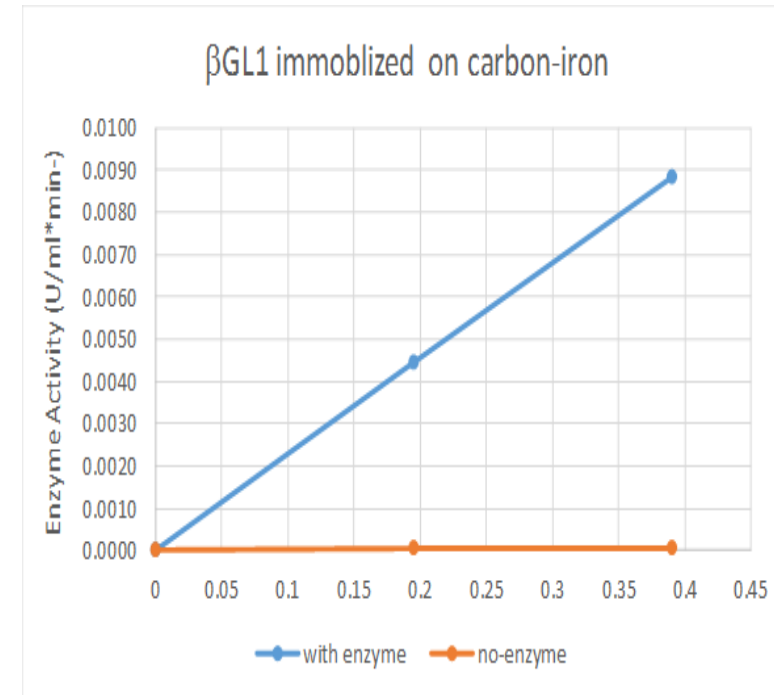
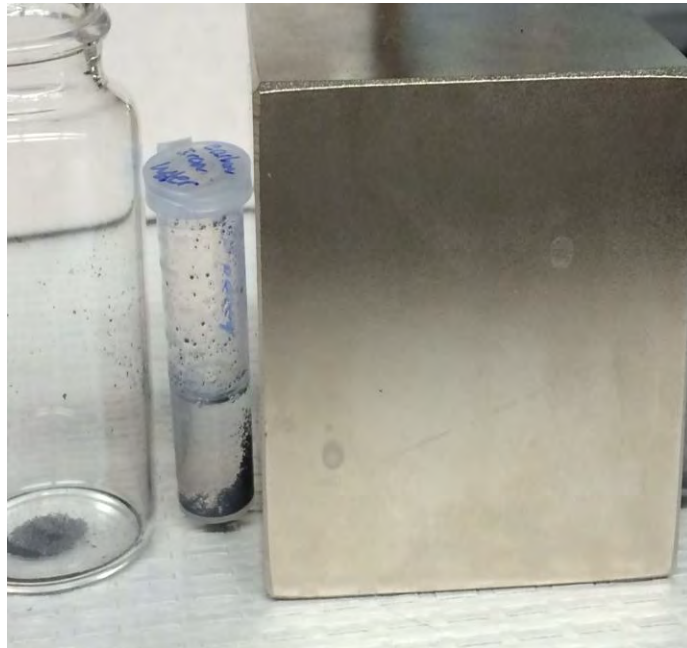


3D rendering of platform model

Continuous-Flow Bioreactor System



Immobilize the Enzymes on Magnetic Particles



Immediate Application In Bioenergy and Bio-based Refinery

1. **Advanced Cellulosic Biofuels**
2. **Biodiesel Production**
3. **Bio-based Product Processing**