2013 DOE Bioenergy Technologies Office (BETO) Project Peer Review

Butanol from Woody Biomass
2.3.2.11

- Date: May 21, 2013
- Technology Area Review: Biochemical Conversion

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- Organization: Oak Ridge National Laboratory

This presentation does not contain any proprietary, confidential, or otherwise restricted information

Goal Statement

This seed project aims to evaluate the fermentation of woody biomass to butanol and associated fermentation products using a multiple biomass sugar-utilizing and hyperbutanol-producing *Clostridium beijerinckii* with the target of producing longer chain alcohols from biomass

Quad Chart Overview

Timeline

- Project start 10/1/2011
- Project end date 6/30/2013
- Percent complete 97%

Barriers

- Barriers addressed
  - Bt-J Catalyst development
  - Bt-K Process integration

Budget

Funding for FY11 $150,000
Funding for FY12 $0
Funding for FY13 $0
Years the project has been funded / average annual funding. 1.5 yr @ $150,000 total

Partners

Informally Prof Hans Blaschek. U of Illinois CU

Project Overview

- Production of butanol as part of the ABE fermentation process has been historically corn or simple sugar-based.
- *Clostridium beijerinckii* BA101 developed at U of Illinois by Hans Blaschek is a multiple biomass sugar-utilizer (including xylose and arabinose) and a hyper-butanol producer.
- A commercial spin-off company has successfully completed a 6000 gallon pilot run using corn glucose feedstocks.
- Research aimed at conversion of lignocellulosic biomass has not been completed with *Clostridium beijerinckii* BA101
- On-going research in the ORNL-led BioEnergy Science Center (BESC) is developing advanced *Populus* and switchgrass feedstocks
- The overall goal is to evaluate *Clostridium beijerinckii* BA101's response to fermentation of *Populus* biomass for butanol production using enzyme based hydrolysis and fermentation approaches.

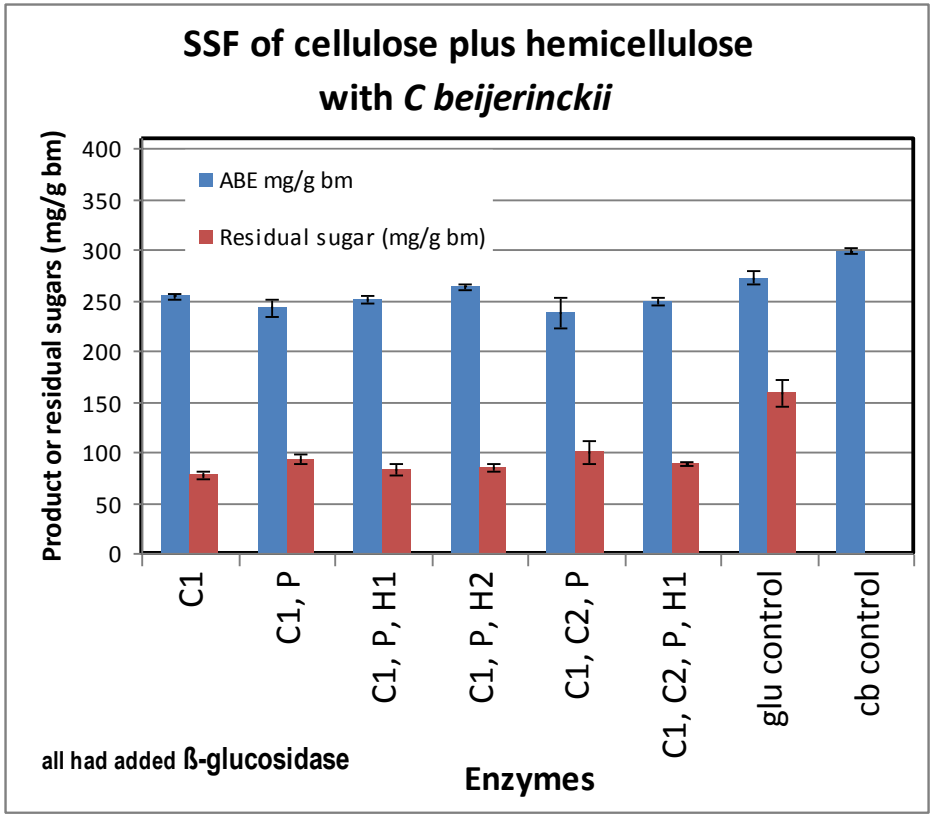
1 - Approach

- Anaerobic fermentation of biomass is well established in the PI's laboratory as part of the BioEnergy Science Center (BESC) research.
- *Clostridium beijerinckii* BA101 can ferment very high levels of glucose and mixed biomass sugars to butanol and acetone.
- Both simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF) will be tested using industrial enzymes for biomass hydrolysis.
- *Populus* sources will be dilute acid pretreated or unpretreated feedstock plus limited pretreatment of BESC *Populus* feedstock
- Different enzyme source and types will be evaluated
- Testing will include impact of increasing the biomass loading on the product titer and overall conversion
- Milestone: determine yield on substrate for 0.1 to 1% *Populus* biomass loading

2-Technical Accomplishments/ Progress/Results

Evaluation of enzymes for SSF approach with *Clostridium beijerinckii* BA101

- SSF tested with addition of multiple enzymes and C *beijerinckii* BA101 using a laboratory biomass source.
- Cellulase, hemicellulase and pectinase enzymes tested
- Substrates: cellulose & hemicellulose or controls
- No inhibition of fermentation with industrial enzymes and lab substrates
- One blend of cellulase, pectinase and hemicellulase was slightly better than cellulase only
- Yield on substrate approached 65%

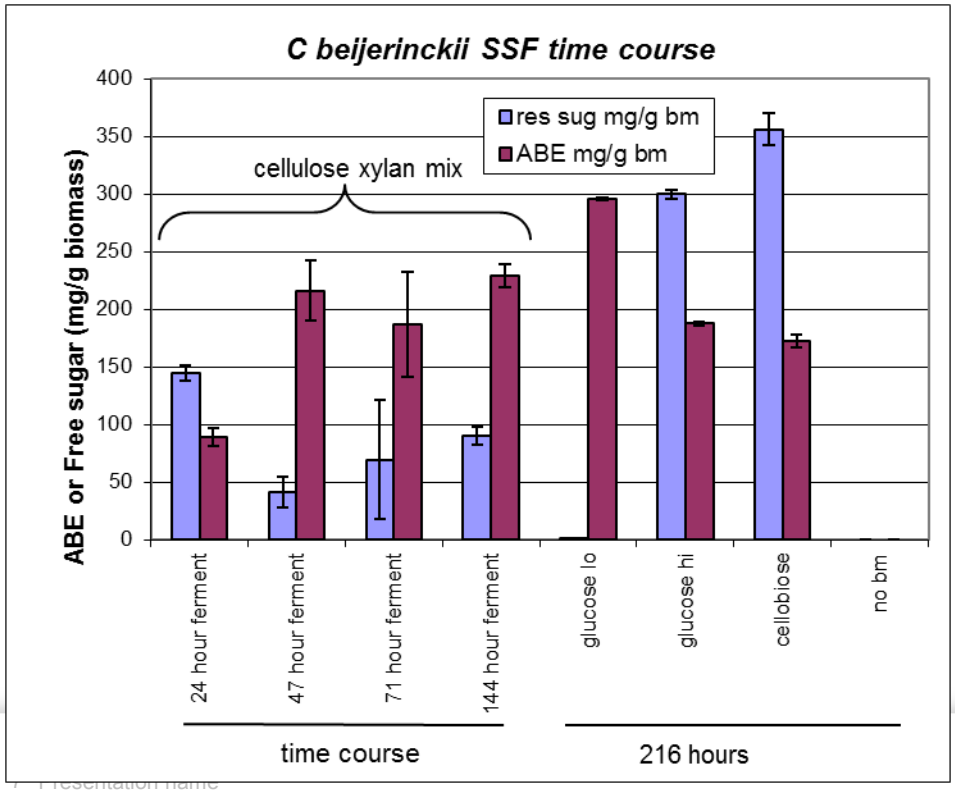


C= cellulase; H=hemicellulase; P=pectinase

2 -Technical Accomplishments/ Progress/Results

Evaluation of SSF biomass conversion with *C beijerinckii* BA101

- SSF proceeds with addition of enzymes and C *beijerinckii* BA101 with a biomass source.
- Testing determined the fermentation did not keep pace with the enzyme activity leading to an ever increasing free sugar level

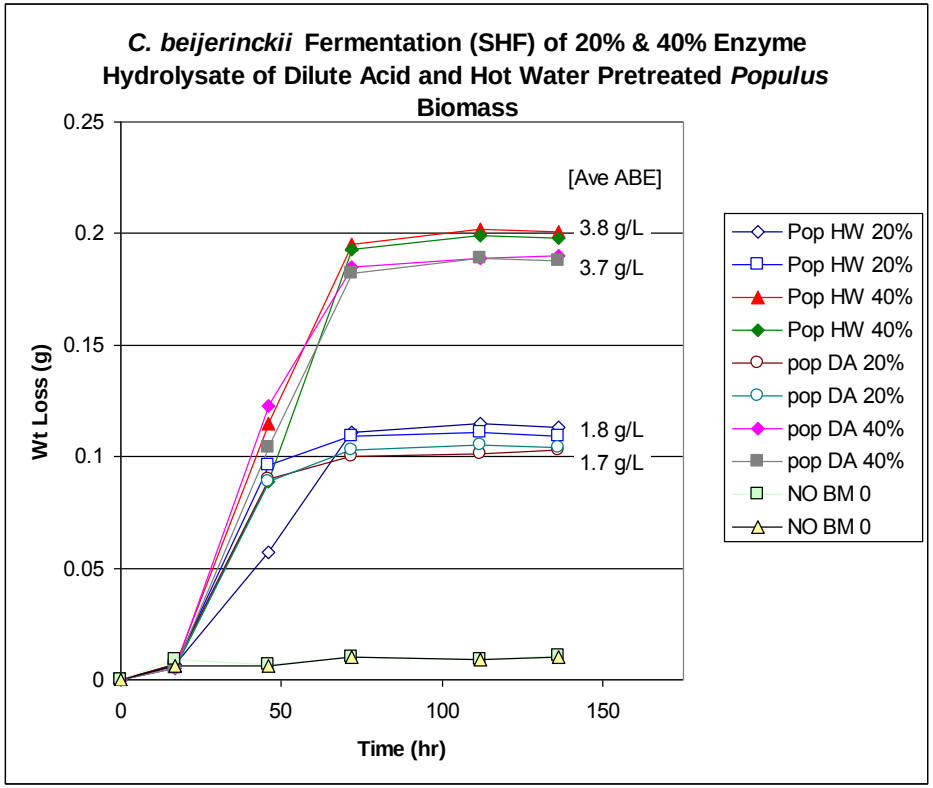


- The lack of fermentation after about 47 hrs indicates butanol fermentation was terminated.
- Biomass loading was low so potentially the SSF approach cannot support continuous fermentation by *C beijerinckii*
- Alternate approach is separate hydrolysis and fermentation

2 - Technical Accomplishments/ Progress/Results (cont'd)

C beijerinckii fermentation of hot water and dilute acid pretreated *Populus* feedstock

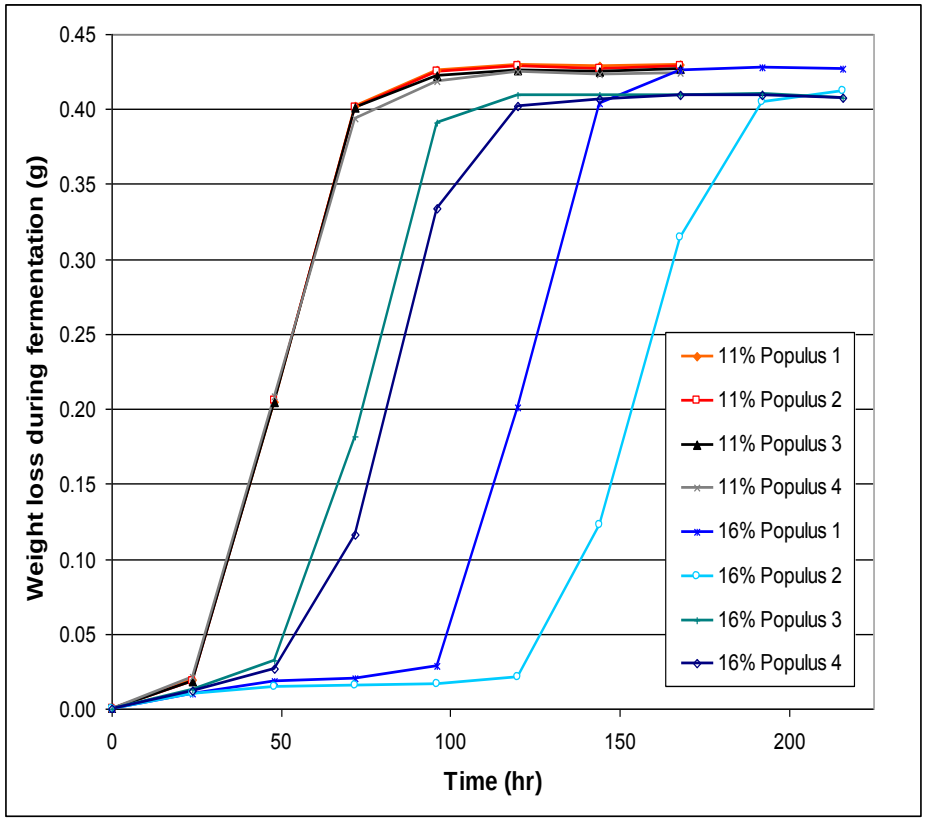
- Pretreated solids hydrolyzed by Novozymes enzymes in SHF mode
  - Soluble sugar levels at 24-25 g/L with 77-82% glucose.
  - Fermentation at two concentrations of hydrolysate (20% or 40%) were complete within 3 days at 85-93% and 87-90% conversion for HW and DA treated biomass materials
  - No inhibitions was found @ 40%
  - Sugars were consumed to less than 0.5 g/L levels



2 - Technical Accomplishments/ Progress/Results (cont'd)

High loading *C beijerinckii* fermentation of hydrolysate from NREL dilute acid pretreated *Populus* feedstock

- Solids hydrolyzed at 11 and 16% loading
- Soluble sugar levels at 49 g/L & 61 g/L respectively.
- 11% loading yielded 0.8% acetone butanol (AB) while 16% loading produced 1.1% AB
- 11% hydrolysate fermented rapidly while the 16% was delayed 1 to 4-5 days
- Sugars were consumed to less than 2 g/L levels



3 - Relevance

- **Drop in fuel potential**: Conversion of *Populus* hydrolysate at high concentration into acetone and butanol by *C beijerinckii* provides an alternative approach to corn-glucose based production.
- **Commercial potential**: Production at over 11 g/L solvents at 90% of theoretical without process optimization suggest significant commercial potential
- **Feedstock availability**: *Populus* species are a well known short rotation woody crops grown in high density forests commercially including ORNL-led BESC partner Greenwood Resources
- **Commercialization***: C beijerinckii* BA101 is being commercialized by Eastman Chemical Company subsidiary Eastman Renewable Materials so a commercial outlet exists for this work.

4 - Critical Success Factors

Clostridium beijerinckii BA101 produces high levels of butanol with biomass hydrolysates.

- Commercialization will require feedstock dependent pretreatment optimization, minimizing industrial enzyme usage, and low cost butanol removal during fermentation.
  - Enzyme optimization will be feedstock and pretreatment dependent.
  - Pervaporization technology under development should be effective on biomass fermentation beer.
- Production of butanol from biomass, including ag residues, will provide an additional source of long chain alcohols for chemical and fuel markets.

5. Future Work

- BETO seed funding permitted the successful evaluation of *C beijerinckii* as a butanol producer from biomass
- The project will be rounded out with the conversion of two naturally occurring BESC-derived *Populus trichocarpa* variants that have significantly different lignin levels and composition to evaluate their potential for drop-in fuels production by SHF processes
- No further work is planned beyond above due to the lack of funding.
- Eastman Renewable Materials will be contacted to provide them with these results.

Summary for Seed Project

1) **Project approach**: Evaluate production of butanol from biomass with existing fermentation technologies
2) **Technical progress**: SHF is superior to SSF; High biomass loadings reached 11 g/L butanol plus acetone at high conversion yields
3) **Project Relevance**: Drop-in fuel butanol can be produced from biomass thus avoiding the food vs fuel issue
4) **Critical Success Factors:** Optimization of pretreatment and enzyme levels with the SHF mode is needed for each feedstock to improve yield on substrate while minimizing costs.
5) **Future Funding**: Funding for completion of item 4 above is needed.
6) **Tech Transfer**: Eastman Renewable Materials will be contacted for potential commercialization of these results