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NATIONAL RENEWABLE ENERGY LABORATORY

Overcoming the Recalcitrance of Cellulosic Biomass by Value Prior to Pulping

Cooperative Research and Development Final Report

CRADA Number: CRD-07-221

NREL Technical Contact: Andrew Lowell

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Cooperative Research and Development Final Report

In accordance with Requirements set forth in Article XI.A(3) of the CRADA document, this document is the final CRADA report, including a list of Subject Inventions, to be forwarded to the Office of Science and Technical Information as part of the commitment to the public to demonstrate results of federally funded research.

CRADA number: CRD-07-221

CRADA Title: Overcoming the Recalcitrance of Cellulosic Biomass by Value Prior to Pulping

Parties to the Agreement: CleanTech Partners

Joint Work Statement Funding Table showing DOE commitment:

Estimated Costs	**NREL** Shared Resources
Year 1	$ 19,368.00
Year 2	$ 30,717.00
Year 3	$ 170,455.00
TOTALS	$ 220,540.00

Abstract of CRADA work:

The Value Prior to Pulping (VPP) project goal was to demonstrate the technical and commercial feasibility of introducing a new value stream into existing pulp and paper mills. Essentially the intent was to transfer the energy content of extracted hemicellulose from electricity and steam generated in the recovery boiler to a liquid transportation fuel. The hemicellulose fraction was extracted prior to pulping, fractionated, or conditioned if necessary, and fermented to ethanol. Commercial adaptation of the process to wood hemicelluloses was a prerequisite for using this less currently valued component available from biomass and wood. These hemicelluloses are predominately glucurono-xylan in hardwoods and galactoglucomannan in softwoods (with a significant softwood component of an arabino-xylan) and will yield fermentation substrates different from cellulose. NREL provided its expertise in the area of fermentation host evaluation using its *Zymomonas* strains on the CleanTech Partner's (CTP) VPP project. The project was focused on the production of fuel ethanol and acetic acid from hemicellulose streams generated from wood chips of industrially important hardwood and softwood species. NREL was one of four partners whose ethanologen was tested on the hydrolyzed extracts. The use of commercially available enzymes to treat oligomeric sugar extracts was also investigated and coupled with fermentation. Fermentations by NREL were conducted with the *Zymomonas mobilis* organism with most of the work being performed with the 8b strain.

The wood extracts hydrolyzed and/or fermented by NREL were those derived from maple, mixed southern hardwoods, and loblolly pine. An unhydrolyzed variant of the mixed southern hardwood extract possessed a large concentration of oligomeric sugars and enzymatic hydrolysis was performed with a number of enzymes, followed by fermentation. The fermentation of the wood extracts was carried out at bench scale in flasks or small bioreactors, with a maximum volume of 500 mL.

Summary of Research Results:

In the investigation of maple extract and its fermentation, an adapted *Z. mobilis* strain was used to convert all of the fermentable sugars in order to achieve an ethanol titer of 27 g/L in a 77% strength extract. With sugar supplementation of the maple extract, in order to imitate a more concentrated extract stream, the maximum ethanol titer achieved by *Z. mobilis 8b* was 78 g/L in a 24.1% total sugar solution. *Z. mobilis 8b* is a glucose-xylose fermenting organism and was unable to ferment other sugars present in the extract.

Mixed southern hardwood and loblolly pine extracts were fermented at strengths of 92% with *Z. mobilis 8b* and produced 22 g/L and 7 g/L, respectively. All fermentable sugars were utilized. In addition to fermenting the mixed southern hardwood extract, enzymatic hydrolysis and fermentation was investigated on an oligomeric form of the same extract. Through a simultaneous saccharification and fermentation (SSF), a maximum titer of 15 g/L of ethanol was achieved with a mixture of commercial enzymes and *Z. mobilis 8b*. Due to the high levels of xylan oligomers and their corresponding acetylation, *Z. mobilis 8b* performed poorly in a medium with such high levels of acetate. The ethanol titer above was achieved by slightly diluting the oligomeric sugar extract and decreasing the enzyme dosing.

Compared to the other partners' fermentative organisms that were evaluated in this project, *Zymomonas mobilis* performed very well. NREL's organism was able to reach very desirable ethanol titers and sugar utilizations from all of the wood extracts investigated. NREL did not create an official technical report or publication from this CRADA. A general technical report is being authored by Barbara Illman of Forest Products Laboratory and will incorporate all of the work performed by multiple parties within this VPP project led by CleanTech Partners. This report is a compilation of the final results, from all involved parties, submitted to CleanTech Partners at the end of the project.

Subject Inventions listing:

N/A

Report Date: 1/30/2012 Responsible Technical Contact at Alliance/NREL: Andrew Lowell

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