Title: Utilization of Lignocellulosic Biofuel Conversion Residue by Diverse Microorganisms

Authors: Caryn S. Wadler[1,5*] (cwadler@wisc.edu), John F. Wolters[2,3,5], Nathaniel W. Fortney[2,5], Kurt O. Throckmorton[1,5], Yaoping Zhang[2,5], Caroline R. Miller[2,3,5], Rachel M. Schneider[2,3,5], Evelyn Wendt-Pienkowski[1,5], Cameron R. Currie[1,5], Timothy J. Donohue[1,2,5], Daniel R. Noguera[2,4,5], Chris Todd Hittinger[2,3.5], and **Michael G. Thomas**[1,5]

Institutions: [1]Department of Bacteriology, University of Wisconsin-Madison, Madison, [2]Wisconsin Energy Institute, University of Wisconsin-Madison, Madison, [3]Laboratory of Genetics, Center for Genomic Science Innovation, J. F. Crow Institute for the Study of Evolution, University of Wisconsin-Madison, Madison, [4]Department of Civil and Environmental Engineering, University of Wisconsin-Madison, Madison, [5]DOE Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison

Project Goals: To offset the costs of biofuel production, we characterized components of lignocellulosic biofuel production waste (Conversion Residue) available for microbes to metabolize into valuable bioproducts and identified *Streptomyces* and yeast species to serve as chassis for future genetic engineering towards this goal.

Abstract: Conversion Residue (CR) is the material remaining after deconstructed lignocellulosic biomass is subjected to microbial fermentation and distillation to remove the biofuel. Current methods of switchgrass hydrolysate fermentation to bioethanol by *Zymomonas mobilis* leave behind about 60% of the organic material from the hydrolysate as a combination of raw plant material, treatment residues, unfermented sugars, bacterial waste products, and cell debris. We analysed several batches of CR generated from AFEX- and enzyme-treated switchgrass hydrolysate fermented by *Z. mobilis* and distilled to remove bioethanol to determine what components could be utilized for a second round of microbial processing to alternate bioproducts. Through a combination of chemical oxygen demand (COD), HPLC, and GC-MS-based assays, we determined that the major components of CR are oligomeric and monomeric sugars from cellulose and hemicellulose and other carbon containing metabolites. We then tested 71 *Streptomyces* species, 163 yeast species, and an aerobic microbial consortium derived from a wastewater treatment plant for their ability to grow on CR. Many of the *Streptomyces* and yeast species were able to grow in CR, with some species capable of utilizing over 40% of the soluble COD. For comparison, the microbial community was able to metabolize about 70% of the soluble COD. HPLC analysis showed that most individual microbes and the community preferentially utilized the monomeric sugars in CR for growth, although there was evidence of further breakdown of oligomeric sugars. These analyses allowed us to identify strains that are good candidate chassis for genetic engineering towards the production of valuable bioproducts. We also developed a synthetic conversion residue (SynCR); a defined medium designed to mimic CR. SynCR allowed us to examine how individual components of CR affect microbial growth and will be a valuable tool for future production of bioproducts.

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