Advanced Biocatalytic Processing of Heterogeneous Lignocellulosic Feedstocks to a Platform Chemical Intermediate (Lactate Ester)

This project combines the pioneering work in lignocellulose conversion by the University of California-Davis (UC-Davis) and industrial partners with the membrane separations advances used by Argonne to produce low cost "green" solvents. The Argonne/UC-Davis teams are focusing on the development of microorganisms capable of mixed fermentation of five and six carbon sugars for integration into the Argonne process

A membrane-based technology developed at Argonne has been use to produce ethyl lactate and other lactate esters at a lower cost using carbohydrates rather than petrochemicals as starting materials. Energy requirements of the new technology are one-tenth those of the traditional process, and salt is eliminated as a processing waste. Ethyl lactate is an excellent solvent that is non-toxic and biodegradable.

The opportunity exists to integrate the production of lactate ester with the availability of low-cost agricultural waste. The California Institute of Food and Agricultural Research has developed processing capabilities to convert the state's highly concentrated feedstock of lignocellulose biomass into inexpensive sugars. A cheaper source of feedstocks would lower the cost of ethyl lactate production and enhance the growth of this expanding worldwide market for less-toxic solvents. Although there are technical challenges to overcome, the project will result in significant energy savings and environmental and economic benefits to California and the nation.

The goals of this project are to use advanced separations technologies to increase the range of feedstocks that can be used in biorefinery systems and to engineer microbes that can handle simultaneous conversions of five and six carbon sugars with simultaneous saccharification and fermentation processes.

Several feedstocks will be evaluated as a source of inexpensive carbohydrates, and they will be adapted to the known requirements of the current lactate ester process. Two approaches will be undertaken: (1) identify feedstock mixtures that are most compatible with six carbon sugars, and

(2) develop fermentations that are capable of using both five and six carbon sugars. Mixed sugars generated from the feedstocks will be exposed to various lactic-acid-producing microorganisms. Productive strains will undergo genetic modification to allow use of both sugars simultaneously. Strains of both parent and genetically modified microbes will be examined for their ability to carry out separate hydrolysis and fermentation, as well as simultaneous saccharification and fermentation. The fermentation process will be integrated with an effective product purification process (such as electrodialysis) to produce a good yield of high-purity lactate esters from biomass. This project is funded by the U.S. Department of Energy, Office of Industrial Technologies (DOE/OIT).

Rice straw waste pretreatment using three separate processes was evaluated for the release of cellulose, pectin, hemicellulose, and total carbohydrates. These materials were then treated with seven commercial preparations of cellulase enzymes. The results showed that the steam exploded and acid pretreatments effectively removed hemicellulose from rice straw, providing a high yield of fermentable sugars. The ammonia pretreatment was distinctly different from other pretreatments in that it did not significantly increase enzymatic digestibility of the rice straw. Three commercial Trichoderma reesei-derived enzyme preparations were more active on pretreated rice straw than to the others tested. Over 15 advanced cellulase preparations and producing strains have been obtained. These will be screened and validated for their ability to produce large quantities of cellulase using a low-cost fermentation culture medium.

Ten strains of Lactobacillus species that are either homofermentative, facultatively heterofermentative, or obligately heterofermentative have been examined for their ability to ferment either five or six carbon sugars and produce lactic acid as one or more fermentation products. One strain of Lactobacillus brevis exhibited co-metabolism of glucose and arabinose sugars; this strain has been chosen for further examination. Efforts have also begun to clone target genes in Lactobacillus that are involved in lactic acid production and production of organic acid side-products.

Lactic acid production was also examined in Escherichia coli. Elimination of the activity of pyruvate:formate lyase in E. coli alters the host's fermentation so that lactic acid is the main product. The resulting strain, called CSM1, carried out a nearly homolactic fermentation of glucose. However, the rate and final product concentration obtained were quite high, comparable to those obtained by strains of Lactobacillus selected as possible hosts for commercial lactic acid production. Both in rich medium and in a defined medium, good yields of lactic acid were obtained..