2000 MAFMA Final Report

Project Title: **Development of a Fungal Fermentation Process for Production of L(+)-Lactic Acid from Corn Milling Byproducts**

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The first objective of this project was to develop a novel fungal fermentation process for direct conversion of corn starch to lactic acid. Development of fermentation processes for converting corn fiber and other corn milling byproducts containing glucose and xylose to lactic acid, propionic acid and butyric acid were also targeted as the second objective in this project. The project was a part of our long-time effort to develop novel fermentation technologies for production of value-added products from agricultural commodities and food processing wastes. Lactic acid is widely used in foods, pharmaceuticals, and in the production of biodegradable poly-lactic acid. Propionate is used as a preservative for food, animal feed, seed, and grain. Fermentation-produced sodium lactate and calcium propionate are currently or can be used as natural preservatives in foods (e.g., processed meats, bakery, and dairy products), and they enjoy premium prices over their chemical counterparts because of consumer's demand for natural foods. Butyric acid is used as a specialty chemical and in perfume. Furthermore, the ethyl esters of these organic acids can be used as green solvent (i.e., ethyl lactate) and fuel additives to gasoline (as a better octane buster than MBTE and ethanol) and biodiesel. Production of these carboxylic acids from food processing byproducts by fermentation is thus of great interest to the food industry. In this project, we have successfully demonstrated the feasibilities of both fermentation processes identified in our objectives and are currently working with EnerGenetics (Keokuk, Iowa) and Environmental Energy, Inc (Blacklick, Ohio) for their commercial development. We have also received additional funding from USDA and DOE to continue the project for its commercialization. Some of the findings from this project have been published in scientific journals. The titles of the journal papers and abstracts are given in the following sections.

Production of L(+)-Lactic Acid from Glucose and Starch by Immobilized Cells of *Rhizopus oryzae* in a Rotating Fibrous Bed Bioreactor, A. Tay and S.T. Yang, Biotechnol. Bioeng., 80:1-12 (2002).

A rotating fibrous-bed bioreactor (RFB) was developed for fermentation to produce L(+)-lactic acid from glucose and cornstarch by *Rhizopus oryzae*. Fungal mycelia were

immobilized on cotton cloth in the RFB for a prolonged period to study the fermentation kinetics and process stability. The pH and dissolved oxygen concentration (DO) were found to have significant effects on lactic acid productivity and yield, with pH 6 and 90% DO being the optimal conditions. A high lactic acid yield of 90% (w/w) and productivity of 2.5 g/L·h (467 g/h m^2) was obtained from glucose in fed-batch fermentation. When cornstarch was used as the substrate, the lactic acid yield was close to 100% (w/w) and the productivity was 1.65 g/L·h (300 g/h m^2). The highest concentration of lactic acid achieved in these fed-batch fermentations was 127 g/L. The immobilized-cells fermentation in the RFB gave a virtually cell-free fermentation broth and provided many advantages over conventional fermentation processes, especially those with freely suspended fungal cells. Without immobilization with the cotton cloth, mycelia grew everywhere in the fermentor and caused serious problems in reactor control and operation, and consequently the fermentation was poor in lactic acid production. Oxygen transfer in the RFB was also studied, and the volumetric oxygen transfer coefficients under various aeration and agitation conditions were determined and then used to estimate the oxygen transfer rate and uptake rate during the fermentation. The results showed that the oxygen uptake rate increased with increasing DO, indicating that oxygen transfer was limited by the diffusion inside the mycelial layer.

Butyric Acid Production from Acid Hydrolysate of Corn Fiber by *Clostridium tyrobutyricum* in a Fibrous Bed Bioreactor, Y. Zhu, Z. Wu and S.T. Yang, , Process Biochemistry, 38:657-666 (2002).

Fermentation kinetics of butyrate production from glucose and xylose by *Clostridium tyrobutyricum* ATCC 25755 immobilized in a fibrous bed bioreactor at pH 6.0 and 37°C were studied. Fermentation with glucose as the main carbon source gave higher cell biomass production and reactor productivity compared with xylose fermentation, probably due to the extra energetic requirement for cell growth on xylose. Acetate formation was reduced as well when xylose was the substrate. However, comparable butyrate concentration of ~40 g/L and butyrate yield of 0.43 g/g were obtained from both glucose and xylose fermentations. In the fermentation of glucose/xylose mixture (1:1), the acid production pattern from xylose did not change in the presence of glucose and no inhibition of xylose uptake was observed. Corn fiber hydrolysis with dilute HCl or H_2SO_4 at 121 ^oC produced ~62% (w/w) fermentable sugars, mainly xylose, glucose, and arabinose. The hydrolysis was not significantly affected by the acids used, their concentrations between 0.1 and 0.5 M, or the treatment time between 15 and 60 min. Butyrate fermentation of corn fiber hydrolysate supplemented with corn steep liquor gave a high butyrate yield of 0.47 g/g carbon source consumed and a reactor productivity of 2.91 g/L·h. This study demonstrates that the low-value corn refining byproducts can be efficiently used for butyrate production.