

Pyruvic acid is a valuable chemical intermediate in the production of fine chemicals used by agrochemical, pharmaceutical, and food industries. In 2005, world-wide production of pyruvic acid was estimated at 11 million metric tons at a bulk price of \$9-11/Kg. Current technology for production of pyruvate is based on conversion from tartaric acid and results in undesirable byproducts. An enzymatic approach to production of pyruvate was developed by cloning the glycolate oxidase (GO) gene from spinach into *Pichia pastoris* (1). GO is a flavoprotein (FMN dependent) which catalyzes the oxidation of lactate to pyruvate with a stoichiometric production of hydrogen peroxide. Hydrogen peroxide can lower GO activity and create non-catalytic byproducts; hence, catalase was also cloned into *P. pastoris* to create a double-transformant designated NRRL Y-21001.

Process development work was performed at the University of Iowa's Center for Biocatalysis and Bioprocessing. High density, double recombinant, *P. pastoris* fermentation (100 g cells/L) was achieved at the 30 L scale. After fermentation, these cells were treated with a proprietary process (2) to enable whole-cell biocatalysis and increase enzyme activity (85 U/g cells‡ for GO, 200 U/g cells for catalase). Current efforts have focused on optimizing these catalytically competent cells in a reaction to generate pyruvate at a high enzyme to product ratio.

Reaction optimization was done at the one-liter scale with a DASGIP Fed-batch Pro system. The DASGIP system contains four parallel reactors with control of temperature, pH, and dissolved oxygen. Oxygen enrichment at large scale would be costly, so air was bubbled through the reaction medium at 1 L/min under maximum stirring. Operating temperatures of 18 °C and 25 °C were tested along with pH values ranging from 5 to 8. Other key variables included: substrate loading, conducting the reaction in water, minimizing enzyme concentration, and maximizing number of recycles. Lactate loading ranged from 0.5 to 4 M, and significant substrate inhibition was observed above 1 M. Based on the above studies, optimum performance was achieved in water at pH 7 with an operating temperature of 18 °C and 1 M substrate loading. Enzyme loading was at 15 g/L for the first 3 cycles. Subsequently, 2 g/L additional enzyme was added every alternate cycle to achieve 10 cycles. Under the above conditions, >90% conversion of lactate to pyruvate was achieved in approximately 11 hours. Based on these parameters, an enzyme to product ratio of 1:38 was achieved.

‡ Enzyme activity needed to catalyze transformation of 1 µmol of substrate per minute.

1. Payne, M. S., Petrillo, K. L., Gavagan, J. E., DiCosimo, R. Wagner, L. W., Anton, D. L., Engineering *Pichia pastoris* for biocatalysis: co-production of two active enzymes, Gene, **194**, 179 (1997).
2. Subramanian, V., Das, S., Glenn, J. H., Patent Pending.