dehydrogenase, and powered by the hydrogenation of the hydrogen carrier nicitinamide adenine dinucleotide (NAD).

The process is continuous, with the cofactor covalently bound to polyethylene glycol and retained with the enzymes in a membrane reactor. In the second step ammonia is linked up by alanine dehydrogenase to give the amino acid and water, and the cofactor regenerated.

Enzyme deactivation (the main problem affecting the economics of biocatalytic processes) may not be a major drawback in scaling up the operation. At pH 9, all the enzymes used are operating at or near their maximum activity. A two-month test run saw no significant activity loss, although enzyme levels were topped up at 40 days.

Costs of the cofactor, originally believed to be critical, were found to be relatively unimportant. Up to 192,000 molecules of product were obtained for every molecule of NAD lost. Yields of 134 g alanine/liter of solution/day were possible, Wandrey said. However, after developing the process with colleagues from the Gesellschaft für Biotechnologische Forschung Braunschweig, he has no plans to scale up. He says he hopes that the major German amino acid supplier, Degussa, may be interested.

There is little commercial scope for developing the process with alanine, a non-essential amino acid. But provided the economics of the different enzymes needed for alternative products are attractive, there may be some future for the process. Methionine, already produced by Degussa and others via a cheap synthetic route, is an unlikely choice. But the market for phenylalanine is expanding because it is used in the sweetener aspartame. Companies in the U.S. and Japan are working on a single-stage enzymatic route in the belief (shared by the recent U.S. Office of Technology report) that, compared with conventional fermentation technology, a high-yield batch process would offset its higher feedstock costs.

—John Bonner

BIOTECH 84

CO-IMMOBILIZATION SPEEDS LACTOSE BREAKDOWN

LONDON—Another novel approach to the exploitation of cheap and abundant lactose in whey and other wastes was described at Biotech 84 by W. Hartmeier from the Technische Hochschule Aachen in West Germany. Hartmeier reported that co-immobilization of yeast or bacterial cells with beta-galactosidase had produced excellent results, and suggested several routes by which they might be improved even further.

The need and opportunity for such innovation stems from inadequacy of organisms like *Candida pseudotropicalis* and *Kluvyeromyces marxianus*. Although superior to brewing yeasts in possessing the beta-galactosidase necessary to break down lactose, these organisms ferment its hydrolysis products far more slowly. Soluble enzyme has been tried, but neither this nor earlier attempts at co-immobilization have proved fully satisfactory.

Hartmeier's team concentrated on two microorganisms—*Saccharomyces cerevisiae* and *Zymomonas mobilis*— and two methods of binding them to beta-galactosidase from *Aspergillus oryzae*. Adapting techniques they have evolved in recent years to link yeast cells with pepsin or glucoamylase (described in *Enzyme Technology*, Springer, 1983), they used glutaraldehyde to envelop every cell. The bacteria were embedded, along with enzyme cross-linked by glutaraldehyde, into alginate beads 2-4 mm in diameter.

Although immobilization caused a 30 percent drop in the yeast's fermentation rate (with glucose as substrate), both procedures resulted in substantial favorable changes in the performance of beta-galactosidase. It was more stable, with a lower activation energy (33.6 kJ/mol as against 45.1 kJ/mol for free enzyme) and K_m value (40.9 mM compared with 69 mM).

Kenneth Baker, Britain's Minister of State for Industry, told senior businessmen at Biotech 84 that they should seek university links and visit campuses at least once every six months. His department, he said, saw its role as encouraging innovation and removing obstacles to entrepreneurs. "Only industry has the insight into the market to pick winners," he said.

As anticipated, batch cultures of co-immobilized *S. cerevisiae* in a stirred tank fermented lactose highly efficiently as a substrate. But the enzyme activity declined rapidly from batch to batch—presumably as a result of shearing caused by stirring and by budding of the cells.

Continuous fermentation in a 314 ml packed-bed reactor proved to be a more stable system. The firmly anchored cells did not grow, Hartmeier reported, and there was no measurable loss of enzyme activity even in cultures maintained for up to three weeks under non-sterile conditions. Catabolite repression meant that the yeast metabolized galactose, released by lactose hydrolysis, only in the absence of glucose and if the yeast was adapted to this sugar.

Batch cultures of immobilized *Z. mobilis* also fermented lactose without metabolizing the liberated galactose. Although the fermentation rate fell considerably during five successive five-hour runs with whey as substrate, there was little loss of activity.

Initial tests with the alginate beads packed into a 65-70 ml column reactor were disappointing. Turbulence and channeling of the circulating liquid may have prevented optimal contact between substrate and the immobilized enzyme-bacteria complex.

But this problem has been overcome in a more recent series of tests with a 350 ml continuous column reactor, packed only half as densely with the alginate beads. In this set-up the biocatalysts remained stable for at least two weeks and probably three, even though maintained under non-sterile conditions.

Hartmeier, formerly head of the Boehringer Ingelheim Company's research laboratories for enzyme studies, believes he has validated the principle of producing ethanol from lactose-containing effluents using enzyme-microbe associations.

He says he might overcome the failure of his yeast and bacterium to ferment galactose either by selecting organisms not subject to catabolite repression, or by using a galactose-adapted yeast for a second stage in the process. From his limited but highly informative experience so far, Hartmeier also feels that attention to reactor design could effect considerable improvements in the productivity of his system.

Biotech 84 was organized by Online Conferences Ltd. Proceedings are available from Online Publications, Pinner Green House, Ash Hill Drive, Pinner, Middlesex, HA5 2AE, U.K.

—Bernard Dixon