

Researchers study lipase applications

Although no one is using lipases on a commercial scale to modify fats and oils, several groups are studying ways to make lipases a profitable method of fat modification. Scientists from industry and academia spoke about some of their work at the AOCS annual meeting in May in New Orleans.

Researchers at Akzo Chemie America in McCook, Illinois, for instance, have developed a patented lipase immobilization system which could make commercial-scale enzymatic hydrolysis as economical as steam splitting.

According to Catherine Brady of Akzo Chemie, initial cost estimates for the enzymatic process compare favorably with the Colgate-Emery steam process. Brady, one of the holders of U.S. Patent 4,629,742, said the company became interested in using enzymes as a low-energy alternative to steam splitting, but found that for the two processes to be equal in cost, soluble lipase prices could not exceed \$5 per pound.

"We had to find a way to immobilize the enzyme to bring the cost of using lipases in phase with steam splitting," Brady said. The group also needed a way to allow the immobilized enzyme to remain as active as it would be in soluble form; immobilization often reduces enzymatic activity enough to require the use of more enzymes, which wouldn't reduce costs, Brady explained.

When the enzyme was immobilized by adsorption from aqueous solutions onto microporous hydrophobic thermoplastic polymers, the lipase maintained high activity. The lipase's half-life and productivity were high enough to make the costs of the two fat-splitting methods comparable, Brady said. When the immobilized enzyme was tested in various reactor configurations such as cocurrent and counter-current fixed beds, stirred tank reactors and horizontal disposed diaphragms, the highest productivity was displayed with the diaphragm. According to Brady, the whole system will be evaluated

during the next few months before the company decides whether it will license the process.

Scientists at Unilever Research in England, meanwhile, are studying the use of lipases in microemulsions to commercially catalyze fats and oils interesterification reactions. According to Unilever scientist Alasdair Macrae, water immiscible organic solvent microemulsion systems may be advantageous in the bioconversion of lipids. Microemulsions are thermodynamically stable. The properties of the microemulsion's aqueous phase can be manipulated by varying the water-surfactant ratio and the chemical nature of the surfactant; microemulsions provide a large interface between the non-polar substrate-containing phase and the aqueous enzyme phase, Macrae said.

In experiments where microemulsions of lipases with phospholipids and hexane were formed, yields of interesterified triacylglycerol were as high as 92%, according to Macrae. Lipases in microemulsions with the anionic surfactant AOT (sodium bis-[2-ethylhexyl]-sulfosuccinic acid) also displayed interesterification activity, Macrae added.

Although the AOT and phospholipid systems have shown the ability to catalyze interesterification reactions, there are problems to solve before the method can be used commercially, Macrae said. For instance, purified lipases and pure phospholipid fractions are essential to successful microemulsions, and the enzymes and surfactants are difficult to recover for reuse. "Also, we still have doubts about lipase stability in microemulsions," Macrae said.

Alexander Klivanov, from the Massachusetts Institute of Technology's Department of Applied Biological Sciences, uses a different approach to alter fats and oils with lipases. Klivanov has found that lipases can carry out highly successful catalysis in completely nonaqueous systems.

"We have found that lipases do

not need water to carry out reactions. We have been able to carry out reactions to produce optically active compounds which would not be feasible in water. By replacing water with non-aqueous solvents, we can catalyze completely novel reactions," he said.

Using this system, lipases show enhanced stability at temperatures as high as 100 C. Their substrate specificities and regio- and stereospecificities also are altered. According to Klivanov, this method could be applied in the production of biosurfactants and peptides which could be useful in the pharmaceutical industry.

Other research reported included work from the Tokyo Institute of Technology and Genencor Inc., a California biotechnology company. The Tokyo group chemically modified lipases with the antipathic synthetic polymer, polyethylene glycol, so that the lipases would be more active in hydrophobic media. The system was found to catalyze various ester syntheses and interesterification reactions. Genencor researchers discussed the company's work in the enzyme engineering of lipases as a means to direct fat modifications (See main article).

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