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Final Report: Engineering Design of Stable Immobilized Enzymes for the Hydrolysis and Transesterification of Triglycerides

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Final Report:
Engineering Design of Stable Immobilized Enzymes for the Hydrolysis and Transesterification of Triglycerides

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Abstract: Enzyme Immobilization. Work will continue in the area of enzymatic transesterification reaction (biodiesel). Both methyl and ethyl esters will be used in this study. Unlike the chemical reaction where methanol has a clear advantage over ethanol, ethanol can be used as easily as methanol in the enzymatic reaction.
Sol/Gel Structure Modification. Work will concentrate on the effect of the vacuum procedure on pore size and distribution for the transesterification reaction. Additives such as glucose have been very effective in the hydrolysis reaction and will be explored further in the transesterification reaction.
Characterization. The developed material will be characterized for the pore size and distribution by electron microscopy and Brunauer-Emmett-Teller (BET) procedures. We also will use scanning electron microscopy and transition electron microscopy methods to investigate the distribution of the immobilized lipases within the supporting matrix.

Description:

Objective:

The objectives of this research project were as follows:

- Catalyst Improvement Strategies—to increase the specific surface area of the immobilized matrix within a specific range of macropore size.
- Materials Characterization—to provide feedback to the catalyst improvement studies regarding pore size distributions, specific surface areas, and enzyme distribution within the alcogel.
- Reactor Studies—to systematically feedback to the catalyst improvement studies on the reactivity, reusability, and stability of the immobilized enzyme.

Summary/Accomplishments:

Enzyme Immobilization

The immobilization of lipase PS from *Pseudomonas cepacia* by entrapment within a chemically inert hydrophobic sol-gel support was investigated first. The gel-entrapped lipase was prepared by the hydrolysis of tetramethoxysilane (TMOS) with methyltrimethoxysilane (MTMS), iso-butyltrimethoxysilane (iso-BTMS), and n--butyltrimethoxysilane (n-BTMS). The immobilized lipase subsequently was used in the hydrolysis of soybean oil to determine its activity, recyclability, and thermostability. The biocatalyst prepared equaled or was better in its hydrolytic activity relative to the free enzyme. The catalytic activity of the entrapped lipase strongly depended on the type of precursor that was used in its preparation. The entrapped lipase within TMOS/iso-BTMS showed the highest activity. The catalytic activity of the immobilized lipase was more pronounced during the earlier stages of the reaction. Thermostability of the lipase was

significantly improved in the immobilized form. The immobilized lipase was stable up to 70°C, whereas, for the free enzyme moderate to severe loss of activity was observed beyond 40°C . The immobilized lipase was consistently more active and stable than the free enzyme. The immobilized lipase also proved to be very stable as it retained more than 95 percent of its initial activity after 12 1-hour reactions.

Enzyme Immobilization—Structure Modification

The structure modification work involved the use of glucose and vacuum. Glucose was introduced with the monomers into the immobilization process, whereas vacuum was applied during the sol-gel aging step. Lipase AY from *Candida rugosa* was used in these experiments. The gel-entrapped lipase was prepared by polycondensation of hydrolyzed TMOS and iso-BTMS. Certain modifications were incorporated into the conventional immobilization procedure that included the use of glucose as additive (procedure 2), the application of vacuum during the drying and aging stages (procedure 3), and the combined use of glucose and vacuum (procedure 4). Procedure 1 was as described earlier for the immobilization of lipase PS. The immobilized lipase was subsequently used in the hydrolysis of soybean oil to determine its stability within the support structure as well as its thermostability. To examine this, the immobilized enzyme was subjected to a period of preincubation at the reaction temperature prior to the experiments. The hydrolysis reaction was then carried out for 1 hour. Results showed that the modified immobilized enzyme initially equaled in its hydrolytic activity relative to free enzyme and retained more than 95 percent of its activity after 120 hours of incubation at 40°C, whereas the free enzyme lost 67 percent of its activity after 24 hours of incubation and lost almost all of its activity after 96 hours of incubation at 40°C. Compared to our initial structure, the activity of the modified structure was higher by more than four-fold. The immobilized enzyme also proved to be very stable, as it retained more than 90 percent of it initial activity after 16 1-hour reactions. Surface characterization studies suggested that the enzyme containing sol-gel particles has amorphous morphology and is void of micro/meso pores.

Enzymatic transesterification of soybean oil with methanol and ethanol also was studied. Of the nine lipases that were tested in the initial screening, lipase PS from *P. cepacia* resulted in the highest yield of alkyl esters. Lipase from *P. cepacia* was further investigated in immobilized form. The gel-entrapped lipase was prepared by polycondensation of hydrolyzed TMOS and iso-BTMS. Using the immobilized lipase PS, the effects of water and alcohol concentration, enzyme loading, enzyme thermal stability, and temperature in the transesterification reaction were investigated. The optimal conditions for processing 10 g of soybean oil were: 35°C, 1:7.5 oil/methanol molar ratio, 0.5 g water, and 475 mg lipase for the reactions with methanol; and 35°C, 1:15.2 oil/ethanol molar ratio, 0.3 g water, 475 mg lipase for the reactions with ethanol. Subject to the optimal conditions, methyl and ethyl esters formation of 67 and 65 mol% in 1 hour of reaction were obtained for the immobilized enzyme reactions. At the same time, the triglycerides reached negligible levels, and the formation of fatty acid, mono- and di-glycerides are 8, 18, 7 mol% and 7, 22, 6 mol% for methanol and ethanol reaction, respectively. The immobilized lipase was consistently more active than the free enzyme. The immobilized lipase also proved to be stable and lost little activity when subjected to repeated uses.

Enzyme Immobilization—Structure Characterization

As was presented earlier, the immobilization of lipases within a chemically inert hydrophobic sol-gel support, which was prepared by polycondensation of hydrolyzed TMOS and MTMS or iso-BTMS, results in heterocatalysts. The heterocatalysts prepared were further characterized by nitrogen adsorption to determine their specific surface area. Solid state nuclear magnetic resonance (NMR) spectroscopy was used to reveal the degree of crosslinking of the sol-gel material. Scanning electron microscopy and atomic force microscopy were used to observe the morphology of the biocatalysts. Transmission electron microscopy and confocal microscopy were used to investigate the enzyme distribution within the sol-gel material. The comprehensive characterization showed that the most active lipase-containing sol-gel was a non-porous amorphous material with enzyme randomly distributed throughout the sol-gel material. The activity of the immobilized enzyme did not correlate to the cross-linking degree or the specific surface area of the sol-gel material. The enhanced activity of the immobilized enzyme was more likely attributed to the massive external surface area of the heterobiocatalyst, the favorable microenvironment of the sol-gel provide for the interfacial enzyme, and interaction between lipase and its hydrophilic support.

Conclusions:

In this research project, the immobilized-enzyme transesterification and hydrolysis of soybean oil was investigated. Lipase PS from *P. cepacia* and lipase AY from *C. rugosa* were immobilized by entrapment within a sol-gel structure that was prepared by polycondensation of hydrolyzed TMOS and iso-BTMS. The immobilized lipase prepared was consistently more active than the free lipase toward the hydrolysis and transesterification of soybean oil. The immobilized lipase also proved to be stable and lost little activity when it was subjected to repeated uses

Publications and Presentations: Total Count: 2

Type	Citation	Journal Searches
Journal Article	Noureddini H, Gao X, Joshi S. Immobilization of *Candida rugosa* lipase by sol-gel entrapment and its application in the hydrolysis of soybean oil. *Journal of the American Oil Chemists' Society* 2003;80(11):1077-1083.	• Dialog Citation Search
Journal Article	Noureddini H, Gao X, Philkana RS. Immobilized *Pseudomonas cepacia* lipase for biodiesel fuel production from soybean oil. *Bioresource Technology* 2005;96(7):769-777.	• Dialog Citation Search

Supplemental Keywords:

sustainable industry, waste, agricultural engineering, bioremediation, environmental engineering, new technology, innovative technology, bioaccumulation, biodegradation, bioenergy, bioengineering, biotechnology, phytoremediation, plant biotechnology, entrapment, immobilization, lipase, sol-gel, hydrolysis, transesterification, soybean oil, Pseudomonas cepacia, Candida rugosa, , Scientific Discipline, Sustainable Industry/Business, TREATMENT/CONTROL, Environmental Engineering, Genetics, New/Innovative technologies, Technology, agricultural oils, bioenergy, bioengineering, biotechnology, catalytic studies, hydrolysis, plant biotechnology, plant genes, reactor studies, soybean, transesterification

Relevant Websites:

http://www.cpbr.org

Progress and Final Reports:

2003 Progress Report
Original Abstract
