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Perspective

Short Note on Enzyme Immobilization

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DESCRIPTION

Enzymes are multipurpose catalysts in the laboratory and on a manufacturing scale. To broaden their applicability in the laboratory and to ensure their (re)use in manufacturing the stability of enzymes can frequently necessitate improvement. Immobilization can address the issue of enzymatic instability. Immobilization can also help to enable the employment of enzymes in dissimilar solvents, at excesses of pH and temperature and exceptionally high substrate concentrations. At the same time substrate-specificity, enantioselectivity and reactivity can be adapted. Enzyme immobilization technology is one of the important modern industrial biotechnologies. Since the commercial use of first immobilized enzymes in the 1960s, enzyme immobilization technologies and theories as well as immobilization resources and chemistry have gained quick improvement. Nowadays, the design of the immobilized enzymes, which suits diverse specific applications, has uncontrolled the traditional trial-and-error method and gradually transited to the rational design, which is categorized by the fact that the enzyme immobilization technology is nowadays used not only to realize the reuse of the expensive enzymes a better control of the procedure, but also to progress the enzyme catalytic functions such as activity, stability, and selectivity. To some extent, the enzyme immobilization technology is becoming an appreciative technology to the genetic engineering. Now, it is becoming increasingly appreciated that the accessibility of a robust immobilized enzyme in the initial stage of procedure improvement will definitively enable early insight into process development and save costs not only in procedure expansion but also in production. However, the lack of rules for selection of the process of immobilization and the performance to be expected of an immobilized enzyme for a definite application of a rational approach to the design of such vigorous immobilized enzymes.

Types of immobilization techniques

- Adsorption/electrostatic interaction
- Entrapment
- Covalent attachment

New generations of biosensors are developing that are based on novel and auspicious transducers such as miniature, reagent less-mediated electrodes, and field effect transistors, piezoelectric and optical devices. Reagent less-mediated biosensors can be made by co-immobilizing both enzymes and mediators onto a miniaturized electrode using electro polymerization, thus improving the sensitivity and speed of the response. Even more promising is the expansion of electrochemical sensors, in which electron transfer is prepared directly from a redox enzyme to an electrode surface via molecular wires. While this has only been reported, so far, for a specific enzyme entrapped in N-methyl pyrrole under definite conditions, the progress of new oriented immobilization techniques, coupled with development in protein engineering, may create direct electron transfer the rule rather than the exemption. Compared to free enzymes in solution, immobilized enzymes are more robust and more impervious to ecological modifications. More importantly, the heterogeneity of the immobilized enzyme systems permits an easy repossession of both enzymes and products, multiple re-use of enzymes, continuous operation of enzymatic procedures, quick termination of reactions, and greater variety of bioreactor designs.

Improvements in current approaches for carrier-based immobilization have been advanced using hetero-functionalized supports that improve the binding efficacy and stability through multipoint attachment. New commercial resins (sepabeads) show better protein binding capacity. Novel approaches of enzyme self-immobilization have been advanced (CLEC, CLEA, spherezyme), as well as carrier materials (dendrispheres), encapsulation (PEI microspheres), and entrapment. Apart from retention, repossession and stabilization, other advantages to enzyme immobilization have developed, such as enhanced enzyme activity, modification of substrate selectivity and enantioselectivity, and multi-enzyme reactions. These advances promise to improve the roles of immobilization enzymes in industry, while opening the door for novel applications.

Bio-mineralized uniform and well-organized calcium carbonate microspheres were manufactured for enzyme immobilization, and the immobilized enzyme was successfully stabilized. The physicochemical parameters of calcium carbonate were studied

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using scanning electron microscopy with energy-dispersive X-ray spectroscopy, particle size analysis, X-ray diffraction analysis, Fourier-transform infrared spectroscopy, and surface area measurement. Furthermore, Barrett-Joyner-Halenda adsorption/desorption analysis exhibited that the calcium carbonate microspheres provided effective mesopore space for enzyme loading. As a model enzyme, Carboxyl Esterase (CE) was entrapped and then cross-linked to form an enzyme structure. In this aggregate, the cross-linked enzymes cannot leach out from mesopores, resulting in enzyme stability. The hydrolytic activities of the free and cross-linked enzymes were analyzed over extensive temperature and pH ranges. The cross-linked enzyme showed better activity than the free enzyme. Furthermore, the immobilized CE was found to be stable for more than 30 days, preserving 60% of its primary activity even after being salvaged more than 10 times. This report is anticipated to be the first demonstration of a stabilized cross-linked enzyme system in calcium carbonate microspheres, which can be applied in enzyme catalyzed reactions intricate in bioprocessing, bioremediation, and bioconversion.