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The Commercial Exploitation of Immobilized Enzymes

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Abstract. The use of enzymes on the industrial scale has been imperfect, in spite of its exceptional catalytic properties. To improve on various properties like stability, activity and inhibition by reaction products, enzymes are immobilized by attachment to an insert. Immobilized enzymes are now being used in almost all sectors of industries including pharmaceuticals, textile, paper and pulp, food and many more. In the future, the immobilized enzyme technology will be a victim of commercial exploitation, both in terms of market and applications. This paper provides a preview on some of the present and future applications of immobilized enzymes.

Keywords: Immobilized enzymes, Applications, Diagnosis, Nano particles, Biodiesel.

1. Introduction

Enzymes are proteins that accelerate the rate of chemical reaction in a cell. But, since they are difficult to separate in a mixture, enzymes are immobilized by physical attachment to a solid support over which a substrate is passed and converted to product. Immobilized enzymes have the ability to improve the activity and stability of the product. These enzymes have non catalytic as well as catalytic functions [1]. Immobilized enzymes can be reused and the product recovery is comparatively easier.

There are different methods of enzyme immobilization and some of them are mentioned in table 1. The application of biocatalysts is limited, primarily because they are expensive, unstable and available in small quantities. Therefore, the development of immobilization technique has helped to overcome some of these problems. Nowadays, immobilized enzymes have various applications in process industries. Some of the major sectors include industrial catalysis, analytical application, medical/therapeutic application and bio - separation.

The use of immobilized enzymes has increased considerably in various other industries like pharmaceuticals, detergents, chemicals and food. Some examples of immobilized enzymes used in industries are listed in table 2. Today, the enzyme market is a significant market estimated at 3.4 billion pounds with an annual growth of 6.5 – 10% [12].

2. Present Applications of Immobilized Enzymes

The possibilities of using immobilized enzymes to carry out desirable reactions are endless. And so, there are numerous applications of immobilized enzymes and some of them are mentioned below.

2.1. Medical/Therapeutics

Immobilized enzymes are presently used in the diagnosis and treatment of various diseases. Some examples of enzymes used in this field are mentioned in table 3.

Immobilized enzymes are also used in biosensors and ELISA for detection of various bioactive substances in the diagnosis of disease states. Biosensors are also used for the removal of waste metabolites.

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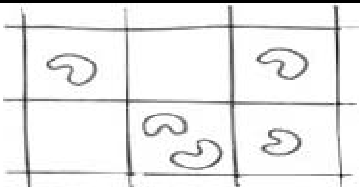
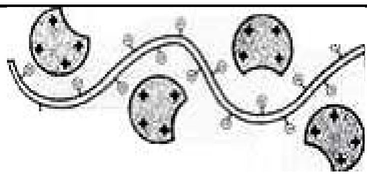
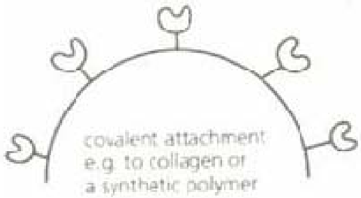
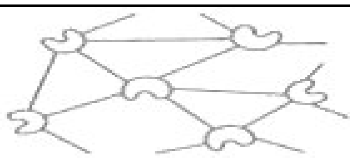
The most widely used biosensors are glucose dehydrogenase based electrodes that have been developed to monitor glucose concentration [8].

Since 1960's, significant efforts have been made to immobilize enzymes in a bioreactor for the correction of inborn errors of metabolism, blood detoxification and cancer treatment [8]. Because of numerous applications in the field of diagnostics and therapeutics, the use of immobilized enzymes can be studied further.

2.2. Food Industry

The application of immobilized enzymes in the food industry is increasing gradually. Food that contains starch can be manufactured with immobilized enzymes so as to introduce sweetness by partial conversion of fructose and to decrease the calorie content by partial conversion to gluconate [10]. Some of the applications in the food industry are:

Table 1: Different methods of immobilization [3, 4, 11]

METHODS	FIGURE	FEATURES
Entrapment		Only for small substrate and product. Enzymes are linked to natural polymers. Slow leakage and restricted mass transfer during catalytic process are problems.
Adsorption		The simplest method and the driving force are hydrophobic interaction and salt bridge. It does not alter the activity of the bound enzyme. Adsorption capacity is small.
Covalent Binding		Functional groups extensively involved are amino, carboxyl and phenolic group of tyrosine. Strong forces stabilize the enzyme. Very little leakage.
Direct cross linking		Solid support is not required. Glutaraldehyde is used to join the enzymes.

Production of high fructose syrup – One of the most studied industrial processes that makes use of immobilized enzymes is the transformation of glucose to fructose. Over the last few years this sector has seen enormous growth because of the controversies regarding the safety of artificial sweeteners. As a result, focus has shifted from cyclamates and saccharine to the use of sucrose and fructose. At present, immobilized glucose isomerase is used to obtain glucose high fructose syrup which contains 42% fructose, 50% glucose and 8% of other sugars [10]. In the future, amyloglucosidase based immobilization process could be used to meet the increased demand of glucose as a substrate for the production of high fructose syrup [10]. The amount of high fructose corn syrup produced over the year is estimated to be 9 million tonnes in Europe and the U.S [10].

Productions of amino acids – Amino acids are extensively used as food additives. In order to lower the production cost, immobilized enzymes are used for the production of amino acids. One of the most interesting features of this field is the use of immobilized whole microbial cell rather than immobilized purified enzymes. To site an example, Ammonium fumarate is used for the production of L – aspartic acid. Another example is the production of L – lysine by hydrolysis of DL – α aminocaptolactum which can easily be synthesized by cyclohexene [10].

2.3. Other Applications

Immobilized enzymes are used on a large scale for the production of detergents. Enzymes like proteinases, amylases and cellulases are immobilized using granulation for the production of detergents. Other applications include waste water treatment, removal of environmental pollutants from animal waste and many more [9].

3. Future Applications of Immobilized Enzymes

In the past few years, the understanding and use of immobilized enzymes has increased on a commercial level. There are various sectors that can benefit from proper research and development on immobilized enzymes. Some future applications have been briefly highlighted below.

Table 2: Industrial Applications of Immobilized Enzymes [5].

Industry	Enzyme Class	Applications
Detergent	Subtilisin	Protein stain removal, Detergent formulations
	Lipase	Fatty acid and oily stain removal
	Cellulase	Colour clarification, cleaning
	Peroxidase	Dye removal
Fuel	Lipases	Biodiesel [FAME] production
	Glycosidase	Saccharification
Food	Glucose Isomerase	Production of HFCS
	β-Galactosidase	Hydrolysis of lactose in dairy products
	Lipases	Dairy, Baking, Fats/Oils
	Transglutaminase	Modify visco-elastic properties, strengthens dough
Pharmaceuticals	Penicillin acylase	Synthesis of 6-APA for production of penicillin
Chemicals	Lipase	Resolution of chiral alcohols and amines

Table 3: Enzymes used in medicine/therapeutics and their application [9].

ENZYME	ENZYME APPLICATION
B – galactosidase	Production of lactose in the small intestine for lactose digestion
Streptokinase	Treatment of thromboembolic disorders
Uricase	Oxidation of urate to allantoin to decrease the urate blood level
Alcohol dehydrogenase	Treatment of liver disorders

3.1. Immobilization Using Nanotechnology

The future of immobilized enzymes is nanostructures which include nanofibres, carbon nanotubes and other nanoparticles. Enzymes such as trypsin are immobilized on the nanostructures using simple adsorption and covalent attachment. This provides a larger surface area resulting in improved enzyme loading, which in turn increases the enzymatic activity per unit mass or volume [7]. The enzymes are immobilized by nanoentrapment, which leads to discrete nanoparticles through polymerization in water phase or water oil interface [7].

Enzyme immobilization using nanoparticles has attracted a lot of attention owing to their porosity and higher surface area. To purge the problem of leaching, covalent linkage has been used which attaches the enzyme molecule to the inner surface of the nanoporous media [7].

Nowadays, Ship - in – a – bottle approach is used for the stabilization of enzyme with a bottle neck structure. This approach prevents the leaching of enzyme thereby improving both the enzymatic loading and enzymatic activity. Another advantage of this technique is that the enzyme coated system could be repeatedly recycled and can be run in a continuous reactor over a long period of time. Nanoporous silica has been successfully employed as a nanoreactor for protein digestion. This has resulted in better digestion of trypsin. Some other enzymes that have been that have been immobilized in nanoparticles are proteases like subtilisin, chymotrypsin, pepsin and papain which have been added into antifouling paints to reduce protein binding on the surface [7].

This promising technology can also be used to craft biofuel cells for the generation of electricity. These cells can be used in low powered sensors, communication devices and medical implants [7]. Nanotechnology may have new application in water remediation. In the future nano scale catalysts may be used for treating contaminants in water. So far, this technique is not commercially viable [9].

The use of nanotechnology for enzyme immobilization will provide new opportunities in the area of enzyme application. In depth knowledge and understanding of the various nanoscale interactions will aid the development of nanobiocatalysis approach, which will lead to numerous applications of enzyme technology.

3.2. Immobilized Lipase for the Production of Biodiesel

Due to the limited energy reserves and increased amount of green house gases from fossil fuels, biodiesel has become a hot topic of discussion in many countries [11]. Most of the biodiesel production is done by chemical methods which involve the use of catalyst such as H_2SO_4 and NaOH. But these methods have some drawbacks such as high energy consumption and significant amount of waste water. Therefore, the use of enzymes is now being exploited. Enzyme lipase is immobilized and used for the production of biodiesel as it provides excellent catalytic activity and stability in non aqueous media, which facilitates the esterification and transesterification process during biodiesel production [11]. Adsorption technique is carried out under mild conditions to immobilized lipase. This process is relatively easy and cheap [11, 13].

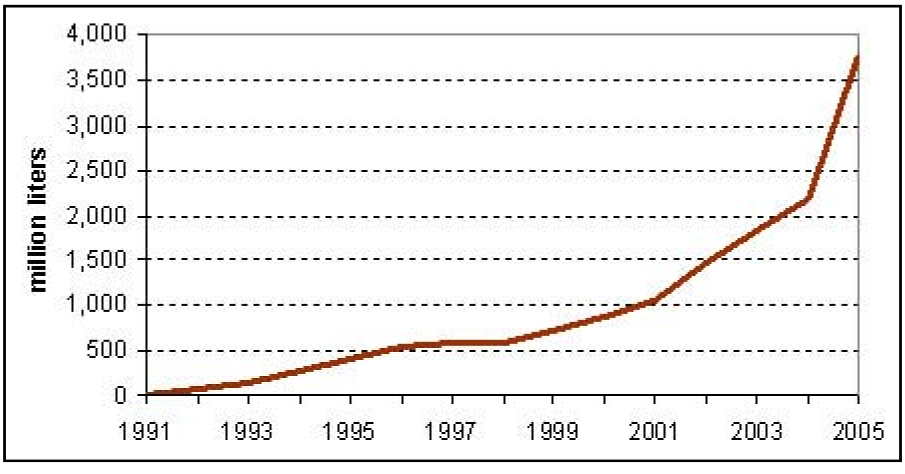


Fig. 1: World biodiesel production [2].

The best studied examples for biodiesel production from sources like soyabean oil, cottonseed oil, and waste oil are Novozyme 435 and Candida sp. 99 – 125[11]. These enzymes are immobilized on a textile membrane, though new techniques of enzyme immobilization are still being developed [11]. Figure 1 shows the production of biodiesel from 1991 – 2005. The production of biodiesel has increased rapidly since 2001 and is expected to reach the 37 billion gallons mark by the end of 2016 [6]. For a better future, further reduction in the cost of biodiesel production and new immobilization techniques with higher activity and stability need to be explored.

3.3. Other Future Prospects

Immobilized enzymes can be of great help in national security, for example, biocatalysts may be incorporated into air filters, masks and clothing to neutralize chemical gases or vapors. These enzymes may also be used in the treatment of pesticide contaminated waste, thereby reducing the impact of pesticides on the environment. Enzymes like glucose oxidase can find application in removal of oxygen from beverages and production of gluconic acid [9].

4. Conclusion

Today, various types of immobilized enzymes are employed in manufacturing food, fuel, clothes, and other products. Despite the momentous advances made in the past decade, there is still scope for improvement. Drawbacks such as enzyme cost, low reaction yield and low biodiversity need to be tackled and worked upon.

In the future, immobilized enzymes are going to play a vital role in various industries including pharmaceuticals, chemicals, food and fuel industry. According to market analysts, the global enzyme market is expected to reach U.S $ 4.3 Billion by 2015 [5]. With the growing interest in the field of immobilized enzymes and advances in biotechnology, the enzyme market is expected to surpass this number in the coming years.

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