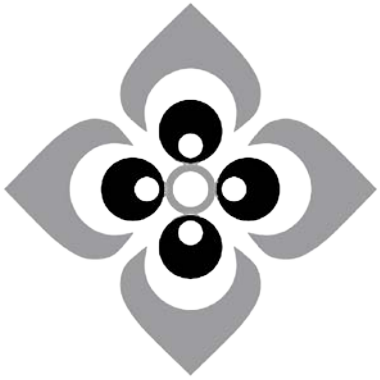

Consortium for Educational Communication

Module
on
Use Of Immobilized Enzymes In Food Industry

By
IRAM ABDULLAH
PhD Research Scholar
Department Of Zoology
University of Kashmir.
Email ID: miriram07@gmail.com
Mobile no. 9858128849

TEXT

Introduction

Enzymes are biological catalysts that promote the transformation of chemical species in living systems. Word "immobilize" means to make anything unable to move at its own. The term immobilized enzymes therefore refers to "Enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously." In other words it is the imprisonment of free or soluble enzymes to different types of supports resulting in reduction or loss of mobility of enzyme that allows its interaction with substrate effectors or inhibitor molecules but it is separate from them". Immobilized enzymes are also sometimes referred to as sound, insolubilized, supported or matrix-linked enzymes.

SALIENT FEATURES OF ENZYME IMMOBILIZATION:-

1. The enzyme phase is called as carrier phase which is water insoluble but hydrophilic porous polymeric matrix, e.g. agarose, cellulose, etc.
2. The enzyme phase may be in the form of fine particulate, membranous, or microcapsule.
3. The enzyme in turn may be bound to another enzyme via cross linking.
4. A special module is produced employing immobilization techniques through which fluid can pass easily, transforming substrate into product and at the same time facilitating the easy removal of catalyst from the product as it leaves the reactor.
5. The support or carrier utilized in immobilization technique is not stable at particular pH, ionic strength, or solvent conditions. Hence, may be disrupted or dissolved releasing the enzyme component after the reaction.

Enzymes have been used in the food industry for many years. They have largely been used as processing aids and they have many attributes that make them fit for this purpose. They are generally non-toxic and speed up chemical reactions with great specificity at low temperatures and pressures and at near-neutral pH. A large industry exists to serve this need across the world. One of the limitations of enzyme application in the food industry is the lack of availability of enzymes with the required properties at an acceptable price. The immobilization technique would enable the reusability of enzymes for tens of times, thus reducing the enzyme and product costs significantly.The history of immobilized enzymes dates back to 1919 when Nelson and Griffin observed that Yeast invertase absorbed on charcoal was able to catalyse the hydrolysis of sucrose. The first industrial use of immobilized enzymes was reported in 1966 by Chibata and coworkers, who developed the immobilization of *Aspergillus oryzae* aminoacylase for the resolution of synthetic racemic D - L amino acids.

Need for enzyme immobiliztion

As enzymes are biological catalysts that promote the rate of reactions but are not themselves consumed in the reactions; they may be used repeatedly for as long as they remain active. However, in most of the processes, enzymes are mixed in a solution with substrates and cannot be economically recovered after the reaction and are generally wasted. Thus, there is an incentive to use enzymes in an immobilized or insolubilized form so that they may be retained in a biochemical reactor for further catalysis.

Techniques for enzyme immobilization

The enzymes can be attached to the support by interactions ranging from reversible physical adsorption and ionic linkages to stable covalent bonds. Although the choice of the most appropriate immobilization technique depends on the nature of the enzyme and the carrier. Several techniques have been used for enzyme immobilization including entrapment, cross-linking, adsorption, or alternatively a combination of these methodologies. Industrial surfaces such as glass beads, nylon-6, chitosan, Eupergit C (epoxy-activated acrylic beads) and agaroses have been variably used for this purpose. However, efficient commercial carriers suitable for the immobilization of enzymes are fairly expensive. Accordingly, the need for a cheaper carrier is still an industrial dream, to compensate the high costs of their entire inputs. Techniques used for the immobilization of enzyme activity may be classified as:

1. Covalent attachment of enzymes to solid supports

In the laboratory a variety of supports have been used, e.g. porous glass and ceramics, stainless steel, sand, charcoal, cellulose, synthetic polymers, and metallic oxides. Enzymes are usually immobilized through their amino or carboxyl groups. In most instances, the immobilization procedure consists of at least two steps: activation of the support and enzyme attachment.

2. Adsorption of enzymes on to solid supports

Ion exchangers readily adsorb most proteins and have therefore been used for enzyme immobilization. This immobilization procedure is simple: an enzyme solution is added to the support, mixed, and surplus enzyme is then removed by washing.

3. Entrapment of enzymes in polymeric gels

In this approach an enzyme is added to a solution of monomers before the gel is formed. Gel formation is then initiated either by altering the temperature or by adding a gel-inducing chemical. As a result, the enzyme becomes trapped in the gel.

4. Cross-linking of enzymes with bifunctional reagents

Among the most popular cross-linkers are glutaraldehyde, dimethyl adipimidate, and aliphatic diamines. The first two directly cross-link enzymes through their 124 PauL B. POULSEN amino groups. Diamines cross-link enzymes through carboxyl groups following activation of these groups with carbodiimides. Cross linking may be both intermolecular and intramolecular.

5. Encapsulation of enzymes/soluble enzymes in semipermeable membrane reactors

In this approach, enzymes are enveloped within various forms of membranes (e.g. between sheets or within hollow capsules or fibres consisting of semipermeable membranes) that are impermeable to enzymes and other macromolecules but permeable to substrates and products of low molecular weight.

Advantages of Immobilized enzymes

Several potential advantages of immobilized enzymes include:

(i) Greater productivity per unit of enzyme since the enzyme is reused.

(ii) Precise control over the reaction, which is often automated and continuous.

(iii) Material handling is minimized.

(iv) Recovery of enzyme free product as product does not contain the biocatalyst.

(v) Ability to terminate reaction at any stage by the removal of insoluble enzyme,

(vi) Enzyme activity may be enhanced and stabilized and

(vii) A unique product may be produced.

(viii) Since the biocatalyst is often used in a reactor, it is fairly easy to automate the process and control the extent of the reaction by simply altering the flow rate through the reactor (residence time).

Use of immobilized enzymes in food industry

There are very few examples of commercial processes that utilize immobilized enzymes for food constituent modifications. The two most successful examples of the use of immobilized enzymes are the production of high-fructose corn syrup and trans-free oils. High fructose corn syrup can only be produced using the immobilized form of glucose isomerase and for immobilized lipases, the enzymes are more stable and active in low aqueous systems when immobilized. Therefore the use of the immobilized form of the enzymes for these processes is economical.

Production of high-fructose corn syrups

One of the most studied industrial processes that makes use of immobilized enzymes is the production of High fructose corn syrup (HFCS). High fructose corn syrup (HFCS) is a liquid alternative sweetener to sucrose that is made from corn, using chemicals and enzymes (α-amylase and glucoamylase) to hydrolyze corn starch to corn syrup containing mostly glucose and a third enzyme (glucose isomerase) to isomerize glucose in corn syrup to fructose to yield HFCS products. The production of high-fructose corn syrup (HFCS) involves the use of immobilized D-glucose isomerase. Glucose isomerase is an intracellular enzyme which is found in several microorganisms and used in the production of fructose from glucose. Fructose syrup is competing with sucrose on the industrial market. Ideally glucose isomerase will produce an equilibrium mixture of glucose and fructose, although in practice (for economic reasons) the mixture will contain about 42% fructose, 52% glucose and 6% dextrins. This mixture is sweeter, light for weight, than glucose and about as sweet as sucrose. The enzymatic production of HFCS is an example where the use of enzymes is more desirable than the use of chemicals. The chemical conversion of starch to HFCS results in production of non-metabolizable sugars, colored products and reduced sweetness. The use of enzymes allows the production of HFCS under ambient pH and temperature, where fewer side products are formed and a higher fructose concentration is achieved.

Lactose hydrolysis

β-galactosidase is widely used in milk industries for hydrolysis of lactose to glucose and galactose. Lactose is the main carbohydrate contained in milk at a concentration between 5% and 10% depending on the source of milk. Lactose can also be found in whey permeate at higher concentrations. The consumption of foods with a high content of lactose presents a medical problem for approximately 70% of the world population, especially in the developing countries, as the naturally present enzyme in the human intestine, loses its activity during lifetime. Undigested lactose in chyme retains fluid, bacterial fermentation of lactose results in production of gases, diarrhoea, and bloating, abdominal cramps after consumption of milk and other dairy products. Unfortunately, there is no cure to lactose intolerance. This fact, together with the relatively low solubility and sweetness of lactose, has led to an increasing interest in the development of industrial processes to hydrolyze the lactose contained in dairy products (milk and whey) with both the free and immobilized conditions. In this respect, a large number of immobilization techniques were reported from time to time. Examples include covalent attachment of lactase to glass, collagen, sepharose, entrapment of enzyme in hollow fiber, polyacrylamide gel, and cellulose acetate and in ionizing radiation induced polymers of acrylate and methyacrylate. Lactase immobilized by binding on cellulose sheets was found to be stable for several months. *Aspergillus* lactase immobilized on controlled pore glass or titanium was reported to be available at a very low cost. The greatest problems associated with

immobilized β-galactosidase for lactose hydrolysis are contamination from microbial growth and cost. Glucose and galactose products hydrolyzed from lactose, have been found to be 4 times sweeter than lactose, more soluble, more digestible and can be consumed by 'lactose intolerant' people. Hydrolysis of lactose present in whey permeate will produce lactose-free syrup, solving an aquatic pollution problem, since whey is commonly thrown in water sources. It is also used as a syrup in dairy and confectionery products.

Production of trans-free oils

Regular soy oil contains approximately 54% linoleic acid (18:2), 23% oleic acid (18:1), 11% palmitic acid (16:0), 8% linolenic acid (18:3), and 4% steric acid (18:0). This fatty acid profile is high in unsaturated fatty acids, which is healthy, but shows poor oxidative stability. To improve oxidative stability, partial hydrogenation is often used but this does produce trans fats, which have adverse health effects. Changing the fatty acid content can be done chemically by interesterification, which is simple and inexpensive but random. No trans fatty acids are produced but the product requires thorough purification to remove by-products after the interesterification reaction. The fatty acid content of triacylglycerols can also be changed enzymatically with lipases in very low aqueous environments. An example reaction utilizing an immobilized lipase, commonly a sn-1,3 specific lipase, in a low aqueous environment with soy oil and free fatty acid (oleic acid) can result in the production of soy oil with a higher oleic acid content with improved oxidative stability. The final oil does need to be deodorized to remove residual free fatty acids. The commercial production of enzymatically interesterified oils for the production of trans-free fats is being done in the USA as well as several other countries. The process is based on Novozymes immobilized lipase system**.** The Novozymes immobilized lipase, Lipozyme TL IM, is a 1,3 specific lipase from *Thermomyces lanuginose* that shows a degree of conversion between 30% and 90%. According to Novozymes association, the immobilized lipase system is economical compared with both partial hydrogenation and chemical interesterification if operating and investment cost are considered. The first commercial production of trans-free oils using Novozyme's immobilized lipase system in the USA was in 2002 by Archer Daniels Midland Company (ADM) in Quincy, Il.

Production of Healthy Cooking Oils.

The Japan based Kao Corp has launched a new diacylglycerol (DAG) product under the name Healthy Econa Cooking Oil which is made enzymatically from natural oil using immobilized lipases. This product is marketed as a healthy oil since DAG aids in the maintenance or loss of weight and fat mass, may lower the level of cholesterol in the body and may help maintain healthy triacylglycerol levels. DAG is digested and absorbed in the small intestine and is consumed as energy without resynthesizing into a neutral fat like conventional oil. As a result, it reduces the level of neutral fat in the blood compared with conventional oil . A variety of Healthy Econa salad and cooking oils are sold in Japan and also used in processed products

such as canned tuna fish, margarine and bread. The US counterpart of Healthy Econa Cooking Oil is EnovaTM oil produced by Archer Daniels Midland (Decatur, Il).

Production of cocoa butter equivalents

Lipases are used to change the melting properties of fats to create a higher value product. Cocoa butter has a melting point of 37 °C which is attributed to its fatty acid content. The major components of cocoa butter are 1(3)-palmitoyl-3(1)-stearoyl-2-mono-olein (POS) and 1,3-distearoyl-2-mono-olein (SOS) which are 52 and 18.4%, respectively, of the total. This sharp melting point is related to consumer acceptance of chocolate. Immobilized lipases are used for the interesterification of palm oil mid-fraction which contains high concentrations of triacylglycerols with palmitic acid in the sn-1 and sn-3 positions and oleic acid in the sn-2 position. In the presence of free steric acid, interesterification of palm oil mid fraction with an sn-1,3 specific lipase results in triacylglycerol composition of POS and SOS which resembles cocoa butter and is used as cocoa butter equivalent in the confectionery industry.

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. e.g 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.

Synthesis of functional oligosaccharides

Immobilized enzymes are used for the synthesis of various oligosaccharides like isomalto-, xylo, fructo- and inulo-oligosaccharides for use in foods. These sugars can act as soluble dietary fibers that are also prebiotics, stimulating the growth of probiotic microorganisms such as Bifidobacterium spp. in the colon and Lactobacillus spp. in the gut. Oligosaccharides derived from starch include malto- and isomalto-oligosaccharides, isomaltose, cyclodextrins and trehalose. Sucrose-derived oligosaccharides include fructo-oligosaccharides, isomaltulose, and glycosylsucrose. Lactose-derived oligosaccharides include galacto-oligosaccharides, lactosucrose, lactulose and lactitol. Enzymes used for the production of functional oligosaccharides are typically microbial enzymes and are used to produce functional oligosaccharides via immobilized purified enzyme, entrapped microbial cells, or conventional batch reactions. Sugar can be converted to isomaltulose (palatinose) and the by-product trehalulose using isomaltulose synthase. Isomaltulose is a low-calorie reducing sugar found naturally in honey. It has several characteristics that are advantageous compared with sucrose including stability in acid solutions, promoting bifidobacteria growth in the human

intestine and it is non-cariogenic. Fructo-oligosaccharides (FOS) are produced from sucrose by the transfructosylation action of fungal beta-fructofuranosidase. FOS are nondigestible sweeteners, which are utilized by intestinal bifidobacteria. Inulin is another source of functional oligosaccharides, which is found in garlic, asparagus root, Jerusalem artichoke, dahlia tubers and chicory roots. Inulin consists of linear 1-2 linked fructose molecules which, when hydrolyzed, yield fructose syrups or oligofructose (inulo-oligosaccharides). Production of inulooligosaccharides can be done with either the immobilized endoinulinase (typically produced by A. niger) or whole cells. Isomalto-oligosaccharides are produced from sucrose using immobilized dextransucrase from Leuconostoc mesenteroides.

Production of jams, jellies and fruit juice

Immobilized pectinases and cellulases are used in preparation of jams, jellies and syrups from fruits and vegetables. The bitterness in citrus fruit juice is mainly due to the presence of naringenin which makes the product unacceptable by some consumers. Immobilized naringinase is used for removal of this bitterness.

As Clarifying agents in beer

Papain is used as clarifying agent. However when this proteolytic enzyme remains in beer for a long time, excess proteolysis occurs and this is undesirable. Utilization of immobilized papain has been studied for controlled treatment to prevent chill-haze in beer.

Tagatose production

D-Tagatose is a naturally occurring monosaccharide, which can be found naturally in small amounts in dairy products and it is 92% as sweet as sucrose with only 38% of the calories. D-Tagatose has generally recognized as safe (GRAS) status in the USA as a sweetener for use in foods. It can be produced from galactose via isomerization under alkaline conditions or can be purified from the mixture of D- and L-tagatose by crystallization. Tagatose can also be formed from galactose using L-arabinose isomerase either directly immobilized onto a support or via the immobilization of cells. In addition, a recent process involves preparing D-tagatose from galactose using an immobilized form of a thermostable L-arabinose isomerase enzyme derived from *Thermotoga neapolitana*.

Production of protein hydrolysates

Immobilized proteases are used for production of bioactive peptides, hydrolysates for nutritional supplements and hypoallergenic infant formulas, or to change the functionality of protein systems. The degree of hydrolysis varies from low hydrolysis to improve functional properties of proteins to extensive hydrolysis for nutritional supplements and hypoallergenic foods. Numerous immobilized enzymes are used for the complete hydrolysis of food proteins for the determination of protein digestibility, which is directly related to protein quality called as

immobilized digestive enzyme assay (IDEA). This IDEA system could also be used to produce free amino acids for use in medical foods. e.g. The immobilized enzymes consisting of trypsin, α-chymotrypsin and carboxypeptidase A are used for enzymatic hydrolysis of casein to obtain a hydrolyzed product with a high ratio of branched-chain amino acids (BCAA) to aromatic amino acids (AAA) which has the potential use as a medical food for patients suffering from hepatic encephalophathies, tyrosinemia and phenylketonuria. The immobilized proteases have been used to hydrolyse casein and predigest soybean protein for use in infant formulas or as a medical food. Many medical foods have been formulated using protein hydrolysates from casein, soy, or wheat for patients with Crohn's disease, ulcerative colitis, short bowel syndrome, food allergies and for infant formula. Bioactive peptides have been identified in protein hydrolysates from milk, fish, corn, eggs and cereals. In addition, immobilized trypsin has been used to liberate phosphopeptides from casein.

Production of flavor compounds

A). Ester flavor synthesis

Fatty acid esters can be synthesized by immobilized lipases. The reaction generally involves esterification of a carboxylic acid (C4 or longer fatty acids) with an alcohol in non-aqueous (heptane or hexane) or low water environments. Various esters can be synthesized for application as flavors or surfactants. Flavor compounds are typically short chain fatty acids and alcohols such as ethyl butyrate (pineapple or strawberry flavor), methyl butyrate (pineapple or apple flavor), butyl butyrate and isobutyl isobutyrate (pineapple flavor), isoamyl isovalerate (apple flavor) and isoamyl acetate/butyrate (banana flavor). Ester production can be done by reacting a fatty acid and alcohol at high temperature in the presence of a metal catalyst. This can lead to undesirable side products, hence the use of immobilized lipases offers synthesis under milder conditions for the production of natural flavors. Other examples of the use of immobilized lipases for flavor ester production via esterification of an alcohol and fatty acid in organic solutions include the production of butyl butyrate from butyric acid and butanol, isoamyl butyrate from butyric acid and isoamyl alcohol, isoamyl isovalerate from isovaleric acid and isoamyl alcohol, isoamyl isobutyrate from isobutyric acid and isoamyl alcohol and isobutyl isobutyrate from isobutyric acid and isobutyl alcohol. For example, Lipozyme IM is used to catalyse a reaction between triacetin as the acyl donor and cis-3-hexen-1-ol in hexane to form a hexyl ester, which has a fruity odor.

B). Aspartame synthesis (artificial sweetener)

Aspartame can by synthesized chemically from aspartic acid and phenylalanine but it results in the formation of an optical isomer that has a bitter taste. Thermolysin is an endopeptidase that catalyses the synthesis of peptides such as the precursor of the artificial sweetener, aspartame. The commercial source of the enzyme is derived from Bacillus thermoproteolyticus. The

advantages to the enzymatic synthesis of aspartame include the fact that the enzyme only recognizes the L isomer of the phenylalanine and the β-carboxyl group of aspartate does not require protection and deprotection to prevent formation of the bitter isomer. In the enzymatic reaction, the N-protected-aspartame derivative produced is insoluble in a biphasic system of water and organic solvent (ethyl acetate or tert-amyl alcohol) and thus shifts the equilibrium to favor synthesis of the peptide. The protecting group can then be removed either chemically or biologically to yield aspartame. Scientists have developed a process using immobilized ThermolaseTM to condense carbobenzoxy-L-aspartate and D,L-phenylalanine methyl ester.