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PATENTS AND LITERATURE
Immobilized Biocatalysts

ROBERT J. LINHARDT

Medicinal and Natural Products Chemistry, College of Pharmacy
University of Iowa, Iowa City, IA 52242

ABSTRACT

Immobilized biocatalysts, including immobilized microbial cells, mammalian cells, and enzymes, have been the object of considerable industrial and academic research. A variety of methods and supports are used for preparing these biocatalysts. Applications for immobilized biocatalysts include the production of chemical, pharmaceutical, and food products in both aqueous and nonaqueous solvents by microbial transformation, bioconversion, and enzymatic reaction. Recent US patents and scientific literature on immobilized biocatalysts are surveyed. A description of these patents and a list of references are given.

INTRODUCTION

The objective of the Patents and Literature Section is to keep readers aware of significant inventions and trends in industrial research, as well as to highlight those areas of research that may lead to new biotechnolog ical opportunities. Three subject areas will be surveyed in 1987: immobilized biocatalysts; monoclonal and immobilized antibodies; and bioassays based on immunological, enzyme, gene probe, and electrochemical methods. The subject of the first Patent and Literature Section of 1987 is Immobilized Biocatalysts.

Patents

This section identifies and gives a brief description of patents from US patent literature from January 1985 to October 1986. The major search

heading was immobilized (minor search headings included adsorbed, entrapped, encapsulated, microencapsulated, bound, and cross-linked) with the cross-terms: enzymes, cells, and microbes. Both patent abstracts and titles were searched. Copies of US Patents can be obtained for $1.50 each from the Commissioner of Patents and Trademarks, Washington, DC 20231.

Amotz, S., Rugh, S., Markussen, E. K., and Thomsen, K.
CARRIER FOR IMMOBILIZING ENZYMES
US 4,572,897, Feb. 25, 1986
Assignee: Novo Industri A/S

Immobilized enzyme granules are prepared by mixing inert filler into a solution or dispersion of water soluble binder, followed by granulating the mixture. Enzyme is immobilized on hydrophilic surfaces of the carrier granules. A water-soluble binder in the granules can be rendered insoluble. Proteins are preferred binder materials, and glutaraldehyde is used for bonding enzyme to the carrier granules and for water insolubilizing the binder.

Bigwood, M. P., and Naples, J. O.
OXIRANE RESINS FOR ENZYME IMMOBILIZATION
US 4,612,288, Sept. 16, 1986
and
US 4,582,860, Apr. 15, 1986
Assignee: Rohm and Haas Co.

Carriers for immobilizing enzymes may be prepared by suspension copolymerizing an oxirane-group-bearing, monovinyl monomer and a major amount of a trivinyl cross-linking monomer having a hydrophilic character, in the presence of a phase separator that does not react with the oxirane group. The resulting carriers, in bead form, have high porosity, high surface area, and pores of diameter sufficient for ready penetration by enzymes and substrates.

Blattler, W. A., Lambert, J. M., and Senter, P.D.
ACID-CLEAVABLE COMPOUND
US 4,542,225, Sep. 17, 1986
and
ACID-CLEAVABLE COMPOUND, USE IN PROTEIN CONJUGATES AND DRUG DELIVERY SYSTEMS
US 4,569,789, Feb. 11, 1986
Assignee: Dana-Farber Cancer Institute, Inc.

Amino-sulfhydryl cross-linking reagents that are cleavable under mildly acidic conditions are described. Methods of making the cross-linkers, as well as using the cross-linkers, are described. Uses include delivery of a biologically active substance across the membranes of selected cells in a

heterogeneous cell population; once inside the cell the active substance is released, intact, by the transient, mild acidity of certain cell structures. Finally, a method of characterizing complex multichain protein structures is also described.

Boross, L. E., Szaj, E., and Kovacs, K.
IMMOBILIZED CHOLINESTERASE ENZYME PREPARATIONS AND A PROCESS FOR THE PREPARATION THEREOF
US 4,556,637, Dec. 3, 1985
Assignee: Reanal Finomvegyszergyar

Immobilized cholinesterases are prepared by treating a polymeric resin, built up from acrylic acid and/or methacrylic acid and acryl amide and/or methacryl amide monomers with an acryl- or allyl-type cross-linking agent and containing at least 0.1 meq/g of carboxylate functional groups. These carboxylate groups are activated with a carbodiimide that is soluble in water or in an organic solvent at temperatures below 0°C. A solution of cholinesterase (pH of 4.5–8.5) is applied to the activated support, washed, and dried.

Boross, L. E., Dar, E. Ivony, K. K., Seres, G. E. Szanj, E., and Szejtli, J.E.
PROCESS FOR THE IMMOBILIZATION OF CYCLODEXTRINE GLYCOSYLTRANSFERASE ENZYME
US 4,593,004, Jun. 3, 1986
Assignee: Reanal Finomvegyszergyar

A process for the preparation of an immobilized cyclodextrine glycosyltransferase is described. Immobilization can be carried out by two methods. In the first, a cyclodextrine glycosyltransferase enzyme is activated with a solution of a carbodimide and is then applied at a pH of 4.5–8.5 to a polysaccharide derivative having >0.1 meq/g of free amino groups. In the second method the enzyme, in a solution of pH 4.5–8.5, is applied to a polymer produced from an acrylic acid and/or methacrylic acid or acrylic amide and/or methacrylic amide monomer by means of a cross-linking agent of the acrylic or allylic type. The polymer has at least >0.1 meq/g of functional carboxy group, which is activated with a solution of a carbodiimide, washed, and dried. The advantage of this process is that the immobilized enzyme is suitable for long lasting application and can be readily used on industrial-scale, continuous production.

Bozzelli, J. W., and Cheng, R. C.
IMMOBILIZATION OF BIOLOGICAL MATTER VIA COPOLYMERS OF ISOCYANATOALKYL ESTERS
US 4,582,805, Apr. 15, 1986
Assignee: The Dow Chemical Co.

The chemical immobilization of biological material, such as bacteria and enzymes, is described. A vinyl addition polymer of an isocyanatoalkyl

ester of an ethylenically unsaturated carboxylic acid is used. The vinyl addition polymer is versatile in that it can be copolymerized with varying amounts and types of comonomers.

Calvo, L. C.
IMMOBILIZED ENZYMES
US 4,556,554, Dec. 3, 1985
Assignee: Germaine Monteil Cosmetiques Corp.

A cosmetic composition is provided for the removal of sebum exudate from the skin. The composition contains immobilized enzymes in cosmetically acceptable vehicles for topical application. The immobilized enzymes are lipolytic lipases, proteolytic proteases, and enzymes for the breakdown of sugar oligomers from glycoproteins in the skin exudates. The enzymes may be present individually or in any desired combination. The enzymes are immobilized by chemical and/or physical means and are released upon application to the skin.

Campbell, D. N., and Schmidt, J. C.
ENZYMATIC TOXIC GAS SENSOR
US 4,525,704, Jun. 25, 1985
Assignee: Allied Corp.

An enzymatic toxic gas sensor having a plurality of parallel planar surfaces and a buffered electrolyte reservoir is described. The buffered electrolyte is conveyed by means of diffusion to dissolve the substrate. The substrate diffuses to an immobilized enzyme, where it is hydrolyzed if the enzyme is active. An electrochemical cell continuously monitors the hydrolyzed substrate concentration, which is an indication of the enzymatic activity and presence of toxic gas. A circuit responds to the current output of the electrochemical cell to indicate the presence or absence of a toxic gas. The shelf life of the sensor is extended by means of a separator for maintaining the enzyme dry and inactive. The enzyme in the preferred embodiment is acetylcholinesterase.

Cannon, J. J.
IMMOBILIZATION OF CATALYTICALLY ACTIVE MICROORGANISMS IN AGAR GEL FIBERS
US 4,578,354, Mar. 25, 1986
Assignee: Pfizer Inc.

A process is described for immobilizing enzyme-containing microbial cells by contacting the cells with an aqueous agar solution containing from 1.0 to 8.0 wt% sulfate and then contacting a solution of inorganic

sodium salts with a stream of the cell mixture. Agar fibers containing cells are thus formed.

Chibata, I., Tosa, T., and Takamatsu, S.
PROCESS FOR PREPARING IMMOBILIZED MICROORGANISM
US 4,526,867, Jul. 2, 1985
Assignee: Tanabe Seiyaku Co. Ltd.

A process for preparing an immobilized microorganism is described in which a microorganism is cultivated in a culture broth. When cultivation is completed, the broth is treated with glutaraldehyde, and the microbial cells are collected from the broth. The microbial cells are mixed with an aqueous solution of a polysaccharide having >10 wt% sulfate and then gelled to entrap the microbial cells. The process is suitable for the industrial preparation of immobilized microorganisms.

Degelaen, J., Loffet, A., and Durieux, J. P.
ENZYMATIC PROCESS FOR THE DETERMINATION OF BETA-LACTAM ANTIBIOTICS
US 4,546,076, Oct. 8, 1985
Assignee: UCB Societe Anonyme

An enzymatic process for the determination of beta-lactam antibiotics in a biological liquid is described using the following steps. (1) The liquid is incubated with D-alanyl-D-alanine-carboxypeptidase produced by *Actinomadura* R 39, immobilized on a water-insoluble support. The beta-lactam antibiotic reacts with the immobilized enzyme to form an inactive and equimolecular enzyme–antibiotic complex. (2) The immobilized enzyme is separated from the liquid and washed. (3) The immobilized enzyme is incubated with a substrate solution to hydrolyze it and form an amount of D-alanine corresponding to the residual enzymatic activity. (4) The amount of D-alanine formed is determined. (5) The determination of step (4) is compared with a standard to obtain the concentration of the antibiotic in the biological liquid. A test set for carrying out this process and composing the necessary reagents is also described.

Freedman, H. H.
CROSS-LINKED GELATIN FOAMS
US 4,530,905, Jul. 23, 1985
Assignee: The Dow Chemical Co.

Gelatins are cross-linked in a nonanhydrous environment to yield water-swellable, essentially water-insoluble foams. The gelatin is contacted with a polyisocyanate between pH 6 and 8 and subjected to a high rate of agitation. The process of this invention can be employed to immobilize proteins, enzymes, antibodies, and the like.

Gardner, D. L.
DUAL MICROCAPSULES AND PROCESS FOR THEIR PREPARATION
US 4,532,123, Jul. 30, 1985
Assignee: Battelle Development Corp.

Dual Microcapsules are disclosed. The outer membrane encapsulates a liquid having one or more smaller microcapsules (Mini-Microcapsules) suspended within it. The Mini-Microcapsules contain a conjugate or a reaction product of a Drug that diffuses into the liquid in which Mini-Microcapsules are suspended. The suspending liquid contains an enzyme that reacts with drug complex or reaction product to regenerate or release the drug. The drug diffuses through the outer membrane into a host.

Hafner, E. W., and Jackson, D. M.
CONSTITUTIVE GLUCOSE ISOMERASE PRODUCER
US 4,532,208, Jul. 30, 1985
Assignee: UOP Inc.

A mutant *Streptomyces coelicolor,* NRRL 15398, produces glucose isomerase constitutively at a level at least as great as the parent does inductively in common growth media. The immobilized isomerase can be effectively used to convert glucose to fructose in a continuous process.

Hagerdal, B. G. R., and Mosbach, K. H.
PREPARATION OF CATALYSTS FOR BIOCHEMICAL CONVERSION REACTIONS
US 4,524,137, Jun. 18, 1985

A catalyst is disclosed that can be used to continuously carry out two or more stages of a biochemical conversion reaction simultaneously in one reaction space. The conversion requires the presence of an enzyme for certain reaction stages and for other reaction stages, the presence of a microorganism. The catalyst consists of solid bodies of one or more polymers of which at least one is cross-linked. At least one enzyme is bound to the polymer material of the solid bodies by covalent bonds, and at least one microorganism is physically entrapped in the three-dimensional structure of the cross-linked polymer of the solid bodies.

Ho, G-H., and Liao, C-C.
MULTILAYER IMMOBILIZED ENZYME COMPOSITIONS
US 4,506,015, Mar. 19, 1985
Assignee: Borden Co. Ltd.

A high-activity, immobilized-enzyme composite is prepared by covalently bonding a second enzyme layer to a first enzyme layer immobilized to a carrier. The silica gel carrier is activated by treatment with a strong base followed by treatment with a strong acid. The first enzyme

layer is covalently bonded to the activated silica gel with an aminosilane and a polyfunctional reactant, and the second enzyme layer is covalently bonded to the first layer with a polyfunctional reactant. Third, fourth, and more successive enzyme layers may be covalently bonded. The composite has high activity per unit volume, superior stability, and good half-life.

Ikeda, M., Sakamoto, S., and Suzuki, K.
MAGNETIC PARTICULATE FOR IMMOBILIZATION OF BIOLOGICAL PROTEIN AND PROCESS OF PRODUCING THE SAME
US 4,582,622, Apr. 15, 1986
Assignee: Fujirebio Kabushiki Kaisha

A magnetic particulate composed of gelatin, water-soluble polysaccharide, sodium polymetaphosphate, and ferromagnetic substance is used as a carrier for immobilization of biological proteins, such as antigens, antibodies, or enzymes. A process of producing the magnetic particulate is also described.

Jao, Y-C., and Good, I. C.
METHOD FOR THE PREPARATION OF SPHERICAL MICROORGANISM CELL AGGREGATES
US 4,543,332, Sep. 24, 1985
Assignee: Miles Laboratories, Inc.

A method for preparing spherically shaped bacterial cell aggregates is described. The cells are flocculated from aqueous medium with a cross-linked polyamine that is the reaction product of an epihalohydrin/polyamine copolymer and a cross-linking agent. Prior to being extruded, a cake having 68–76 wt% water is produced by filtration of the flocculated cells and is ground into particles <60 mesh. Spheronizing is with a plate rotating at a tangential velocity of 4.5–12 m/s within a cylinder containing the plate. Toughness of the spherical aggregates produced can be increased by the addition of a binder after filtration and before extrusion. During spheronizing, fines may be produced. These fines can be recycled by mixing them with the wet filter cake and binder before extrusion.

Jarvis, A. P., and Lim, F.
METHOD OF CULTURING ANCHORAGE-DEPENDENT CELLS
US 4,495,288, Jan. 22, 1985
Assignee: Damon Biotech, Inc.

A method is described for growing anchorage-dependent cells: Cells of the type that normally undergo mitosis only when anchored on a substrate, e.g., fibroblasts or epithelial cells. The method involves the steps of encapsulating a seed culture of the cells within a semipermeable membrane and suspending the capsules in a growth medium. The interior

surfaces of the capsule membrane and/or collagen enclosed within the capsules serve as a substrate for the cells. The ratio of the available substrate surface area to the volume of the culture may be large, allowing the cells to be grown substantially throughout the volume of the culture medium.

Jarvis, A. P.
PROCESS FOR RECOVERING NONSECRETED SUBSTANCES PRODUCED BY CELLS
US 4,582,799, Apr. 15, 1986
Assignee: Damon Biotech, Inc.

A process for recovering nonsecreted substances produced by cells is described. The process eliminates some of the high-molecular-weight contaminants, simplifying the purification process. The cells are encapsulated within a semipermeable membrane having properties that permit rapid passage of the relatively low-molecular-weight substances of interest, but retard or prevent passage of higher-molecular-weight contaminants. The encapsulated cells are suspended in a culture medium and undergo normal cell growth and mitosis. The encapsulated cell culture grows to substantially fill the capsules, but not rupture them. The cell membrane is then lysed without disrupting the capsule membrane. The permeability of the capsule membrane is such that the substances of interest diffuse rapidly through the capsule membrane into the extracapsular fluid while the higher-molecular-weight contaminants and cell fragments are retained within the capsule. The process is particularly useful for obtaining low- to moderate-molecular-weight substances produced by prokaryotic, genetically modified organisms because the crude product can be collected free of high-molecular-weight pyrogenic contaminants.

Karasawa, Y., and Takata, Y.
MALTOSE SENSOR
US 4,547,280, Oct. 15, 1985
Assignee: Hitachi, Ltd.

A method is described for assaying maltose to quantitatively determine amylase. An enzyme membrane having immobilized alpha-glucosidase and glucose oxidase is used to improve an enzyme electrode where a hydrogen peroxide electrode with a palladium cathode is used for assaying maltose.

Kasahara, Y., Suzuki, H., and Ashihara, Y.
IMMUNOASSAY METHOD USING TWO IMMOBILIZED BIOLOGICALLY ACTIVE SUBSTANCES
US 4,582,792, Apr. 15, 1986
Assignee: Fujirebio Kabushiki Kaisha

A biologically active composition with two immobilized phases, an immobilized antigen or antibody, and an immobilized enzyme, enzyme inhibitor or activator is described that can be used to measure an antigen or antibody using a simple procedure with high sensitivity.

Katz, E., Benedicktus, J. J., Knarr, E. L., and Scallet, B. L.
IMMOBILIZED GLUCOSE ISOMERASE CONTAINING MICROBIAL CELLS
US 4,604,354, Aug. 5, 1986
Assignee: Busch Industrial Products Corp.

An immobilized glucose isomerase having increased productivity and stability is prepared by mixing a smectite filler and 50–100-mesh, granular-activated carbon with flocculated cells of *Actinoplanes* sp. and forming the resulting mixture into discrete particles.

Keyes, M. H., and Vasan, S.
PROCESS FOR THE PRODUCTION OF MODIFIED PROTEINS AND PRODUCT THEREOF
US 4,609,625, Sep. 2, 1986
Assignee: Owens-Illinois, Inc.

A process is described for chemically modifying naturally occurring proteins to produce enzyme-like, modified proteins. The process involves partially denaturing a cofactor containing holoprotein by removal of the cofactor to produce a partially denatured apoprotein. The partially denatured protein is contacted with an inhibitor of a selected model enzyme and cross-linked. The resultant protein product is an enzyme-like, modified protein having the catalytic characteristics of the model enzyme whose inhibitor is contacted with the partially denatured apoprotein.

Kuu, W. Y.
IMMOBILIZED BIOCATALYSTS
US 4,518,693, May 21, 1985
Assignee: Research Corp.

Biocatalysts, such as microbial cells, are immobilized by forming spherical gel beads containing the microbial cells from a hydrogel, such as agar or carrageenan. The beads are incubated for a time sufficient to permit the microbial cells to produce carbon dioxide to decrease resistance of the beads to diffusion. A monomer, cross-linking agent, and accelerator are diffused into the beads, which are then contacted with a polymerization initiator. The polymerized monomer prevents breakup characteristic of hydrogels containing growing microbial cells. This method is particularly suitable for the immobilization of microbial cells for use in fermentation to produce ethanol.

Malloy, T. P., and DeFilippi, L. J.
SURFACE-MODIFIED ELECTRODES
US 4,581,336, Apr. 8, 1986
Assignee: UOP Inc.

Surface-modified electrodes with an enzyme immobilized on a support are described. The support consists of at least a monolayer coating of a carbonaceous pyropolymer possessing recurring units containing at least carbon and hydrogen atoms composited on a high-surface-area, refractory, inorganic oxide. The coated support is then treated by impregnation with a water-soluble polyamine followed by contact with a solution of a molar excess of a bifunctional monomeric material to form a copolymer that provides pendant bonding sites. The copolymer is entrapped and adsorbed in the pores of the support material. The treated support is then contacted with an excess of an enzyme to effect the conjugate attachment of the enzyme to the treated support. The immobilized enzyme will act as a working electrode in the presence of a predetermined substrate, such as glucose, to provide electrical energy.

Manecke, G., and Klussmann, U.
POLYMER-CONTAINING BIOCATALYST
US 4,546,078, Oct. 8, 1985
Assignee: Schering Aktiengesellschaft

Microorganisms, such as *Arthrobacter simplex, Aspergillus ochraceus, Bacillus sphaericus, Curvularia lunata, Flavobacterium dehydrogenans, Mycobacterium* sp., or *Saccharaomyces uvarum,* are immobilized on a copolymer of acrolein and 1-vinyl-2-pyrrolidine, cross-linked by reaction with an alkylenedioxydiamine. This biocatalyst is used in the preparation of steroids.

Metcalfe, L. D. and Frank, D.
IMMOBILIZATION OF PROTEINS ON POLYMERIC SUPPORTS
US 4,539,294, Sep. 3, 1985
Assignee: Akzona Inc.

A protein is immobilized on a porous, polymeric support by a first soaking in a dilute, long-chain cationic solution and a second soaking in a dilute, aqueous protein solution. The long-chain cationic is a nitrogen compound, such as a diamine, having at least one alkyl or alkenyl group containing at least eight carbon atoms. The immobilization of a protein, such as catalase or the two-enzyme system of catalase and glucose oxidase, is described.

Meyers, W. E., and Beck, L. R.
METHOD AND DEVICE FOR CELL CULTURE GROWTH
US 4,546,083, Oct. 8, 1985
Assignee: Stolle Research & Development Corp.

A cell-culture device is described for the cultivation of animal, plant, microbiological, or artificial cells. The device involves a three-dimensional arrangement of fibers within a housing arranged to provide maximum exposed fiber surface flow channel diameter while also reducing the tortuosity of the flow path. Cells are bound to the fibers to allow them to contact nutrient fluid solution to remove any substances originating in the cells, such as viruses and pharmaceuticals.

Miyashiro, Y., Ogawa, M., Yamazaki, Y., and Igarasi, S.
POLYSACCHARIDE BEADS
US 4,493,894, Jan. 15, 1985
Assignee: Takeda Chemical Industries, Ltd.

A matrix composed of a water-insoluble, beta-1,3-glucan gel in the shape of beads with diameters within the range of about 5–1000 μm is prepared by dispersing an alkaline aqueous solution of a water-soluble, beta-1, 3-glucan in a water-immiscible organic solvent, and adding an organic acid to the resultant dispersion. The matrix is useful as carrier materials for immobilized enzymes, affinity chromatography, gel filtration, ion exchange, and other applications.

Miyata, T., and Namiki, S.
SUBSTRATE CONSISTING OF REGENERATED COLLAGEN FIBRILS AND METHOD OF MANUFACTURING SAME
US 4,565,580, Jan. 21, 1986
Assignee: Koken Co. Ltd.

A substrate consisting of regenerated collagen fibrils is used in the form of a bead or microsphere. These fibrils consist of irregularly entangled regenerated collagen fibrils, each having a diameter of 10–1000 μm. An aqueous solution between the regenerated collagen fibrils makes the content of the regenerated collagen fibrils 20–0.01 wt%. An acidic aqueous collagen solution is dispersed in a water-immiscible organic solvent in the form of numerous droplets to form an emulsion, and the droplets are then coagulated by addition of a water-miscible organic solvent and an alkali to the emulsion. Alternatively, a neutral collagen solution is dispersed in a water-immiscible organic solvent in the form of numerous droplets to form an emulsion, and the droplets are then coagulated by raising the temperature of the emulsion to 30–40°C. The collagen beads or microspheres prepared by the methods described above may be cross-linked by hexamethylenediisocyanate or glutaraldehyde. The substrate can be used for cell culture or for measuring adhesion activity of blood platelet.

Nees, S.
PROCESS FOR THE CULTIVATION OF MATRIX-BOUND BIOLOGIC CELL SYSTEMS
US 4,542,101, Sep. 17, 1985

A process is described for cultivation of matrix-bound biologic cell systems on microcarrier particles within a replenishable nutrient medium. A step for providing the controlled displacement of a culture vessel and its contents, to effect uniform cell exposure to nutrient material, is also described.

Niiyama, Y., Mori, J., and Sugahara, K.
ELECTROCHEMICAL SENSOR HAVING AN IMMOBILIZED ENZYME MEMBRANE
US 4,579,642, Apr. 1, 1986
Assignee: Hitachi, Ltd.

A recess is formed in the bottom of a cylindrical vessel and a hole for the liquid-junction is formed at the center of the recess, to which is joined a resilient, ammonium-selective membrane. Since this membrane has a thickness larger by about 0.1 mm than the depth of the recess, the ammonium-selective membrane protrudes from the bottom of the vessel. A polyester woven fabric on which urease is immobilized is attached to the vessel covering the ammonium-selective membrane. Since the immobilized enzyme membrane is applied with an appropriate tension by the slightly protruded ion selective membrane, bondability between the two membranes is improved.

Noll, L. A.
CELL CULTURE USING A MONOLITHIC SUPPORT
US 4,514,499, Apr. 30, 1985
Assignee: Corning Glass Works

An immobilized cell composite used in cell culture is described. The composite is comprised of: (a) a high-surface-area monolithic support having a number of parallel channels passing through it. The channels have walls formed of an insoluble, nontoxic composition and the support has at least about 20 channels/in^2 of cross-sectional area; and (b) a population of plant or animal tissue cells anchored to the porous channel walls.

Pierce, P. J.
THE USE OF GALLIUM AND INDIUM SALTS FOR THE IMMOBILIZATION OF PROTEINS
US 4,551,431, Nov. 5, 1985
Assignee: Phillips Petroleum Co.

A dry, stable, particulate hydrous gel/protein composite is prepared by coprecipitation of hydrous aluminum, gallium, or by alkaline indium gel, (i.e., aluminum hydroxide gel) and a protein, separating, and drying. The dried composite can be ground and sized.

Pinnavaia, T. J., Mortland, M. M., and Boyd, S. A.
CLAY–ENZYME COMPLEXES AND METHOD FOR PREPARING SAME
US 4,605,621, Aug. 12, 1986
Assignee: Michigan State University

Immobilized enzymes are prepared by coupling an enzyme to an organoclay. The immobilized enzymes are enzyme–organoclay complexes in which the binding is substantially pH independent.

Reischl, A.
FILLER-CONTAINING POLYURETHANE (UREA) COMPOSITIONS PREPARED FROM PREPOLYMERS REACTED WITH WATER IN THE PRESENCE OF LIGNITE OR PEAT
US 4,608,397, Aug. 26, 1986
Assignee: Bayer Aktiengesellschaft

A process is described for the production of polyurethane (urea) compositions that contain lignite and/or peat bound in an abrasion-proof manner, which are modified cationically and have a very high water absorbability. The compositions are produced by reacting isocyanate-terminated prepolymers, which are cationically modified and have a functionality of more than 2.1, with more than the stoichiometric quantity of water in the presence of lignite and/or peat and optionally in the presence of organic and/or inorganic fillers and biomasses (living cells, living bacteria, or enzymes). The corresponding polyurethane compositions contain up to 95% by weight of lignite and/or peat in the filler-containing polyurethane (urea) composition and are already swollen from production. The water absorbability value is from 33 to 97 wt%. The compositions containing biomasses can be used in microbial synthesis for the production of complicated organic compounds or as carriers for the growth of plants.

Rembaum, A., and Yen, R. C. K.
HYBRID MICROSPHERES
US 4,534,996, Aug. 13, 1985
Assignee: California Institute of Technology

Substrates composed of inert, synthetic, organic resin beads or sheets, such as polystyrene, are coated with a covalently bound layer of polyacrolein by irradiation of a solution of acrolein or other aldehyde with high-intensity radiation. Individual microspheres are formed that attach to the surface to form the aldehyde-containing layer. The aldehyde groups can be converted to other functional groups by reaction with materials such as hydroxylamine. Adducts of proteins, such as antibodies or enzymes, can be formed by direct reaction with the surface aldehyde groups.

Robertson, J. S., LiPuma, M. M., and Gross, S. E.
PREPARATION OF CATALYST SUPPORTS AND MATERIALS PRODUCED THEREBY
US 4,581,338, Apr. 8, 1986
Assignee: Manville Service Corp.

A spherical catalyst support is prepared by: (a) forming a mixture composed of: (i) 30–85 wt% diatomite, (ii) 15–40 wt% solvent, (iii) 0–15 wt% fluxing agent, and (iv) 0–15 wt% organic burnout material; (b) forming this mixture into spherical balls; (c) calcining these balls at a temperature in the range of about 700–2300°F for 10–45 min; and (d) depositing at least one catalytically active substance on the surface of spherical support. The spherical support produced by the above process is especially useful as a support for immobilizing enzymes and microbial cells.

Rohrbach, R. P., Maliarik, M. J., and Malloy, T. P.
PRODUCTION OF HIGH-SUGAR SYRUPS
US 4,511,654, Apr. 16, 1985
Assignee: UOP Inc.

The preparation of syrups that contain a high glucose or maltose content is effected by a two-step process. In the first step, starch that has been pretreated with alpha-amylase to adjust the dextrose equivalent is treated with immobilized amyloglucosidase or beta-amylase. The contact time is adjusted by changing the residence time and the liquid hourly space velocity to provide a conversion of from 50 to 85%, to avoid the formation of undesired products. The partially hydrolyzed reaction mixture from the enzyme treatment is then passed through an ultrafiltration membrane where the permeate having high glucose or maltose content is recovered, while the retentate is recycled for mixture with the partially hydrolyzed reaction mixture or for further treatment with the immobilized enzyme.

Rohrbach, R. P.
SUPPORT MATRIX AND IMMOBILIZED ENZYME SYSTEM
US 4,525,456, Jun. 25, 1985
Assignee: UOP Inc.

An ion exchanger matrix for immobilizing enzymes consisting of a functionalized polyethylenimine deposited on a core support is described. This matrix may be prepared from a variety of core supports since the functionalized polyethylenimines of this invention show excellent adhesive properties even to smooth surfaces. Such support matrices are particularly useful when the enzyme to be immobilized has a limited half-life. The functionalized polyethylenimine is a carboxylic acid amide of polyethylenimine, a sulfonic acid amide of polyethylenimine, or a polyalkylated polyethylenimine.

Roland, J. F.
IMMOBILIZED ENZYME SYSTEMS
US 4,585,738, Apr. 29, 1986
Assignee: Kraft, Inc.

Immobilized enzyme systems that contain a tea polyphenol–enzyme adduct and the methods for preparing and using such immobilized enzyme systems are described.

Rosevear, A., and Lambe, C. A.
PRODUCTION OF CHEMICAL COMPOUNDS WITH IMMOBILIZED PLANT CELLS
US 4,578,351, Mar. 25, 1986
Assignee: United Kingdom Atomic Energy Authority

Plant cells containing intracellular chemicals are induced to excrete these compounds by maintaining the cells at a high cell density. This high call density is maintained by immobilizing the cells in a modified polyacrylamide gel containing polyacrylamide and a minor amount of xanthan gum or sodium alginate. Vinca plant cells can be immobilized to produce the chemical compounds, ajmalicine and serpentine. Alternative to immobilizing the cells, a sufficiently high cell density can be provided by maintaining the cells in a permeable enclosure, such as a woven nylon bag.

Sakata, C., and Imai, H.
METHODS OF IMMOBILIZING MICROORGANISMS
US 4,547,463, Oct. 15, 1985
Assignee: Nippon Oil Company, Ltd.

A method of immobilizing microorganisms is described. A suspension of living microbial cells are brought into contact with a water-insoluble copolymer containing structural units derived from 2-acrylamidoethanesulfonic acid. The microorganism becomes adsorbed on the copolymer, and the water-insoluble copolymer having the microorganism adsorbed are brought into contact with a nutrient medium for cultivation to increase the number of cells present on the copolymer.

Szaj, E., Kiss, J. E., Ivony, J. E., Huber, I. E., Boros, L. E., and Dar, E.
IMMOBILIZED AMINOACYLASE ENZYME
US 4,608,340, Aug. 26, 1986

Immobilized aminoacylase having high specific activity is obtained by covalent binding of aminoacylase to a partially hydrolyzed Akrilex P-type acrylamide-*N,N*-methylene-*bis* (acrylamide) copolymer. Covalent bonding is carried out by partially hydrolyzing the copolymer with a base or an acid to form carboxy groups, activating the carboxy groups with a carbodiimide and coupling aminoacylase to the activated carboxy

groups. The aminoacylase may be isolated from mammal kidneys by forming an aqueous kidney extract, heat treating the extract, and isolating aminoacylase from the heat-treated extract.

Tennent, D. L., and Sharma, B. P.
IMMOBILIZED ENZYME COMPOSITE/PROCESS USING A MICA CARRIER
US 4,522,924, Jun. 11, 1985
Assignee: Corning Glass Works

An immobilized enzyme composite having a mica carrier is described. The composite consists of a mixture of an enzyme and a water-swelling mica (i.e., fluorohectorite, boron fluorophlogopite, hydroxyl boron phlogopite) and solid solutions of at least one mica and a structurally compatible species (i.e., talc, fluorotalc, polylithonite, fluoropolylithonite, phlogopite, and fluorophlogopite).

Turkov, E., and Stamberg, J. E.
PROTEOLYTIC, DRY BIOPOLYMERIC COMPOSITION FOR TREATMENT OF WOUNDS, AND METHOD OF USING SAME
US 4,613,502, Sep. 23, 1986
Assignee: Ceskoslovenska Akademie Ved.

A wound cover in the form of a powder or a dry powdery fluid that is useful in covering and treating of ulcerous and necrotic wounds is described. It consists of animal or fungous chitin and chitosan in a powdered form of particle size 0.01–0.3 mm or of cross-linked dextran in the form of spheric particles of diameter 0.05–0.5 mm and of an immobilized protease. Enzymes are chemically bonded to the structure of the biopolymeric carrier and provide cleaning of the wound by dissolution of undesirable protein material, in particular fibrin, necrotic tissues, components of pus, and the like. The adsorption and regeneration effects of powder act to provide suction of exudate and purulent matter infected with bacteria into interstitial capillary space. This cover acts by fast cleaning of necrotic defects and speeds up the granulation and healing of the wound and can be utilized in pharmaceutical production.

von Blucher, H., von Blucher, H., and de Ruiter, E.
YARN HAVING SPECIFIC PROPERTIES
US 4,610,905, Sep. 9, 1986

Yarns that are sheathed with active ingredients, such as adsorbents, fireproofing agents, ion-exchangers, decontaminating agents for chemical combat agents, catalysts, or fixed enzymes are described. The active ingredients are adhered to the surface of the yarn or embedded in a binding agent. The sheathing may be additionally braided or flocked.

Wolfe, S., Westlake, D., and Jensen, S.
STABLE EPIMERASE REAGENT, CYCLASE REAGENT, AND RING EXPANSION REAGENT FOR CELL-FREE PRODUCTION OF CEPHALOSPORINS
US 4,536,476, Aug. 20, 1985
Assignee: Queen's University at Kingston

Cyclase, epimerase, and a ring expansion enzyme are isolated separately from a cell-free extract of a prokaryotic beta-lactam producing organism to provide separate and stable enzyme reagents for commercial production of cephalosporins from peptide precursors. The enzymes may be immobilized on a suitable support and the production of cephalosporins may be carried out continuously.

Wood, L. L., and Calton, G. J.
IMMOBILIZED CELLS FOR PREPARING PHENYLALANINE
US 4,600,692, Jul. 15, 1986
Assignee: Purification Engineering, Inc.

A process is described for preparing phenylalanine by contacting phenylpyruvic acid or phenylpyruvate with immobilized whole cells having transaminase activity in the presence of an amine donor. The cells are preferably immobilized with a polyazetidine polymer. Ruptured or permeabilized cells, with the enzyme in the free or immobilized state, may also be used. The preparation of phenylalanine from cinnamic acid using immobilized cells having phenylalanine ammonia lyase activity is also described.

Yoshioka, T., Teramoto, K., and Shimamura, M.
ENZYME REACTION METHOD FOR ISOMERIZATION OF GLUCOSE TO FRUCTOSE
US 4,563,425, Jan. 7, 1986
Assignee: Toray Industries, Inc.

A carrier-bound metal ion, iron, is particularly suitable for inhibiting deactivation of glucose isomerase when isomerizing glucose to fructose. Glucose isomerase life is remarkably prolonged by contacting carrier-bound iron ions with a glucose substrate solution prior to isomerizing with glucose isomerase.

Literature

This section surveys the literature in the area of immobilized biocatalysts published from January 1985 to October 1986. This section is not intended to be all encompassing and lists only some of the major articles and reviews that appeared during this period.

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