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Patents and Literature

ROBERT J. LINHARDT

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

The objective of this 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 biotechnological opportunities. This issue on enzymes and cells in organic solvents and supercritical fluids is the first subject area covered in 1986. New subject areas that will be examined in later issues include: Applications of polysaccharides; protein engineering; DNA probes for clinical applications; mammalian cell culture; and microbial transformations. In each new issue, a new subject area will be introduced with a review of recent patents and literature.

Enzymes and Cells in Organic Solvents and Supercritical Fluids

Patents

This section identifies and gives a brief description of patents from the US patent literature from January 1980 to October 1985. The major search heading was Enzymes and Cells, with the cross-terms: *organic solvents* and *supercritical fluids*. No patents or literature were recovered under the cross-term *supercritical fluids*. Both US 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.

*Author to whom all correspondence and reprint requests should be addressed.

Carleton, K. L., and Rowland, J. P.
MULLS CONTAINING CHAIN STRUCTURE CLAY SUSPENSION AIDS
US 4,264,466, Apr. 28, 1981
Assignee: The Proctor and Gamble Co.

Liquid mulls having improved physical stability consisting of a liquid phase and a dispersed solid phase, are described. The liquid phase contains a major proportion of a nonionic surfactant and optionally contains a minor proportion of a nonaqueous solvent. The dispersed solid phase is a particulate material that is insoluble in the liquid phase of the mull. The inclusion of chain-structure-type clays in the composition unexpectedly aids the physical stability of the mull, so insoluble particulate materials, such as builders having ordinary particle sizes, may be incorporated into the compositions. Preferred compositions are substantially anhydrous to allow the optional inclusion of water-sensitive detergency adjuvants, such as enzymes or bleaches, into the compositions. The compositions may also contain 0–25% of a further dispersion aid selected from anionic surfactants, cationic surfactants, zwitterionic surfactants, and hydrotropic materials. Such mulls have utility, for example, as detergent compositions.

Daniels, M. J., and Farmer, D. M.
IMMOBILIZATION OF ENZYMES
US 4,421,850, Dec. 20, 1983
Assignee: Tate and Lyle Ltd.

An immobilized enzyme product is produced by contacting an inert particulate support with an aqueous enzyme solution containing at least 25% dissolved solids and with a water-miscible organic solvent and crosslinking the enzyme to insolubilize the enzyme on the support as a gel containing 50–90% water. The water-miscible organic solvent is in substantial excess to the amount of water mixed with the support in the process. The solvent may be contacted with the support before or after contacting with the enzyme solution. The resultant immobilized enzyme product has a bulk volume 5–300% greater than the bulk volume of the support material. At least a portion of the gel is external to the support and constitutes at least 3% by volume of the immobilized enzyme product.

Ehrenthal, I., and Miner, K. E.
IMMOBILIZATION OF GLUCOSE ISOMERASE
US 4,208,482, Jun. 17, 1980
Assignee: Anheuser–Busch, Inc.

Immobilized glucose isomerase is prepared by mixing whole microbial cells containing glucose isomerase with agar, combining the resultant

mixture with an organic solvent, recovering discrete particles of agar gel containing the entrapped whole microbial cells, and drying the agar gel particles. The immobilized glucose isomerase has long half-life stability when used in a column in a continuous process to convert glucose to fructose.

Johansen, J. T.
PROCESS FOR RECOVERING CU,ZN-SUPEROXIDE DISMUTASE FROM YEAST
US 4,340,675, Jul. 20, 1982
Assignee: De Forenede Bryggerier A/S

Cu,Zn-superoxide dismutase is recovered from yeast by plasmolysis with a small amount of ether or any other water-immiscible, organic solvent and subsequent autolysis in water at a temperature of 25–50°C and pH 5–9, following which the precipitate is removed and the superoxide dismutase purified and isolated from the residual liquid by chromatography on carboxymethyl cellulose at pH 4.7–5.5. The amino acid sequence of Cu,Zn-superoxide dismutase from *Saccharomyces cerevisiae* is given.

Maurukas, J.
PROCESS-STABLE COENZYME NAD SOLUTION
US 4,218,536, Aug. 19, 1980

A stable oxidized coenzyme NAD reagent is described that is ready for use in photometric assay of body fluids. The reagent is prepared by dissolving the coenzyme NAD in a neutral organic solvent, such as glycol (antifreeze). The product is a liquid, easily measured by volume for carrying out analytical operation, and can be stored in liquid condition at low temperatures without freezing and thawing.

McCollough, G. T., Esders, T. W., and Lynn, S. Y.
PROCESS FOR THE RECOVERY OF INTRACELLULAR ENZYME
US 4,275,166, June 23, 1981
Assignee: Eastman Kodak Co.

A process for the recovery of an intracellular enzyme from an aerobic soil microorganism is described. The recovery method is carried out by (a) forming an aqueous suspension of microbial cells containing the desired intracellular enzyme, (b) disrupting the microbial cells in the suspension to release the enzyme from the cells, and (c) before, during, or after step (b), introducing a water-miscible organic solvent into the suspension to form a mixture of the organic solvent and the enzyme-containing suspension. The desired enzyme is retained in the liquid phase of the mixture formed in step (c) and undesired cellular components, such as other microbial cell proteins, precipitate.

Modrovich, I. E.
STABILIZED LIQUID ENZYME COMPOSITIONS FOR DIAGNOSTIC DETERMINATIONS
US 4,310,625, Jan. 12, 1982

A method to stabilize labile enzymes for long term use in biological diagnostic determinations is described. A solution of the enzyme is formed in an aqueous media containing at least 20% organic solvent, such as 30% aqueous propane diol, in the presence of a small amount of polymer, such as 0.1% gelatin, and then diluting the media at least 20 times with water while maintaining the polymer concentration at a minimum of 0.01%. This liquid enzyme composition can be stored for extended periods without loss of significant enzyme catalytic activity. Stability is further enhanced by including from 1 to 18% of salts and 0.1% bacteriastatic agents in the liquid enzyme composition. The liquid enzyme composition can further contain an enzyme substrate and buffer salts.

Modrovich, I. E.
STABILIZED LIQUID ENZYME AND COENZYME COMPOSITIONS
US 4,271,264, June 02, 1981

Stabilized liquid enzyme and coenzyme compositions are described for use in biological diagnostic determination of glucose. A mixture is prepared containing an aqueous vehicle, a nicotinamide adenine dinucleotide coenzyme, a nucleotide, an enzyme including hexokinase and/or glucose-6-phosphate dehydrogenase, and an organic solvent to stabilize the enzyme, coenzyme, and nucleotide. The compositions have a pH of 6.0–8.5 and may contain a bacteriostat, such as azide. The preferred organic solvent is a polyol. Alternatively, a two-reagent liquid enzyme and coenzyme composition is prepared in which the enzyme is stabilized in one solution and the coenzyme and nucleotide stabilized in another, and the two solutions combined when used. In the enzyme-containing solution, an ammonia salt can be used as an alternative to the organic solvent to stabilize the enzyme. The compositions exhibit excellent shelf life, and the container in which the compositions are stored can be repeatedly opened for use without any substantial degradation of the enzyme, coenzyme, or nucleotide.

Modrovich, I. E.
STABILIZED LIQUID ENZYME AND COENZYME COMPOSITION
US 4,277,562, Jul. 07, 1981

A multireagent for use in stabilized liquid form is described. It contains three separate reagent solutions that can be combined in proper amounts to form a working composition for a biological-diagnostic determination. The first reagent solution contains an aqueous vehicle, organic solvent,

and coenzyme; the second, an aqueous vehicle, organic solvent, enzyme, and substrate; and the third, an aqueous vehicle, buffering agent, and an additional component, such as a sulfhydryl compound.

Modrovich, I.E.
STABILIZED LIQUID ENZYME AND COENZYME COMPOSITION
US 4,250,254, Feb. 10, 1981

Stabilized liquid enzyme and/or coenzyme compositions for use in biological diagnostic determinations are described. The compositions contain an enzyme and/or coenzyme, an organic solvent, such as a polyol, and an aqueous vehicle. The compositions may also contain a polymer and a bacteriostat. The compositions exhibit excellent shelf life, and the container in which a composition is stored can be repeatedly opened for use without any substantial degradation of the enzyme and/or coenzyme.

Oyama, K., Nishimura, S., Nonaka, Y., Hashimoto, T., and Kihara, K.
METHOD FOR MANUFACTURING DIPEPTIDES
US 4,284,721, Aug. 18, 1981
Assignee: Sagami Chemical Research Center

An improved method for manufacturing dipeptides from an N-substituted aspartic acid and a phenylalanine lower alkyl ester is described. The two starting materials are allowed to react with each other in the presence of an immobilized metalloproteinase in an organic solvent immiscible with water. The enzyme can be recovered for reuse. The loss of materials resulting from hydrolysis is the phenylalanine lower alkyl ester is reduced, so that use of the phenylalanine lower alkyl ester in a nearly stoichiometric quantity suffices for the reaction to ensure an improved yield and reduction in cost of industrial production.

Sakimae, A., and Onishi, H.
PREPARATION OF IMMOBILIZED ENZYMES OF MICROORGANISMS
US 4,276,381, Jun. 30, 1981
Assignee: Mitsubishi Rayon Company, Ltd.

Immobilized enzymes or microorganisms are prepared by dispersing lumps of ice containing an enzyme or microorganism in an organic solvent containing a dissolved water-insoluble high-molecular-weight substance. The organic solvent is then removed to entrap the ice lumps in the water-insoluble high-molecular weight substance. Deactivation of the enzyme or microorganism by the organic solvent is prevented by the enzyme or microorganism being entrapped in the lumps of ice.

Schutt, H.
STEREOSELECTIVE RESOLUTION OF PHENYLGLYCINE DERIVATIVES WITH ENZYME RESINS
US 4,439,524, Mar. 27, 1984
Assignee: Bayer Aktiengesellschaft

A process is described for the stereoselective resolution of D,L-phenylglycine derivatives by specific, enzymatic hydrolysis of the ester (or amide) groups of *N*-acyl-L-phenylglycine esters (or amides). The D-phenylglycine esters (or amides) are next separated from the *N*-acyl-L-phenylglycines, and then the ester (or amide) groups of the D-enantiomers and the acyl groups are subjected to acid hydrolysis. Enzyme bound to carrier is allowed to act on the *N*-acyl-D-phenylglycine esters (or amides) in an inert two-phase mixture consisting or water-immiscible organic solvent and water.

Stekolnikov, L. I., Sevastyanov, B. A., Shilov, G. G., Belousov, A. A., and Mamonov, N. D.
MEHOD FOR PREPARING ENZYMATIC COMPOSITION FOR ACCELERATION OF AGING OF MEAT PRODUCTS
US 4,195,097, Mar. 25, 1980
Asssignee: Vsesojuzny Nauchno–Issledovatelsky Institut Myasnoi Promyshlennosti

A method is described for preparing an enzymatic composition for acceleration of aging of meat products. A separate dissolution of an enzyme, hyaluronidase, and a prolongator of biological effect, serum albumin (pH 2–2.5), are prepared. Hyaluronidase, 4–6 parts by weight, and serum albumin, 0.5–1.5 parts by weight, are used. The aqueous solution of the enzyme is mixed with the aqueous solution of the prolongator, and they chemically react to give a suspension comprised of an aqueous solution of the desired product with suspended particles of the unreacted ingredients. After mixing of the aqueous solutions, the desired product is isolated by separation of the aqueous solution from particles of the unreacted ingredients and precipitation of the desired product from aqueous solution by means of an organic solvent at a volumetric ratio between solvent and the solution of 4–10:1. The aqueous solution is previously cooled to a temperature of 4–6°C, and the precipitate of the desired product is then separated from the liquid phase and dried in succession with acetone and ethyl ether. The enzymatic composition prepared according to the present invention has certain advantages over previous compositions employed for treatment of meat products. It is stable upon long-term storage and retains its activity unchanged at 18–20°C for 2 yr and longer.

Literature

This section surveys the literature in the area of enzymes and cells in organic solvents and supercritical fluids published from Jan. 1980–Oct.

1985. This lists all articles and reviews that appeared during this time period in Chemical Abstracts and Medline databases.

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