APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY 8, 69–84 (1983)

PATENTS AND LITERATURE

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. Four major areas of biochemistry will be covered corresponding to enzymes, cells, bioproducts, and nucleic acids. The patent section will briefly cover each area in most issues of the journal. The literature section will focus on one area per issue.

PATENTS

This section will identify patents and published patent applications from the international patent literature. The title, name(s) of the inventor(s), the patent number, the date of filing, the asignee, and a short description of the invention will be given. Copies of US patents can be obtained for $1.00 each from the Commissioner of Patents and Trademarks, Washington, DC 20231.

ENZYMES

Thermostable Lactase Derived from *Bacillus*

M. E. Long; C. K. Lee
US 4,323,651 (Oct. 2, 1980)
R. J. Reynolds Tobacco Company

When cultivated in a suitable nutrient medium, *Bacillus coagulans* NRRL B-8100 produces a thermostable lactase.

Enzymatic Method for Improving the Injectability of Polysaccharides

W. L. Griffith; A. L. Compere; J. W. Holleman
US 4,326,037 (Nov. 1, 1979)
The United States of America, Department of Energy

An endoenzyme is used to hydrolyze sugar linkages and thereby decrease the tendency of a polysaccharide to plug a porous medium. The partially hydrolyzed poly-

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saccharides are used as thickening agents to recover oil from subterranean formations.

Method of Producing a Plaque Dispersing Enzyme

L. G. Simonson; B. L. Lamberts
US 4,328,313 (Feb. 9, 1981)
United States of America, Secretary of the Navy

A seed culture of *Pseudomonas sp.* isolate NRRL B-12324 is inoculated into a defined medium containing about 0.1% of alpha-1,3-linked "limit glucan" substrate. After growth takes place, alpha-1,3-glucanase can be recovered from the medium and used as an oral therapeutic agent.

Lactase Preparation

D. M. Fenton
US 4,329,429 (Mar. 24, 1980)
Pfizer Inc.

Lactase-containing yeast cells are contacted iwth an alkyl alcohol, or a diethyl ketone, and the lactase is extracted by using an aqueous solution in the pH range of 5.5 to 8.0.

Enzymatic Conversion of Red Cells for Transfusion

J. Goldstein
US 4,330,619 (Aug. 14, 1980)
New York Blood Center

B-type erythrocytes at a pH of 5.7–5.8 are reacted with alpha-galactosidase for a sufficient period of time to convert the cells to type O, which are useful in transfusion therapy.

Stabilization of Peroxidase

E. C. Dawson; J. D. H. Homan; B. K. Van Weemen
US 4,331,761 (Jan. 4, 1980)
Akzona Inc.

Peroxidase compositions containing polyvalent ions of groups 3 and 4 of the periodic table, preferably those of Al, Zn, Mg, Fe, and Cu are stable to low enzyme concentrations and relatively stable to freeze-drying.

Process for the Production of Ascorbate Oxidase

E. Matsumura; H. Ishikawa; H. Misaki
US 4,331,763 (Dec. 16, 1980)
Toyo Jozo Kabushiki Kaisha

Ascorbate oxidase is produced by alkaline extraction from plants of the genus *Sechium*. The extract is subjected to several purification procedures and the resulting enzyme has a pH optimum of 7 and a molecular weight of 100,000.

Thermal Stable Beta-Galactosidase

M. W. Griffiths; D. D. Muir; J. D. Phillips
US 4,332,895 (Jun. 5, 1979)
National Research Development Corp.

New strains of *Bacillus stearothermophilus* produce beta-galactosidase that is suitable for use at 65°C. For hydrolysis of lactose, either the immobilized partially purified enzyme or enzyme-containing whole cells is used.

Process for Obtaining Cholesterol Oxidase

H. Gauhl; G. Schawohl; H. Seidel; K. Beaucamp
US 4,334,023 (May 7 1980)
Boehringer Mannheim GmbH

Members of the genera *Streptomyces* and *Arthrobacter* produce cholesterol oxidase at high levels of activity in culture supernatants without the addition of an inducer.

Preparation and Crystallization of Fraction I Protein from Plant Sources

S. Johal
US 4,334,024 (Nov. 3, 1980)
Unassigned

Ribulose 1,5-bis-phosphate carboxylase is purified and recovered in crystalline form by treating an aqueous plant extract with polyethylene glycol and adding magnesium chloride to enhance crystal formation. The pure crystals which separate out are washed and dried and are greater than 90% enzyme.

Saccharification of Starch Hydrolyzates

B. E. Norman
US 4,335,208 (Mar. 11, 1980)
Novo Industri A/S

Starch is saccharified at pH 3–5 in the presence of a glucoamylase and an acidic isoamylase to convert the starch hydrolyzate to a glucose syrup.

Process for the Production of Galactose Oxidase

O. Terada; K. Aisaka
US 4,335,213 (Jul. 2, 1980)
Kyowa Hakko Kogyo Co., Ltd.

Giberella fujikuroi or *G. zeae* is cultured in nutrient medium and galactose oxidase is recovered from the culture liquor.

Enhanced Immobilization of a Glucose Isomerase

J. R. Teague; A. L. Huebner
US 4,337,172 (Jan. 15, 1981)
UOP Inc.

Pretreatment of support matrices with divalent magnesium ions enhances the immobilization of glucose isomerase by increasing the half life of the enzyme on the column and decreasing the time needed for immobilization.

Immobilization of Biocatalysts

D. F. Hershberger; M. M. Sternberg
US 4,337,313 (Dec. 8, 1980)
Miles Laboratories, Inc.

Enzymes or microorganisms are reacted with tannin, a long-chain polyamine, a crosslinking agent, and a cationic flocculating agent. The resultant product is separated from the reaction mix and can be used in a column bed reactor.

Method of Producing an Immobilized alpha-Glucosyl Transferase Useful in the Production of Palatinose from Sucrose

J. Shimizu; K. Suzuki; Y. Nakajima
GB 2 082 591 A (Aug. 13, 1981) UK Patent Application
Mitsui Sugar Co. Ltd.

Bacterial cells containing alpha-glucosyl transferase are entrapped in calcium alginate granules and then treated with polyethyleneimine and glutaraldehyde. The enzyme immobilized in this manner is packed in a column and sucrose is passed through at high velocity resulting in the efficient production of palatinose.

CELLS

Biotransformations Using Methane-Utilizing Bacteria

I. J. Higgins
US 4,323,649 (Feb. 28, 1980)
Imperial Chemical Industries Ltd.

Complex cyclic organic compounds such as l-phenylheptane and *m*-chlorotoluene are partially degraded by reaction with cells or cell extracts containing a methane mono-oxygenase and/or a dehalogenase enzyme. The preferred microorganism is *Methylosinus trichosporium* strain OB3b (NCIB 11131).

Microbial Insecticide

K. D. Spence; R. E. Andrews
US 4,325,937 (Mar. 5, 1981)
Battelle Development Corp.

A viral, bacterial, or fungal insect pathogen is embedded in a microbead containing protein and nucleic acid. The microbead structure shields the microbial agent from sunlight-induced inactivation.

Fermentation Process for Producing Higher Plant Cells

E. Peel; C. C. Dalton
US 4,326,034 (Aug. 8, 1979)
The British Petroleum Company Ltd.

Higher plant cells (e.g., cells of *Spermatophyta*) that require a carbohydrate source are induced to develop an enhanced capacity for photosynthesis in the absence of

carbohydrate by cultivating them in the presence of light, oxygen, and carbon dioxide and gradually reducing the quantity of readily usable carbohydrate in the media.

Aerobic Submerged Fermentation of Sporulating, Ectomycorrhizal Fungi

J. H. Litchfield; W. T. Lawhon, Jr.
US 4,327,181 (May 15, 1980)
Battelle Development Corp.

Cultures of ectomycorrhizal fungi can be grown by aerobic submerged fermentation at about pH 4–7 on a vermiculite carrier. Such large-scale production is suitable for direct inoculation of tree roots or inoculation of soil prior to planting.

Bacillus Stearothermophilus Strain UK 788 and Process for Producing a Useful Enzyme

H. Nakajima; K. Nagata; M. Kageyma; T. Suga; T. Suzuki; K. Motosugi
US 4,331,762 (Nov. 21, 1980)
Unitika Ltd.

Bacillus stearothermophilus strain UK 788 is several times larger than wild-type strains and has an easily breakable cell wall. These properties permit isolation of useful heat-resistant enzymes on an industrial scale.

Microcapsule Containing a Microorganism and a Process for Its Production

T. Sozzi; A. Schrenk; M. Buhler
US 4,332,790
Societe d'Assistance Technique pour Produits Nestle SA

A microcapsule is prepared by coating lactobacilli and/or other intestinal microorganisms with a fat that is solid at body temperature.

Process for the Production of an Insulin-Producing Cell Line of Pancreatic Beta Cells

R. A. Rosenberg
US 4,332,893 (Jun. 13, 1980)
Unassigned

Insulin-producing pancreatic beta cells are infected with a Rous sarcoma virus containing a temperature-sensitive lesion in the viral transforming gene.

Media Manipulation for Preparing Biologically Active Hollow Mycelial Pellets

C. S. Gong; L. F. Chen; G. T. Tsao
US 4,332,903 (Aug. 1, 1980)
Purdue Research Foundation

Fungal spores are inoculated under conditions limiting growth to formation of tiny cell aggregates of less than 2 mm. Thereafter, the aggregates are subjected to conditions supporting vigorous vegetative growth to produce hollow pellets having a porous webbed mycelial layer and a hollow core.

Biochemical Treatment by Microorganic Method

R. Kurane; T. Suzuki; Y. Takahara
US 4,332,904 (Apr. 17, 1979)
Agency of Industrial Science & Technology,
Ministry of International Trade & Industry

Nocardia erythropolis is cultivated in a vessel on a hydrophilic support. An aqueous dispersion of a phthalate ester is passed through the vessel and is biochemically modified as it passes through the packed bed.

Preparation of Porous Active Yeast Granules

F. J. Carduck; D. Kloetzer; G. Veldman
US 4,335,144 (Jan. 19, 1981)
Deutsche Hefewerke GmbH

Moist yeast having a solids content of 30–40% is mixed with a gas and the resulting mixture is extruded through orifices while allowing the gas to escape from the exudate to form pores. The porous yeast granules may be dried to produce active dry yeast granules that are capable of rapid rehydration and regeneration.

Method of Growing Anchorage-Dependent Cells

W. R. Tolbert; M. M. Hitt; J. Feder; R. C. Kimes
US 4,335,215 (Aug. 27, 1980)
Monsanto Co.

Anchorage-dependent cells are grown in agitated microcarrier suspension in which the cells and microcarriers are aggregated by periodic, temporary residence in a separate compartment where they are subjected to a gentle tumbling action.

Coimmobilizates from Fermentable Yeasts with Coupled Enzymes as Well as Their Production and Use

W. Hartmeier
GB 2 077 291 A (Jun. 5, 1981) UK Patent Application
Boehringer Ingelheim International GmbH

Dehydrated yeast cells are contacted with an enzyme precipitating solution that causes the enzymes to stick to the yeast cell, but does not inactivate the cell's fermentative action. A crosslinking agent is then used to produce firm bonding between the enzymes and the yeast cells. The yeast–enzyme coimmobilizates are valuable in wine production.

BIOPRODUCTS

Extraction Process

P. J. Senior; L. F. Wright; B. Alderson
US 4,324,907 (Feb. 22, 1980)
Imperial Chemical Ind.

Poly(beta-hydroxybutyric acid) is separated from bacterial cells by drying the cells with a heated gas and then extracting them with a partially halogenated solvent such as chloroform.

Process for the Production of L-Aspartic Acid

H. Yukawa; T. Nara; Y. Takayama
US 4,326,029 (Aug. 11, 1980)
Mitsubishi Petrochemical Co., Ltd.

A microorganism from the genus *Brevibacterium* that is resistant to alpha-aminobutyric acid produces aspartic acid from fumaric acid and ammonium.

Production of Ethanol from Sugar Cane

F. W. Hayes
US 4,326,036 (Oct. 16, 1980)
Unassigned

Chopped and shredded sugar cane is passed through several digesters that contain hemicellulases, cellulases, and cultures that produce ethanol.

Semicontinuous Method for Production of Xanthum Gum Using *Xanthamonas Campestris* ATCC 31061

W. P. Weisrock
US 4,328,310 (Apr. 16, 1981)
Standard Oil Co.

Xanthum gum is produced by continual withdrawal of a portion of the fermentation medium, recovery of xanthan from the withdrawn portion, and continual addition of fresh, sterile medium to the residual medium.

Fermentative Preparation of L-Isoleucine

M. H. Updike; G. J. Calton
US 4,329,427 (Oct. 6, 1980)
W. R. Grace and Co.

A mutant of *Brevibacterium thiogenitalis* ATCC 31723 produces L-isoleucine when cultured under aerobic conditions in the presence of a post-threonine precursor of L-isoleucine.

Microbial Heteropolysaccharide

R. G. Cox; D. C. Steer
US 4,329,448 (Jul. 7, 1980)
Lever Brothers Co.

Biopolymer PS 87 contains glucose, galactose, mannose, glucuronic acid, and fucose. It is synthesized by a strain of *Bacillus polymyxa* and its pseudoplastic properties make it useful as a thickener.

Isolation of Microbial Protein with Reduced Nucleic Acid Content

G. R. Lawford; P. N. Lewis
US 4,330,464 (Jul. 9, 1981)
George Weston Ltd.

Microbial cells are disrupted and the protein and nucleic acid-containing fraction is treated with a chelating agent and passed through an anion exchange column that selectively adsorbs the nucleic acid.

Conversion of Guar Gum to Gel-Forming Polysaccharides by the Action of alpha-Galactosidase

R. L. Whistler
US 4,332,894 (Aug. 15, 1980)
Purdue Research Foundation

Alpha-D-Galactopyranosyl groups are removed from guar gum by controlled hydrolysis with alpha-galactosidase. This process results in high viscosity polysaccharides with the ability to form gels of different strengths.

Method of Producing L-Glutamic Acid by Fermentation

H. Nakazawa; I. Yamane; E. Akutsu
US 4,334,020 (Jun. 6, 1980)
Ajinomoto Company Inc.

A mutant of the genus *Brevibacterium* or *Corynebacterium* resistant to compounds having vitamin P activity produces L-glutamic acid in the culture liquid upon aerobic cultivation.

Process for the Production of High Fructose Syrups and Ethanol

R. E. Heady
US 4,335,207 (Jun. 3, 1980)
CPC International Inc.

Fructose polymers and ethanol are produced by a two-step process that involves contacting a sucrose solution with a fructosyl transferase enzyme and then fermenting the resultant reaction product with a yeast preparation that does not ferment or hydrolyze fructose polymers, but will ferment the glucose that is formed concurrently to ethanol.

Process for the Preparation of L-Tryptophan by Enzyme

Y. Assai; M. Shimada; K. Soda
US 4,335,209 (Apr. 25, 1980)
Mitsui Toatsu Chemicals, Inc.

Indole and DL-serine and D-serine are reacted with a serine racemizing enzyme and tryptophan synthetase to produce L-tryptophan.

Microbiological Process for the Production of Poly(beta-Hydroxybutyric Acid)

K. A. Powell; B. A. Collinson
US 4,336,334 (Feb. 21, 1980)
Imperial Chemical Industries Ltd.

When grown on methanol under aerobic conditions, *Methylobacterium organophilum* strains NCIB 11482 to 11488 produce high molecular weight poly(beta-hydroxybutyric acid).

Fermentation Process

W. C. Muller; F. D. Miller
US 4,336,335 (May 22, 1980)
National Distillers & Chemical Corp.

Heated carbon dioxide gas is passed through a fermentation medium to simultaneously vaporize and carry off oxygenated hydrocarbon fermentation products such as ethanol, butanol, or acetone. The products can be separated from the carrier gas in a scrubbing unit.

Preparation of L-Methionine by Fermentation

H. Iizuka (First Author)
Japan Kokai Tokkyo Koho 56-127097 (A)
Patent Application No. 55-30707 (Mar. 11, 1980)
Mitsubishi Yuka K.K.

Bacteria from the genus *Acinetobacter*, for example, *A. calcoaceticum* YK-1013, produce L-methionine when cultured aerobically on a medium containing ethanol as a carbon source.

Preparation of Fructose

Y. Nakamura
Japan Kokai Tokkyo Koho 56-134995 (A)
Patent Application No. 55-36347 (Mar. 21, 1980)
Noguchi Kenkyusho

Glucose isomerase converts D-glucose to D-fructose in an aqueous solution that contains sulfolane, dimethyl sulfone, or 3-methylsulfone.

NUCLEIC ACIDS

Deoxyribonucleic Acid Synthesis Using Binding Protein Extracted from Chick Embryo Fibroblasts

P. P. Hung; S. G. Lee
US 4,331,589 (Apr. 16, 1981)
Abbott Laboratories

Single-stranded RNA is copied into its complementary DNA by reverse transcription using binding protein.

Protein Synthesis

M. Ptashne; G. D. Lauer; T. M. Roberts; K. C. Backman
US 4,332,892 (Jan. 10, 1980)
President and Fellows of Harvard

A portable promoter containing no protein translational start site but consisting of a Shine-Dalgarno sequence and a transcription initiation site is inserted upstream from a translational start site of the gene to be expressed. When inserted in a suitable plasmid, the hybrid fused gene is capable of producing native, unfused, prokaryotic and eukaryotic protein in bacteria.

Hybrid Plasmid and Process of Making Same

F. Reusser
US 4,332,898 (Jun. 16, 1980)
The Upjohn Company

Hybrid plasmid pUC1021 is constructed from *Bacillus megaterium* chromosomal DNA and plasmid pBR322.

Construction of Co-Integrate Plasmids from Plasmids of Streptomyces and Escherichia

J. J. Manis; K. Highlander
US 4,332,900 (Oct. 1, 1980)
The Upjohn Company

Plasmid pUC1019 is obtained by covalent linkage of a BclI restriction endonuclease fragment of the *Streptomyces espinosus* plasmid pUC6 with a

BamHI endonuclease site of the *Escherichia coli* plasmid pBR322. Plasmid pUC1024 is obtained by restructuring pUC1019.

Fused Gene and Method of Making and Using Same

T. J. Silhavy; H. A. Shuman; J. Beckwith; M. Schwartz
US 4,336,336 (Jan. 12, 1979)
President and Fellows of Harvard College

A gene for a bacterial protein that is normally transported to the membrane or outside the cell is joined to a bacterial gene coding for a protein that is normally found in the bacterial cytoplasm. The cytoplasmic protein can thereby be transported to the periplasmic space or outside the cell surface in the form of a hybrid protein after infection, transformation, or transduction of bacterial cultures.

Novel Recombinant Plasmid Having Gene of Human Fibroblastic Interferon Messenger RNA

H. Sugano (First Author)
Japan Kokai Tokyo Koho 56-131598 (A)
Patent Application No. 55-33931 (Mar. 19, 1980)
Gan Kenkiyuukai

Human fibroblastic interferon mRNA is prepared from human fibroblasts. The mRNA is used as a template to synthesize double-stranded DNA with reverse transcriptase and the DNA is inserted into a vector DNA to produce the recombinant plasmid.

A Method of Producing a Polypeptide Product and a Plasmidic Expression Vehicle Therefor, a Method of Creating an Expression Plasmid, a Method of Cleaving Double-Stranded DNA, and Specific Plasmids

D. G. Kleid, D. G. Yansura, H. L. Heyneken; G. F. Miozzari
GB 2 073 203 A (Mar. 23, 1981) UK Patent Application
Genentech, Inc.

Plasmids are modified to contain a tryptophan promoter–operator system from which the attenuator region has been deleted. Cells containing the plasmids are grown to industrial levels in the presence of tryptophan that represses the expression of inserted genes. When the tryptophan is withdrawn, the pathway is derepressed and the desired gene product is produced efficiently in high yield.

Literature Survey

The objective of the literature survey is to make a thorough, recent review of publications in one specific area. This issue's survey will be on bioproducts. Future surveys will be on nucleic acids, enzymes and cells. The articles will be chosen for their impact on current biotechnology processes and for their potential to break new ground that may lead to new applications. The entries are listed in alphabetical order by the first author's name.

BIOPRODUCTS

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2. The Potential Use of *Dunaliella* for the Production of Glycerol, beta-Carotene and High-Protein Feed, A. Amotz and M. Avron, *Environ. Sci. Res.* **23,** 207–214 (1982).
3. Continuous Ethanol Production and Cell Growth in an Immobilized-Cell Bioreactor Employing *Zymomonas mobilis*, E. Arcuri, *Biotechnol. Bioeng.* **24,** 595–604 (1982).
4. Algae Mass Cultivation. Production and Utilization, E. Becker, *Process Biochem.* **16,** 10–14 (1981).
5. Glycerol Production by Immobilized Cells of *Saccharomyces cerevisiae*. B. Bisping and H. Rehm, *European J. Appl. Microbiol. Biotechnol.* **14,** 136–139 (1982).
6. An Efficient Synthesis of High-Molecular-Weight NAD(H) Derivatives Suitable for Continuous Operation with Coenzyme-Dependent Enzyme Systems, A. Bueckmann, M. Kula, R. Wichman and C. Wandrey, *J. Appl. Biochem.* **3,** 301–315 (1981).
7. A New Method to Prepare Amides by Bioconversion of Corresponding Nitriles, K. Bui, A. Arnaud, and P. Galzy, *Enzyme Microbiol. Technol.* **4,** 195–197 (1982).
8. Ethanol Production from Pentoses by Immobilized Micoorganisms, L. Chiang, H. Hsiao, M. Flickinger, F. Chen, and G. Tsao, *Enzyme Microbiol. Technol.* **4,** 93–95 (1982).
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10. Serotonin and Histamine Production by Carcinoid Cells in Culture, M. Debons-Gullemin, J. Launay, A. Roseto, and J. Peries, *Cancer Res.* **42,** 1513–1516 (1982).
11. Production of Ammonium Dependent on Basic L-Amino Acids by *Anacystis nidulans*, E. Flores, A. Herrero, and M. Guerrero, *Arch. Microbiol.* **131,** 91–94 (1982).
12. Isolation and Properties of Bitter-Sensitive Proteins via Affinity Chromatography, I. Gatfield, *Flavour 81*, 3rd Weurman Symp. 385–393 (1981).
13. Isolation of New Cellulolytic Anaerobic Bacteria with the Purpose of Specifying and Improving the Biochemical and Microbiological Aspects of Fermentations for Production of Methane, Ethanol, Butanol, and Acetone, R. Gay and H. Petitdemange, *Entropie* **98,** 47–54 (1981).
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15. Methane and Ethanol Production via a Biological Route Using Biomass: Potentials and Technological Concepts, G. Goma and T. Yameogo, *Entropie* **98,** 112–122 (1981).

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17. Enzymatic Hydrolysis of Plant Extracts Containing Inulin, J. Guiraud and P. Galzy, *Enzyme Microbiol. Technol.* **3,** 305–308 (1981).
18. L-Lysine Production by Mutants of *Bacillus licheniformis,* H. Hagino, S. Kobayashi, K. Araki, and K. Nakayama, *Biotechnol. Lett.* **3,** 425–430 (1981).
19. Sugar Nucleotides from Suspension-Cultured Soybean Cells, T. Hayashi and K. Mutsuda, *Agric. Biol. Chem.* **45,** 2907–2908 (1981).
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