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 every issue 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 assignee, and a short description of the invention will be given. Copies of US patents can be obtained for 50ϕ each from the Commissioner of Patents and Trademarks, Washington, DC 20231.

ENZYMES

Process for the Production of Fructose Transferase Enzyme

J. A. Smith; S. J. Luenser US 4,309,505 (May 19, 1980) CPC International Inc.

When cultured in a medium containing 16–24% sucrose, yeast extract, and inorganic nitrate, *Aureobasidium pullulans* generates a high yield of fructosyl transferase that can be recovered without need to remove black pigment or viscous polysaccharide.

Preparation of Cheese with Microencapsulated Enzymes

N. F. Olson; E. L. Magee
US 4,310,554 (Jul. 10, 1979)
Wisconsin Alumni Research Foundation

An aqueous enzyme extract of cheese flavoring microorganisms is emulsified to a water-in-oil emulsion that forms microcapsules when dispersed in milk. After coagulation, the curd is cured to produce the cheese flavors generated by the extracts in the microcapsules.

Enzymatic Microbiological Process for Producing Optically Active Aminoacids Starting from Hydantoins and/or Racemic Carbamovl Derivatives

R. Olivieri; A. Viglia; L. Degen; L. Angelini; E. Fascetti US 4,312,948 (May 11, 1979) Snamprogetti S.p.A.

Enzymic complexes obtained from *Agrobacterium* (NRRL B 11291) produce D-amino acids from racemic mixtures of their *N*-carbamyol derivatives of the corresponding hydantoins.

Thermophilic Collagenases, Thermophilic Bacteria Capable of Producing Thermophilic Collagenases, and Process for Producing Said Collagenases

N. Miwa; Y. Masuda; S. Kawarabuki; T. Sai; T. Saito US 4,315,988 (Mar. 30, 1979) Mitsubishi Petrochemical Co., Ltd.

A thermophilic bacterium in the genus *Bacillus* produces a collagenase that does not lose activity when maintained for 1 h at a temperature of 60°C in the presence of calcium ions at a pH in the range of 6.5–8.5.

Glucose Analyzer Membrane Containing Immobilized Glucose Oxidase

M. G. Busby; D. W. Hartwig US 4,317,879 (May 7, 1979) Airco, Inc.

Glucose oxidase is covalently bound to a Teflon-like membrane in a mixture of paraformaldehyde and bovine serum albumin after the membrane has been etched to produce a hydrophilic surface.

Process for the Production of Fructose Polymers and High Fructose Syrups

R. Heady US 4,317,880 (Jun. 3, 1980) CPC International Inc.

Fructose polymers are prepared by the action of fructosyl transferase and glucose isomerase enzyme preparations on a sucrose-containing substrate under appropriate reaction conditions. The fructosyl transferase enzyme is derived from *Pullularia pullulans*, ATCC No. 9348.

Process for Producing Cyclodextrins

Y. Yagi; K. Kouno; T. Inui US 4,317,881 (Mar. 26, 1980) Sanraku-Ocean Co., Ltd.

Strains belonging to the genus *Micrococcus* produce an enzyme that can be used to regulate the ratio of α -cyclodextrin to β -cyclodextrin.

Starch-Degrading Enzymes from Cladosporium Resinae

J. J. Marshall US 4,318,989 (Jun. 16, 1980) Lifeline Products, Inc.

A culture filtrate of *Cladosporium resinae*, ATCC No. 20495, has been found to contain a mixture of at least four enzymes that can convert starch and pullulan into glucose. The enzymes have been identified as a maltase, an α -amylase, and two glucoamylases.

Separation of Proteases from Fluids

A. R. Thomson; B. J. Miles; J. C. Caygill; D. J. Moore US 4,318,990 (May 15, 1980)
United Kingdom Atomic Energy Authority

A porous inorganic material, such as porous titania particles, is used to sorb sulfhydryl proteases (papain, bromelain, ficin, etc.) from fluids. The proteases are recovered from the inorganic support by elution with plant juice.

Coenzyme Immobilized Electrode

K. Nakamura; S. Nankai; T. Iijima US 4,321,123 (Oct. 6, 1980) Matsushita Electric Industrial Co.

The activity of enzyme or substrate concentrations is measured electrochemically by immobilizing the coenzyme alone or the coenzyme and enzyme together by covalent coupling to the electrode.

Glucokinase

Y. Ooshima (First Author) Jpn Kokai Tokkyo Koho 56-124383 (A) Application Number 55-26847 Mitsubishi Kasei Kogyo K. K.

Thermus thermophilus produces a glucokinase that is extracted from the cultured cells and separated by affinity chromatography using D-glucosamine as a ligand. The enzyme phosphorylates the 6 position of glucose, does not act on pentoses, and has an optimum reaction temperature of 60–70°C, and an optimum pH of 8–8.5.

CELLS

Lipid Vesicles Bearing Carbohydrate Surfaces as Lymphatic Directed Vehicles for Therapeutic and Diagnostic Substances

J. D. Baldeschwieler; R. C. Gamble; M. R. Mauk; T. Y. Shen; M. M. Ponpipom US 4,310,505 (Nov. 8, 1979)
California Institute of Technology

Phospholipid vesicles are prepared that contain a cell-surface receptor selected from synthetic sugar and amino sugar derivatives of cholesterol, and an effective amount of radioactive tracer, or cytotoxic or therapeutic agent as part of the vesicles.

Anucleated Live E. Coli Vaccine

G. G. Khachatourians US 4,311,797 (Sept. 18, 1979) University of Saskatoon

A metabolically active, nonreproductive, anucleated strain of *E. coli* is mixed with a K99+ enteropathogenic strain. After incubation of the mixed cells in a culture medium, the minicell-producing cells that contain the K99 plasmid are separated from the other cells and cultivated in a second culture medium.

Method for the Production of Yeast on Ethanol and Means Therefor

P. Zaffaroni; A. Senni; L. Formiconi US 4,317,884 (Sept. 11, 1978) Snamprogetti S.p.A.

Yeast strain NRRL-Y11119 uses ethanol as a carbon and energy source and generates high quality protein-containing biomass.

Microbiological Process for Removing Non-Ionic Surface Active Agents, Detergents, and the Like from a Wastewater and Microorganism Capable of Same

*J. E. Blair; L. T. Davis*US 4,317,885 (Jun. 12, 1980)
Sybron Corporation

Under aerobic conditions, a novel microbial strain of *Pseudomonas fluorescens* degrades and removes anionic and/or non-ionic surface active agents that are present in wastewater.

Process for Biological Denitrification of Effluents

B. Cabane; J. VergnaultUS 4,318,988 (Dec. 21, 1979)

PCUK Produits Chimiques Ugine Kuhlmann

Selected high performance denitrifying bacteria fixed on a neutral support are used to denitrify effluents containing up to 10 g/L of nitric nitrogen.

Separation of Uranium by Biosorption

B. Volesky; M. Tsezos US 4,320,093 (Nov. 13, 1979) Unassigned

Biomass derived from fermentation of the fungal microorganism *Rhizopus* arrhizus is used to extract uranium or thorium cations from aqueous tailings generated in the ore extraction process.

Process for the Production of Carrier Particles from Yeast Cells and for Producing Diagnostic Agents Therefrom and Test Kits Containing Such Agents

F. G. Bull US 4,320,194 (Jul. 28, 1980) Gist-Brocades N. V.

Yeast cells are stabilized by crosslinking the proteins of the cell cytoplasm and treating the cell wall carbohydrates by reaction with an epoxide. The resultant cells may be further treated by dyeing, esterification, or etherification, and activation with cyanuric chloride followed by reaction with the protein or other material to be carried.

Method for Processing Waste

R. A. Messing US 4,321,141 (Apr. 11, 1980) Corning Glass Works

Biodegradable organic wastes are passed through a hydrolytic redox bioreactor and an anaerobic bioreactor, with each bioreactor containing immobilized microbes. The biochemical oxygen demand of the waste can also be determined using similar bioreactors.

Pulsed Voltammetric Detection of Microorganisms

J. E. Ahnell US 4,321,322 (Jun. 18, 1980) Unassigned

An electroanalytical cell that employs the growth medium as the electrolyte and analyte is used to detect microorganisms as a consequence of the depletion of oxygen in the medium caused by aerobic metabolism.

Preparation of Spherical Shaped Mycelial Pellets

L. F. Chen; C. S. Gong; G. T. Tsao US 4,321,327 (Dec. 6, 1979)
Purdue Research Foundation

Spores of a mycelial microorganism are mixed with a cellulose derivative or with agar. After a precipitation step, the resulting porous beads containing the spores are incubated in a culture medium.

Acquisition of Genetically Recombined Strain

R. Katsumata (First Author) Jpn Kokai Tokkyo Koho 56-109587 (A) Application Number 55-13403 Kyowa Hakke Kogyo K. K.

Bacteria belonging to the genus *Corynebacterium* are converted into protoplasts and then fused by adding polyethylene glycol. The recombined strains have acquired double mutations in both analog resistance and auxotrophy.

BIOPRODUCTS

Antitumor Agent from Human Serum and Process

S. Green US 4,309,418 (Mar. 25, 1980) Sloan-Kettering Research Institute for Cancer

Human serum contains an antitumor agent that can be purified and has the characteristics of protein with a molecular weight above 150,000 that is stable at 90°C for 1 h and inhibits the growth of HeLa tumor cells.

Fructose Production

R. S. Leiser
US 4,310,628 (Mar. 14, 1980)
A. E. Staley Manufacturing Company

A highly concentrated monosaccharide feed syrup essentially free of Co^{2+} is continuously passed through a bed of immobilized glucose isomerase from *Bacillus* at a temperature of 55–61°C and a pH of 7.0–7.5.

Anaerobic Digestion of Waste and Biomass by Use of Lactobacillus Culture Additives

P. A. Fedde; S. Ghosh; M. P. Henry; D. L. Klass US 4,314,904 (Dec. 12, 1979)
Transagra Corporation

A nonviable lactobacillus fermentation product is added to an anaerobic digester system and generates a greater than normal digestion rate.

Process for Recovering Interferon

Y. Uemura; H. Arimura; H. Morise; S. Funakoshi; T. Suyama US 4,314,935 (Aug. 6, 1980)
The Green Cross Corporation

Interferon of human origin can be adsorbed onto water-insolubilized heparin and then eluted with an aqueous solution of an inorganic salt.

Fermentation Process

N. W. Lützen US 4,316,956 (Feb. 6, 1980) Novo Industri A/S

An aqueous slurry of granular starch is fermented by an ethanol-producing microorganism in the presence of alpha-amylase and glucoamylase; the process is characterized by low levels of dextrin and fermentable sugars in solution in the fermentation broth and the enzymes are recovered for use in a subsequent fermentation.

Preparation of 2,5-Diketogluconic Acid

D. A. Kita; D. M. Fenton US 4,316,960 (Apr. 10, 1980) Pfizer Inc.

Acetobacter cerinus produces 2,5-diketogluconic acid when cultivated in a medium containing 20–30% glucose and choline.

Microbiological Production of Protein

D. G. MacLennan; J. C. Ousby; T. R. Owen; D. C. Steer US 4,317,843 (Aug. 30, 1976) Imperial Chemical Industries

Bacteria belonging to the genera *Pseudomonas, Microcyclus*, and *Hyphomicrobium* produce protein compositions and other fermentation products suitable for use in human and animal foods when cultured on a variety of sources of assimilable carbon, especially oxygenated hydrocarbons.

β-Carotene Producing Strains of the Fungus *Phycomyces Blakesleenus*

F. J. M. Araujo; I. L. Calderon; I. L. Diaz; C. Olmedo US 4,318,987 (Nov. 16, 1979) Unassigned

By genetic manipulations, strains of the fungus *Phycomyces blakesleeanus* have been generated that produce β -carotene in a proportion of 2.5% of dry weight. This is 1000 times more than the natural microorganisms would produce under the same conditions.

Process for Selectively Extracting Dyestuffs Contained in Cyanophyceae Algae, the So-Extracted Dyestuffs and their Use, Particularly in Foodstuffs

M. Rebeller; P. Yout; D. Lonchamp US 4,320,050 (Apr. 4, 1980) Institut Français du Petrole

Phycocyanine, a blue pigment, is extracted from *Spirulina* species of cyanophyceae algae by a three-step process involving calcium ions, an alkaline aqueous phase, and ultrafiltration.

Fiber Production From Continuous Cultivation of Microorganisms

R. L. Mynatt US 4,320,198 (Oct. 15, 1979) Unassigned Sphaerotilus natans is cultured on a pitted metallic plate supplied with a flowing nutrient substrate and the pellicle growth is harvested to produce cellulose-containing materials.

Method for Making Collagen Sponge for Medical and Cosmetic Uses

A. Berg; Z. Eckmayer US 4,320,201 (Sept. 2, 1980)

An alkaline proteases from *Bacillus* is used to produce an insoluble collagen sponge with a velour-like surface.

Process for Making Glucosone

J. A. Maselli; R. O. Horwath US 4,321,324 (Jun. 18, 1980) Standard Brands Incorporated

Glucose is oxidized with glucose-2-oxidase to glucosone and the hydrogen peroxide produced is reacted with an alkene to form oxygenated products of the alkene.

Preparation of L-Phenylalanine

E. Gotou (First Author) Jpn Kokai Tokkyo Koho 56-121492 (A) Application Number 55-24519 Ajinomoto K. K.

Variant strains belonging to the genus *Brevibacterium* or *Corynebacterium* require glutamic acid for growth and have the ability to produce L-phenylalanine when cultivated in an ordinary liquid culture medium.

Preparation of D-Ribose by Fermentation

K. Satou (First Author)

Jpn Kokai Tokkyo Koho 56-113297 (A)

Application Number 55-17105

Ajinomoto K. K.

A variant of the genus *Corynebacterium*, which is incapable of growing in a medium containing D-ribose as the sole carbon source, produces D-ribose when cultured in a conventional medium.

NUCLEIC ACIDS

Oligonucleotides Useful as Adaptors in DNA Cloning, Adapted DNA Molecules, and Methods of Preparing Adaptors and Adapted Molecules

R. J. Wu; C. P. Bahl; S. A. Narang US 4,321,365 (Oct. 19, 1977) Research Corporation

An adaptor molecule useful for attaching DNA to a cloning vehicle that comprises a double-stranded oligodeoxyribonucleotide having at one end a protruding nucleotide sequence that is the recognition site for a restriction enzyme.

Process for Transducing Escherichia Coli K12 x1776

C. L. Hershberger US 4,322,497 (Jun. 2, 1978) Eli Lilly and Company

A method of transducing genetic markers into strains of E. coli K12 $_{\chi}$ 1776 when they are in the stationary growth phase.

Adrenocorticotropin-Lipotropin Precursor Gene

J. D. Baxter; J. L. Roberts; P. H. Seeburg; H. M. Goodman US 4,322,499 (Dec. 22, 1978)
The Regents of the University of California

A recombinant DNA plasmid or bacteriophage transfer vector has a cDNA sequence part of which is the endorphin gene cDNA sequence.

Preparation of L-Glutamic Acid by Fermentation Method

T. Tsuchida (First Author) Jpn Kokai Tokkyo Koho 56-117795 (A) Application Number 55-22062 Ajinomoto K. K.

Chromosomal DNA from a strain having high L-glutamic acid producing ability is ligated to a plasmid or phage vector and used to transform an *E. coli* strain. A strain resistant to *p*-fluorophenylalanine, *S*-(2-aminoethyl)-cystein, 2-thiazolealanine, and 1,2,4-triazolealanine is used as the host cell.

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 cells. Further surveys will be on bioproducts, nucleic acids, and enzymes. 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.

CELLS

- Steroid Transformation with Immobilized Microorganisms. V. Continuous Side Chain Cleavage of a Cholesterol Derivative with Immobilized Mycobacterium phlei Cells, P. Atrat, E. Hueller, and C. Hoerhold, Eur. J. Appl. Microbiol. Biotechnol. 12, 157–160 (1981).
- 2. Biotransformation of Monoterpenes by Mentha Cell Lines: Conversion of Menthone to Neomethone, D. Aviv, E. Krochmal, A. Dantes, and E. Galun, *Planta Med.* 42, 236–243 (1981).
- 3. Plant Cell Cultures and Their Biotechnological Potential, W. Barz and B. Ellis, *Ber. Dtsch. Bot. Ges.* **94**, 1–26 (1981).
- 4. Covalent Stabilization of Alginate Gel for the Entrapment of Living Whole Cells, S. Birnbaum, R. Pendleton, P. Larsson, and K. Mosbach, *Biotechnol. Lett.* 3, 393–400 (1981).
- 5. Use of Immobilized Cells, I. Chibata and T. Tosa, *Annu. Rev. Biophys. Bioeng.* **10**, 197–216 (1981).
- 6. Immobilization of *Brevibacterium flavum* Cells on Collagen for the Production of Glutamic Acid in a Recycle Reactor, A. Constantinides, D. Bhatia, and W. Vieth, *Biotechnol. Bioeng.* **23**, 899–916 (1981).
- 7. Microcarrier Culture: Applications in Biologicals Production and Cell Biology, C. Crespi, T. Imamura, P. Leong, R. Fleischaker, H. Brunengraber, W. Thilly, and D. Girad, *Biotechnol. Bioeng.* 13, 2673–2690 (1981).

- 8. Direct Delignification of Untreated Bark Chips with Mixed Cultures of Bacteria, A. Deschamps, J. Gillie, and J. Lebeault, *Eur. J. Appl. Microbiol. Biotechnol.* 13, 222–225 (1981).
- Serine-Selective Membrane Probe Based on Immobilized Anaerobic Bacteria and a Potentiometric Ammonium Gas Sensor, C. Di Paolantonio, M. Arnold, and G. Rechnitz, Anal. Chim. Acta 128, 121–127 (1981).
- Immobilization of Escherichia coli Cells Containing Aspartase EC-4.3.1.1. Activity
 with Polyurethane and Its Application for L-Aspartic Acid Production, M. Fusee, W.
 Swann, and G. Calton, Appl. Environ. Microbiol. 42, 672–676 (1981).
- 11. Acetic Acid Production by Immobilized Acetobacter Cells, C. Ghommidh, J. Navarro, and G.Durand, *Biotechnol. Lett.* 3, 93–98 (1981).
- Production of Anchorage-Dependent Cells—Problems and Their Possible Solutions,
 M. Jensen, *Biotechnol. Bioeng.* 13, 2703–2716 (1981).
- Continuous Production of Steroid Glycoalkaloids by Immobilized Plant Cells, V. Jirku, T. Macek, T. Vanek, V. Krumphanzl, and V. Kubanek, Biotechnol. Lett. 3, 447–450 (1981).
- 14. Amperometric Determination of Ammonium Gas with Immobilized Nitrifying Bacteria, I. Karube, T. Okada, and S. Suzuki, *Anal. Chem.* **53**, 1852–1854 (1981).
- 15. The Isomerization of p-Glucose into p-Fructose Catalyzed by Whole-Cell Immobilized Glucose Isomerase. The Dependence of the Intrinsic Rate of Reaction on Substrate Concentration, pH, and Temperature, A. Kikkert, K. Vellenga, H. De Wilt, and G. Joosten, *Biotechnol. Bioeng.* 23, 1087–1101 (1981).
- 16. Immobilization of Microbial Cells in Polyurethane Matrixes, J. Klein and M. Kluge, *Biotechnol. Lett.* 3, 65–70 (1981).
- 17. Application of Microbial Cells as Multistep Catalysts in Potentiometric Biosensing Electrodes, R. Kobos and H. Pyon, *Biotechnol. Bioeng.* 23, 627–633 (1981).
- 18. Cell Immobilization, A. Koshcheenko, *Itogi Nauki Tekh.*, *Ser. Mikrobiol.* 11, 55–117 (1981).
- 19. Plant Protoplast Agglutination and Immobilization, P. Larkin, Recent Adv. Phytochem. 15, 135–160 (1981).
- 20. Ethanol Production from Whey with Immobilized Living Yeast Kluyveromyces fragilis, Y. Linko, H. Jalanka, and P. Linko, Biotechnol. Lett. 3, 263–268 (1981).
- Use of Immobilized Cells of *Rhizopus nigricans* for the 11-AlphaHydroxylation of Progesterone, I. Maddox, P. Dunhill, and M. Lilly, *Biotechnol. Bioeng.* 23, 345–354 (1981).
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- 29. Immobilization of the Methanogenic Bacterium *Methanosarcinia barkeri*, P. Scherer, M. Kluge, and J. Klein, *Biotechnol. Bioeng.* 23, 1057–1066 (1981).
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- 32. Growth of *Curvularia lunata* Spores into Mycelial Form within Various Gels, and Steroid 11-Beta-Hydroxylation by the Entrapped Mycelia, K. Sonomoto, M. Hoq, A. Tanaka, and S. Fukui, *J. Ferment. Technol.* **59**, 465–469 (1981).
- 33. Oxygen Transfer in Broths of Plant Cells at High Density, H. Tanaka, *Biotechnol. Bioeng.* 24, 425–442 (1982).
- 34. Decomposition of Malic Acid in Red Wine by Immobilized Microbial Cells, A. Totsuka and S. Hara, *Hakko Kogaku Kaishi* **59**, 231–237 (1981).
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- 36. Bacterial Organisms Suitable for Filamentous Cell Immobilization, W. Tso and W. Fung, *Biotechnol. Lett.* 3, 421–424 (1981).
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- 38. A Bioreactor for Continuous Treatment of Waste Waters with Immobilized Cells of Photosynthetic Bacteria, M. Vincenzini, W. Balloni, D. Mannelli, and G. Florenzano, *Experientia* 37, 710–712 (1981).
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