Microbiological Process Report

Production of Microbial Enzymes and Their Applications

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Enzymes are biocatalysts produced by living cells to bring about specific biochemical reactions generally forming parts of the metabolic processes of the cells. Enzymes are highly specific in their action on substrates and often many different enzymes are required to bring about, by concerted action, the sequence of metabolic reactions performed by the living cell. All enzymes which have been purified are protein in nature, and may or may not possess a nonprotein prosthetic group.

The practical application and industrial use of enzymes to accomplish certain reactions apart from the cell dates back many centuries and was practiced long before the nature or function of enzymes was understood. Use of barley malt for starch conversion in brewing, and of dung for bating of hides in leather making, are examples of ancient use of enzymes. It was not until nearly the turn of this century that the causative agents or enzymes responsible for bringing about such biochemical reactions became known. Then crude preparations from certain animal tissues such as pancreas and stomach mucosa, or from plant tissues such as malt and papaya fruit, were prepared which found technical applications in the textile, leather, brewing, and other industries. Once the favorable results of employing such enzyme preparations were established, a search began for better, less expensive, and more readily available sources of such enzymes. It was found that certain microorganisms produce enzymes similar in action to the amylases of malt and pancreas, or to the proteases of the pancreas and papaya fruit. This led to the development of processes for producing such microbial enzymes on a commercial scale.

Dr. Jokichi Takamine (1894, 1914) was the first person to realize the technical possibility of cultivated enzymes and to introduce them to industry. He was mainly concerned with fungal enzymes, whereas Boidin and Effront (1917) in France pioneered in the production of bacterial enzymes about 20 years later. Technological progress in this field during the last decades has been so great that, for many uses, microbial cultivated enzymes have replaced the animal or plant enzymes. For example, in textile desizing, bacterial amylase has largely replaced malt or pancreatin. At present, only a relatively small number of microbial enzymes have found commercial application, but the number is increasing, and the field will undoubtedly be much expanded in the future.

Production of Microbial Enzymes

Enzymes occur in every living cell, hence in all microorganisms. Each single strain of organism produces a large number of enzymes, hydrolyzing, oxidizing or reducing, and metabolic in nature. But the absolute and relative amounts of the various individual enzymes produced vary markedly between species and even between strains of the same species. Hence, it is customary to select strains for the commercial production of specific enzymes which have the capacity for producing highest amounts of the particular enzymes desired. Commercial enzymes are produced from strains of molds, bacteria, and yeasts as shown in table 1.

Up until less than 10 years ago, commercial fungal and bacterial enzymes were produced by surface culture methods. Within the past few years, however, submerged culture methods have come into extensive use. Descriptions of processing methods for preparing industrial microbial enzymes have been published (Underkofler, 1954; Hoogerheide, 1954; Forbath, 1957).

TABLE 1

Some commercial enzymes and source microorganisms

Source	Enzyme	Microorganism
Fungal	Amylases Glucosidases Proteases	*Aspergillus oryzae* *Aspergillus flavus* *Aspergillus niger*
	Pectinases	*Aspergillus niger*
	Glucose oxidase Catalase	*Penicillium notatum* *Aspergillus niger*
Bacterial	Amylases Proteases Penicillinase	*Bacillus subtilis*
Yeast	Invertase	*Saccharomyces cerevisiae*
	Lactase	*Saccharomyces fragilis*

[1] Presented at Symposium, Society for Industrial Microbiology, Storrs, Connecticut, August, 1956.

For fungal enzymes, modifications of Dr. Takamine's original mold bran process have usually been employed. In this process, the mold is cultivated on the surface of a solid substrate. Takamine used wheat bran and this has come to be recognized as the most satisfactory basic substrate although other fibrous materials can be employed. Other ingredients may be added, such as nutrient salts, acid or buffer to regulate the pH, soy bean meal or beet cosettes to stimulate enzyme production. In one modification of the bran process, the bran is steamed for sterilization, cooled, inoculated with the mold spores, and spread out on trays (Underkofler *et al.*, 1947; Forbath, 1957). Incubation takes place in chambers where the temperature and humidity are controlled within limits by circulated air. It may be stated that instead of trays for incubation, Takamine, as well as other producers, at one time used slowly rotating drums. Generally tray incubation gives more rapid growth and enzyme production.

Bacterial enzymes have been and are also produced by the bran process. However, until recently the process originally invented by Boidin and Effront (1917) was most extensively employed (Wallerstein, 1939). In this process, the bacteria are cultivated in special culture vessels as a pellicle on the surface of thin layers of liquid medium, the composition of which is adjusted for maximum production of the desired enzyme. Different strains of *Bacillus subtilis* and different media are employed, depending on whether bacterial amylase or protease is desired.

The submerged method was originally developed and first extensively employed for production of penicillin and other antibiotics. So much has been written recently about submerged culture of molds and bacteria that it is unnecessary to go into detail here. In the laboratory, submerged cultures are grown in shake flasks or in aerated tubes or flasks. Commercially, deep tanks are employed which have provision for introduction of sterile air and for vigorous agitation. The amount of air, degree of dispersion of air, and amount of agitation are dependent variables. For effective results the air must be dispersed in very fine bubbles throughout the mass of culture liquid. Fine aeration through porous substances may be used to produce high dispersion. Most manufacturers, however, depend upon efficient agitators to break up the air into the requisite small bubbles.

Either surface or submerged culture methods currently may be employed for most microbial enzymes production. Usually different cultures must be used for maximum enzyme yields by the two methods, although there are exceptions to this rule. There are advantages and disadvantages to each method, some of which are shown in table 2. Which method is used for a particular commercial enzyme will be dictated by plant equipment, convenience, relative yields, and application.

Recovery of the enzyme generally depends upon precipitation from an aqueous solution, although some enzymes may be marketed as stabilized solutions. In the bran process, the enzyme is extracted from the koji (the name given to the mass of material permeated with the mold mycelium) into an aqueous solution by percolation. In the liquid processes, the microbial cells are filtered from the beer. The enzyme may be precipitated by addition of solvents, such as acetone or aliphatic alcohols, to the aqueous enzyme solution, either directly or after concentration by vacuum evaporation at low temperature. The precipitated enzyme may be filtered and dried at low temperature, for example in a vacuum shelf dryer. The dry enzyme powders may be sold as undiluted concentrates on a potency basis or, for most applications, may be diluted to an established standard potency with an acceptable diluent. Some common diluents are salt, sugar, starch, and wheat flour. Most commercial enzymes are quite stable in the dry form, but some require the presence of stabilizers and activators for maximum stability and efficiency in use.

In theory, the fermentative production of microbial enzymes is a simple matter, requiring an appropriate organism grown on a medium of optimum composition under optimum conditions. The stocks in trade of microbial enzyme manufacturers are thus the selected cultures, the composition of media, and the cultural conditions, all of which are usually held confidential. In practice, enzyme manufacturers suffer the same difficulties in fermentation, frequently in even greater degree, as antibiotics producers. Total loss of fermentation batches may result from contamination, culture variation, failure of cultural control, and other like causes. Furthermore, knowledge and careful application of the best methods for recovery, stabilization, and

TABLE 2

Comparison of surface and submerged processes

Surface	Submerged
Requires much space for trays	Uses compact closed fermentors
Requires much hand labor	Requires minimum of labor
Uses low pressure air blower	Requires high pressure air
Little power requirement	Needs considerable power for air compressors and agitators
Minimum control necessary	Requires careful control
Little contamination problem	Contamination frequently a serious problem
Recovery involves extraction with aqueous solution, filtration or centrifugation, and perhaps evaporation and/or precipitation	Recovery involves filtration or centrifugation, and perhaps evaporation and/or precipitation

storage of such delicate biological entities as the labile enzymes presents a constant challenge.

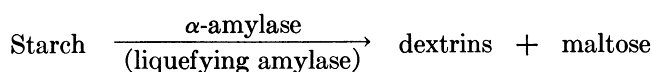
Applications of Microbial Enzymes

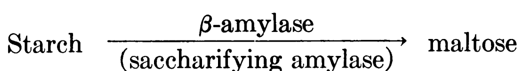
Uses of microbial enzymes in food, pharmaceutical, textile, paper, leather, and other industries are numerous and are increasing rapidly. The more important current uses are listed in table 3. Most of the industrially important microbial enzymes, with two major exceptions at present, are hydrolases, which catalyze the hydrolysis of natural organic compounds.

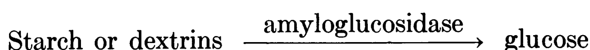
Carbohydrases

Carbohydrases are enzymes which hydrolyze polysaccharides or oligosaccharides. Several carbohydrases have industrial importance, but the amylases have the greatest commercial application.

The various starch-splitting enzymes are known as amylases, the actions of which (Kerr, 1950; Myrbäck and Neumüller, 1950; Meyer and Gibbons, 1951; Bernfeld, 1951) may be expressed in greatly simplified form as follows:







The terms "liquefying" and "saccharifying" amylases are general classifications denoting the principal types of amylase action. β-Amylase, which is not of microbial origin, is a true saccharifying enzyme, forming maltose directly from starch by cleaving disaccharide units from the open ends of chains. The α-amylases from different sources usually have good liquefying ability, but may vary widely in saccharifying ability and thermal stability. Amyloglucosidase is a saccharifying enzyme unique in that it attacks starch and 1,4-linked glucose oligosaccharides with direct formation of glucose. A range of amylases, suitable for almost any kind or extent of starch conversion, is now available from microbial sources.

Bacterial amylase preparations generally remain operative at considerably higher temperature than do fungal amylases, and at elevated temperatures give rapid liquefaction of starch. A significant application of the bacterial enzyme is in the continuous process for desizing of textile fabrics (Gale, 1941; Wood, 1947). Another is in preparing modified starch sizing for textiles (Gale, 1941) and starch coatings for paper (Gale, 1941; Schwalbe and Gillan, 1957).

High temperature stability is also important in the brewing industry where microbial amylases have found use in supplementing low diastatic malt, and especially for initial liquefaction of adjuncts such as rice and corn grits (Schellhas, 1956). Additional specific uses for bacterial amylase is in preparing cold water dispersible laundry starches (Pigman *et al.*, 1952) and in removing wall paper.

Fungal amylases possess relatively low thermal stability but act rapidly at lower temperatures and produce good saccharification. An enormous potential use for fungal amylase is as a saccharifying agent for grain alcohol fermentation mashes. At least two alcohol plants in this country regularly use fungal amylase for this purpose. It has been repeatedly shown that use of fungal amylases results in better alcohol yields than with malt conversion (Underkofler *et al.*, 1946; U. S. Department of Agriculture, 1950).

An extremely important use for fungal amylases is in conversion of partially acid hydrolyzed starch to sweet syrups. Acid hydrolysis is a random action whereas enzymic hydrolysis is a patterned one. By proper control of the type and proportion of enzymes used (α-amylase, amyloglucosidase, maltase) syrups of almost any desired proportions of glucose, maltose, and dextrins may be produced (Dale and Langlois, 1940; Langlois, 1953). Crystalline glucose will probably soon be manufactured by amyloglucosidase conversion of starch, in competition with the conventional acid hydrolysis process.

Amylases find extensive use in baking. Use of fungal amylase by the baker to supplement the diastatic activity of flour is common practice. The fungal amylase has the advantage of low inactivation temperature. This permits use of high levels of the amylase to improve sugar production, which increases gas formation and improves crust color, without danger of excessive dextrinization of the starch during baking (Johnson and Miller, 1948, 1949; Harrel *et al.*, 1950; Reed, 1952a; Miller *et al.*, 1953; Pence, 1953).

The first industrial manufacture of fungal enzyme, Takadiastase, in this country was for a pharmaceutical digestive aid, and this continues to be a major application (Beazell, 1942).

Other applications of microbial amylases where both fungal and bacterial enzymes are utilized are in processing cereal products for food dextrin and sugar mixtures and for breakfast foods, for preparation of chocolate and licorice syrups to keep them from congealing, and for recovering sugars from scrap candy of high starch content. Fungal amylases are also used for starch removal for flavoring extracts and for fruit extracts and juices, and in preparing clear, starch-free pectin. Microbial amylases are used for modifying starch in vegetable purees, and in treating vegetables for canning (Bode, 1954).

Several disaccharide-splitting carbohydrases have

TABLE 3

Uses of industrial enzyme preparations

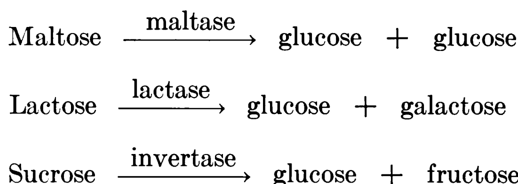
Industry	Application	Enzyme	Source*	Extent of Use†
Baking and milling	Bread baking	Amylase	*Fungal*, malt	2
		Protease	Fungal	1
Beer	Mashing	Amylase	*Malt*, bacterial	1
	Chillproofing	Protease	*Papain*, bromelain, pepsin, fungal, bacterial	1
	Oxygen removal	Glucose oxidase	Fungal	3
Carbonated beverages	Oxygen removal	Glucose oxidase	Fungal	3
Cereals	Precooked baby foods	Amylase	Malt, fungal	2
	Breakfast foods	Amylase	Malt, fungal	2
	Condiments	Protease	Papain, bromelain, pepsin, fungal, bacterial	2
Chocolate, cocoa	Syrups	Amylase	Fungal, bacterial	2
Coffee	Coffee bean fermentation	Pectinase	Fungal	2
	Coffee concentrates	Pectinase, hemicellulase	Fungal	2
Confectionery, candy	Soft center candies and fondants	Invertase	Yeast	2
	Sugar recovery from scrap candy	Amylase	Bacterial, fungal	3
Dairy	Cheese production	Rennin	Animal	1
	Milk, sterilization with peroxide	Catalase	Liver, bacterial	3
	Milk, prevention of oxidation flavor	Protease	Pancreatin	2
	Milk, protein hydrolyzates	Protease	Papain, bromelain, pancreatin, fungal, bacterial	2
	Evaporated milk, stabilization	Protease	Pancreatin, pepsin, bromelain, fungal	4
	Whole milk concentrates	Lactase	Yeast	3
	Ice cream and frozen desserts	Lactase	Yeast	3
	Whey concentrates	Lactase	Yeast	2
	Dried milk, oxygen removal	Glucose oxidase	Fungal	3
Distilled beverages	Mashing	Amylase	*Malt*, fungal, bacterial	1
Dry cleaning, laundry	Spot removal	Protease, lipase, amylase	*Bacterial*, pancreatin, fungal	1
Eggs, dried	Glucose removal	Glucose oxidase	Fungal	1
	Mayonnaise, oxygen removal	Glucose oxidase	Fungal	4
Feeds, animal	Pig starter rations	Protease, amylase	Pepsin, pancreatin, bromelain, fungal	3
Flavors	Removal of starch, clarification	Amylase	Fungal	3
	Oxygen removal	Glucose oxidase	Fungal	3
Fruits and fruit juices	Clarification, filtration, concentration	Pectinases	Fungal	1
	Low methoxyl pectin	Pectinesterase	Fungal, vegetable	2
	Starch removal from pectin	Amylase	Fungal	2
	Oxygen removal	Glucose oxidase	Fungal	4
Leather	Bating	Protease	*Bacterial*, pancreatin, fungal	1
	Unhairing	Protease, mucolytic	Bacterial, fungal, pancreatin	4
Meat, fish	Meat tenderizing	Protease	*Papain*, bromelain, fungal	2
	Tenderizing casings	Protease	Papain, bromelain, fungal	3
	Condensed fish solubles	Protease	Papain, bromelain, bacterial	2
Paper	Starch modification for paper coating	Amylase	*Bacterial*, malt	2
Starch and syrup	Corn syrup	Amylase, dextrinase	Fungal	1
	Production of glucose	Amylase, amyloglucosidase	Fungal	3
	Cold swelling laundry starch	Amylase	Bacterial	2
Pharmaceutical and clinical	Digestive aids	Amylase	*Fungal*, pancreatin	1
		Protease	*Papain*, pancreatin, bromelain, pepsin, fungal	1
		Lipase	Pancreatin	3
		Cellulase	Fungal	3
	Wound debridement	Streptokinase-streptodornase, trypsin, bromelain	Bacterial, animal, plant	1

TABLE 3—*Continued*

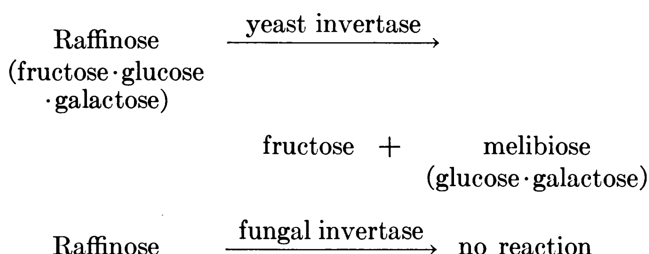
Industry	Application	Enzyme	Source*	Extent of Use†
	Injection for bruises, inflammation, etc.	Streptokinase, trypsin	Bacterial, animal	2
	Paper test strips for diabetic glucose	Glucose oxidase, peroxidase	Fungal, plant	2
	Varied clinical tests	Numerous	Plant, animal, microorganisms	3
Photographic	Recovery of silver from spent film	Protease	Bacterial	1
Textile	Desizing of fabrics	Amylase	*Bacterial*, malt, pancreatin	1
		Protease	Bacterial, fungal, pancreatin	1
Vegetables	Liquefying purees and soups	Amylase	Fungal	3
	Dehydrated vegetables, restoring flavor	Flavor	Plants	4
Wine	Pressing, clarification, filtration	Pectinases	Fungal	2
Miscellaneous	High test molasses	Invertase	Yeast	1
	Resolution racemic mixtures of amino acids	Protease	Fungal	4
	Wall paper removal	Amylase	Bacterial	3

* Where one of optional sources predominates it has been italicized.
† 1 General and extensive industrial use.
2 Industrial use by some manufacturers.
3 Limited industrial use.
4 Laboratory or experimental use only.

considerable importance. For the purpose of demonstrating analogous action, the three enzymes, maltase, lactase, and invertase may be considered together:



These enzymes all attack their corresponding disaccharides with the formation of two molecules of monosaccharide. All may be obtained from fungal and bacterial sources, but invertase and lactase are obtained commercially from yeasts. Yeast and fungal invertases both hydrolyze sucrose, but differ in the nature of their actions. Yeast invertase is a fructosidase, attacking the fructose end, whereas fungal invertase is a glucosidase, attacking the glucose end of the sucrose molecule. This may be demonstrated by comparing activities against certain tri- and tetra-saccharides. For example, yeast invertase splits raffinose into fructose and melibiose, but there is no reaction with fungal invertase since glucose is not terminal in the raffinose molecule:



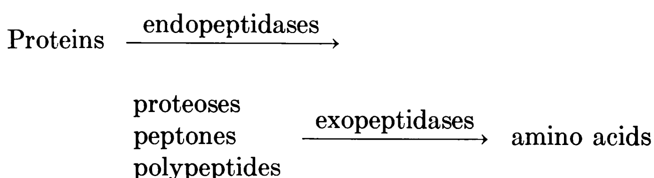
Invertase (Neuberg and Roberts, 1946; Neuberg and Mandl, 1951) is employed in manufacturing artificial honey, and particularly for invert sugar which is much more soluble than sucrose. Hence, a very large use of crude invertase is to prevent crystallization in high test molasses. The high solubility of invert sugar is also important in the manufacture of confectioneries, liqueurs, and ice cream where high sucrose concentrations would lead to crystallization. Invertase is also used in the preparation of chocolate coated, soft cream center candies. Molding and coating are carried out while the contents are firm, after which invertase action yields a smooth, stable cream.

Lactase (Reed, 1952b) may be employed in preventing lactose crystallization in ice cream, which causes "grainy" or "sandy" ice cream. Lactase also prevents lactose crystallization in both whole milk and whey concentrates.

Maltase, while not marketed as such, plays an important role, as mentioned above, in the preparation of sweet syrups by the enzymic degradation of starch.

Proteases

Industrially available proteolytic enzymes produced by microorganisms are usually mixtures of endopeptidases (proteinases) and exopeptidases. In overly simplified form the action of the proteases may be formulated:



In addition to microbial proteases, the plant proteases bromelin, papain, and ficin, and the animal proteases, pepsin and trypsin, have extensive industrial application. Because of the complex structures and high molecular weights of proteins made up of some 20 different amino acids, enzymic proteolysis is extremely complicated. Most proteases are quite specific with regard to which peptide linkages they can split (Smith, 1951). Hence, it is necessary to select the appropriate protease complex or combination of enzymes for specific applications. Usually this can only be determined by trial and error methods. By means of such experimentation, however, many and diverse uses have been found for the various proteases. With proper selection of enzymes, with appropriate conditions of time, temperature, and pH, either limited proteolysis or complete hydrolysis of most proteins to amino acids can be brought about.

Microbial proteolytic enzymes from different fungi and bacteria are available. Most fungal proteases will tolerate and act effectively over a wide pH range (about 4 to 8), while with a few exceptions, bacterial proteases generally work best over a narrow range of about pH 7 to 8.

Fungal protease has been used for centuries in the Orient for the production of soy sauce, tamari sauce, and miso, a breakfast food (Hoogerheide, 1954). In these usages, soybeans or other grains are steamed and inoculated with spores of *Aspergillus flavus-oryzae* or *Aspergillus tamarii*. After maximum enzyme production has taken place, the koji is covered with brine and enzymatic digestion allowed to take place. Limited use is made of this process for making soy sauce in this country also. In these uses, no attempt is made to separate the enzymes from the producing organisms. For most industrial applications, the microbial proteases are extracted from the growth medium as described in an earlier section of this paper.

One of the largest uses for fungal protease is in baking bread and crackers (Johnson and Miller, 1949; Pence, 1953; Miller and Johnson, 1955). The proper amount of protease action reduces mixing time and increases extensibility of doughs, and improves grain, texture, and loaf volume. However, excess of protease must be avoided, and the time for enzyme action and quantity of enzyme used must be carefully controlled by the baker or sticky, unmanageable doughs will result.

Cereal foods are also treated with proteolytic enzymes to modify their proteins, resulting in better processing, including improved product handling, increased drying capacity, and lower power requirements.

To prevent development of undesirable haze in beer and ale when these beverages are cooled, proteolytic enzymes are added during the finishing operation to "chillproof" these beverages. Chillproofing agents contain pepsin, papain, bromelin, fungal and bacterial proteases in various combinations, and digest enough of the protein to prevent formation of haze (Wallerstein, 1956).

Proteolytic enzymes are used for tenderizing meats, and animal casings for processed meats. Consumer products contain papain and bromelin as active agents. Recent work at the American Meat Institute (Wang and Maynard, 1955) has shown that various proteolytic enzymes preferentially attack different meat tissues. Recent practical tests have indicated that combinations of plant, fungal, and bacterial proteases have an advantage over any single enzyme for meat tenderizing.

Protein hydrolyzates for condiments and special diets, and for animal feeds, are obtained by extensive enzymatic hydrolysis of plant, meat and fish, and milk proteins. Enzymatic processing has the advantage over acid or alkaline hydrolysis of proteins in the simple equipment employed and the lack of destruction or racemization of amino acids.

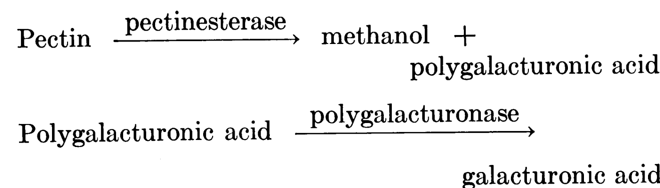
Pharmaceutical and clinical applications for fungal proteases include their use in digestive aids, and for bacterial proteases (streptokinase-streptodornase) in debridement of wounds and by injection to relieve inflammation, bruises, and blood clots.

Bacterial enzymes are used throughout the dry cleaning industry (Ferracone, 1951). Dry cleaning solvents will not remove proteinaceous stains, such as milk, egg, and blood, from clothing. Digesters containing bacterial proteases are used to solubilize such stains during the dry cleaning operation without damaging the fabric. A somewhat similar application is the use of bacterial proteases for desizing and degumming textiles.

Other major industrial applications of bacterial proteases include bating and unhairing of hides for leather manufacture (Wallerstein Co., 1929), and for recovering silver from photographic film by enzyme digestion and solubilization of the gelatin coating.

Pectinases

The pectolytic enzymes are another important group of enzymes of microbial origin (Kertesz, 1951; Lineweaver and Jansen, 1951). The two well recognized types of pectolytic enzymes are pectinesterase and polygalacturonase, the actions of which in overly simplified form are:



Most commercial pectin enzymes are mixtures of these and probably other enzymes. An excellent review of the rather complex nature of pectolytic enzymes has

recently been published by Demain and Phaff (1957).

Pectins are colloidal in nature, making solutions viscous and holding other materials in suspension. Pectinesterase removes methyl groups from the pectin molecules exposing carboxyl groups which in the presence of bi- or multivalent cations, such as calcium, form insoluble salts which can readily be removed. At the same time, polygalacturonase degrades macromolecular pectin, causing reduction in viscosity and destroying the protective colloidal action so that suspended materials will settle out.

Extensive use of pectolytic enzymes is made in processing fruit juices. Addition of pectic enzymes to grapes or other fruits during crushing or grinding results in increased yields of juice on pressing. Wine from grapes so treated will usually clear faster when fermentation is complete, and have better color.

Most consumers prefer clear fruit juices. The cloud, such as in fresh cider, is usually material held in suspension by pectin and filtration is difficult, if not impossible. The safest way to accomplish pectin removal without affecting color or flavor is to treat the juice with a pectic enzyme. Juice for jelly manufacture is frequently depectinized since more uniform jelly can be achieved when a standard amount of pectin is added in controlled amounts. The variable quality and quantity of the natural pectin in the juice does not then interfere.

Pectic enzymes are necessary for making high density fruit juice concentrates or purees. If apple juice is concentrated to 72° Brix without removal of the naturally occurring pectin, a gel will result rather than the desired liquid concentrate. In most cases, juices are depectinized and filtered before concentration, but in others the pectinase is allowed to act while the juice is being concentrated.

Another use for pectic enzymes is in removing the gelatinous coating from coffee beans (Johnston and Kirby, 1950). Natural fermentation produced by microorganisms on the beans formerly was used for this purpose but sometimes gives unpredictable results.

Other Hydrolytic Enzymes

Other useful hydrolytic enzymes include cellulase, hemicellulase, and pentosanase. Partly due to lack of commercial availability of high-potency products, particularly for cellulase, they are not yet of major industrial importance.

There are numerous potential uses for cellulase, such as in tenderizing cellulosic food products and recovering cellulosic wastes. Manufacturers are actively seeking more active cellulases, and large-scale uses must await their availability.

Hemicellulases are active in hydrolyzing certain gums. One industrial application is in preventing gelation in coffee concentrates, where pectic enzymes are not effective (Reich *et al.*, 1957). Other uses are in partially degrading various natural gums to reduce the viscosity of solutions of these gums.

Simpson (1955) has reported increased yields of high quality starch from wheat by use of pentosanase.

Lipases are produced by numerous organisms but have little industrial application, despite the importance of fats in foods.

Nonhydrolytic Enzymes

Only two nonhydrolytic enzymes at present have large-scale industrial applications, glucose oxidase and catalase (Snyder, 1953).

Glucose oxidase is of fungal origin, and acts in the presence of oxygen to convert glucose to gluconic acid and hydrogen peroxide. It is highly specific and oxidizes only β-D-glucose.

$$\underset{\text{(glucose)}}{C_6H_{12}O_6} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \underset{\text{(gluconic acid)}}{(C_6H_{12}O_7} + H_2O_2$$

Catalase, which is also present in commercial fungal glucose oxidase preparations, acts on hydrogen peroxide to yield water and oxygen.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

The net reaction of the glucose oxidase-catalase enzyme system therefore results in one-half mole of oxygen being consumed for each mole of glucose oxidized.

$$2C_6H_{12}O_6 + O_2 \xrightarrow{\text{glucose oxidase-catalase}} 2C_6H_{12}O_7$$

The glucose oxidase-catalase system is used commercially both for removing glucose and for removing oxygen. An interesting application is also its use as a test reagent since it is specific for glucose. This suggestion was first made by Keilin and Hartree (1948), and it has had considerable use in laboratories for this purpose as a quantitative measure of glucose in the presence of other sugars (Whistler *et al.*, 1953; Froesch and Renold, 1956). Commercial application is in the form of paper test strips for diabetic patients, which indicate the presence of glucose in the urine by a color change when the strip is dipped into the sample (Hunt *et al.*, 1956; Adams *et al.*, 1957). Numerous other uses for these test strips for qualitative detection of glucose are also possible.

Extensive industrial use is made of glucose oxidase in desugaring eggs before they are dried (Baldwin *et al.*, 1953). Such removal of glucose greatly enhances shelf life of dried egg products by preventing the occurrence of "browning" and other deteriorative processes.

The problem in marketing of certain canned foods and drinks is oxygen rather than glucose. In the case of

liquid products, glucose oxidase and a little glucose are simply dissolved in them before packing. Residual oxygen in the cans is thus removed by action of the glucose oxidase. For example, in canned soft drinks, the three major changes which may occur are loss of color, alteration of flavor, and can corrosion. Different flavors vary in their susceptibilities to these changes which may be traced to "head space" oxygen remaining in the can. Addition of small amounts of glucose oxidase to canned soft drinks has been shown (Barton *et al.*, 1955) to greatly enhance the keeping quality and diminish can corrosion of susceptible canned beverages.

With cheese, glucose oxidase and glucose are coated on the inside of the wrapper where it contacts the cheese (Sarett and Scott, 1956).

Following the same principles of application, many other uses for glucose oxidase become possible in packaged foodstuffs where the presence of glucose or of oxygen in the food or container presents a deterioration hazard.

A recently patented (Scott, 1956) deoxygenation packet holds tremendous potential for future application. These packets are made of a film, such as polyethylene, which is impermeable to water, but allows the diffusion of oxygen. They contain glucose oxidase-catalase, along with glucose and appropriate buffers. When placed in sealed containers the packets rapidly take up the residual oxygen, leaving an atmosphere free of oxygen. The Quartermaster Food and Container Institute (Kurtz and Yonezawa, 1957) have reported special effectiveness of the packets in protecting dried and dehydrated products containing fats and other oxygen-sensitive materials. These enzyme packets may prove to be a practical solution in packaging dried foods and other items which now have short shelf life due to fat rancidity or other oxidative changes.

Catalase, essentially free of other enzymes, may readily be obtained from bacteria (Herbert and Pinsent, 1948), and also from animal sources. Cold-sterilization of milk for cheese processing, now under consideration, will provide an industrial outlet for catalase. Hydrogen peroxide is added to the milk to sterilize it, and catalase is used to remove the residual hydrogen peroxide before further processing the milk into cheese.

Future of Industrial Enzymes

Industrial uses of enzymes have increased greatly during the past few years. Prospects are excellent for continuing increased usage of presently available enzymes in present applications, and in new uses, and of new enzymes for many purposes.

Enzymes have several distinct advantages for use in industrial processes:

1. They are of natural origin and are nontoxic.
2. They have great specificity of action; hence can bring about reactions not otherwise easily carried out.
3. They work best under mild conditions of moderate temperature and near neutral pH, thus not requiring drastic conditions of high temperature, high pressure, high acidity, and the like, which necessitate special expensive equipment.
4. They act rapidly at relatively low concentrations, and the rate of reaction can be readily controlled by adjusting temperature, pH, and amount of enzyme employed.
5. They are easily inactivated when reaction has gone as far as desired.

Because of these inherent advantages, many industries are keenly interested in adapting enzymatic methods to the requirements of their processes. Examples of some applications under intensive investigation include unhairing of hides for leather, protection of foods and other materials against oxidation, resolution of racemic mixtures of amino acids, and restoration of flavor to dehydrated or canned foods.

Another recent application of enzymes has been in clinical test reagents. Additional developments in this field can be expected.

Clinical application of enzymes has been developing also. Proteolytic enzymes are used for debridement of wounds, and promising clinical results have been reported by injection of certain enzymes such as streptokinase, crystalline trypsin, and chymotrypsin. Since many physical ailments result from derangement of metabolic enzyme systems, increased therapeutic use of enzymes, presently unpredictable, may be expected. For clinical and therapeutic uses, highly purified and perhaps crystalline enzymes will be necessary. Availability of high purity enzymes on an industrial scale is just beginning, and rapid advances in this field may be expected.

Currently much enzyme research is underway by various industries including enzyme manufacturers. Such research is devoted to finding new and improved methods for using enzymes, to improving yields of industrial microbial enzymes, and to finding new enzymes for industrial purposes. Continually increasing usage of old and new enzymes will result from such research.

Summary

The processes for industrial production of microbial enzymes by surface and submerged procedures have been reviewed.

A table listing current industrial uses of enzymes has been presented and the major uses of the microbial carbohydrases (amylases, invertase, lactase and maltase), the proteases, the pectinases, glucose oxidase and catalase have been described.

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Microbiological Process Report

The Persistence and Biological Effects of Antibiotics in Soil[1,2]

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The use of antibiotics in sprays and dusts applied to agricultural crops for the control of plant diseases has given rise to questions of immediate and practical importance. This review summarizes information on the fate of antibiotics that reach the soil, their persistence and susceptibility to chemical and microbiological degradation, and their effects on microbiological processes related to soil fertility and crop production. The influence of antibiotics on seed germination and plant growth is discussed briefly. The ecological significance of antibiotic production under natural conditions is not considered since it was the subject of recent reviews by Brian (1949, 1957).

THE PERSISTENCE OF ANTIBIOTICS IN SOIL

The inactivation of antibiotics in soil may be the result of one or more of three distinct processes: (a) intrinsic chemical instability of the antibiotic molecule; (b) adsorption on soil clay minerals and organic matter; and (c) microbiological degradation.

The inactivation of such antibiotics as penicillin, viridin, gliotoxin, frequentin, and albidin may be partially or wholly explained by their intrinsic chemical instability in aqueous solution at the pH of the soil tested (Jefferys, 1952; Wright, 1954). The rapid inactivation of cycloheximide, gladiolic acid, and penicillin in sterilized soil under pH conditions favorable to stability (Gottlieb *et al.*, 1952; Jefferys, 1952) suggests that these antibiotics are subject to undefined chemical transformations. It is possible that in such cases the antibiotic is hydrolyzed or oxidized chemically with some soil constituent acting as catalyst.

The adsorption of antibiotics by soil was noted by various investigators (Waksman and Woodruff, 1942; Pramer and Starkey, 1950a; Winter and Willeke, 1951; Gregory *et al.*, 1952; Hessayon, 1953) and studied extensively (Siminoff and Gottlieb, 1951; Gottlieb *et al.*, 1952; Gottlieb and Siminoff, 1952; Martin and Gottlieb, 1952; Martin and Gottlieb, 1955). Basic antibiotics are adsorbed by clay minerals and soil organic matter, whereas neutral and acidic antibiotics are not adsorbed to any significant extent. Amphoteric antibiotics will act as either an acid or base depending on their isoelectric point and the pH of the soil. Since the pH of the soil is usually lower than the isoelectric point of the antibiotic, these substances behave as basic compounds in most cases.

The adsorption of antibiotics by clay minerals results in expansion of the crystal lattice and flocculation of the clay. Although the biological activity of adsorbed antibiotics may be reduced (Skinner, 1956), it should not be concluded that the adsorption is irreversible and the inactivation permanent. Siminoff and Gottlieb (1951) showed that adsorbed streptomycin entered into base-exchange reactions and was to a limited extent replaceable by the dyes, methylene blue and janus green. Likewise, Ark and Alcorn (1956) reported that the addition of dipotassium phosphate, peptone, or certain other substances to a bentonite-streptomycin

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