FOOD BIOTECHNOLOGY

TECHNIQUES AND APPLICATIONS

Gauri S. Mittal

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PREFACE

The importance of biotechnology techniques for research, development and education needs very little discussion. Although biotechnology techniques have been used in food processing since ancient times, intensive research and study in this area have only been initiated during recent years. These new techniques are being used in many food processes, and will be used on a wider scale in the future to improve food quality, safety, nutritional value and palatability, and to develop new food products.

Many books have been published in recent years on food biotechnology, based on papers presented in various regional, national and international conferences. In addition to these, many books are available on biotechnology techniques, but each book only covers a particular field, e.g., tissue culture, genetic engineering, and protein engineering. The present book fills the gap and illustrates recent biotechnology techniques with applications in food processing.

This book introduces food scientists and engineers to biotechnology techniques in food processing and production. The book is divided into eleven chapters, with a glossary of important terms used in this book. Chapter 1 introduces the subject of food biotechnology, its importance and present trends. The genetic engineering principles, including recombinant DNA techniques, are discussed in Chapter 2. This chapter introduces the reader to the basics of restriction enzymes, DNA cloning, RNA analysis, DNA sequencing, and cloning in yeast. This chapter concludes with techniques on gene transfer into mammalian cells. In the remainder of the book, I do not merely discuss techniques, but I attempt to demonstrate their applications. Chapter 3 surveys plant tissue or cell culture techniques including embryogenesis, organogenesis, and protoplast fusion.

Chapter 4 presents microbial synthesis and production techniques, and thus deals with the single-cell protein, single-cell oil, antimicrobial substances, and cloning. Mutagenesis and protein engineering techniques are covered in Chapter 5. Various types of mutagenesis – random, *in vitro*, site-directed, and oligonucleotide-mediated are explained, and their techniques presented. The chapter concludes with a discussion of the techniques of protein engineering. Needless to say, this field has a great potential for future expansion.

Chapter 6 provides an in-depth view of the immobilization techniques for

enzymes and cells. This concentrates upon immobilization, but use and selection of enzymes for food processing, and reactors are also discussed. Techniques of biosensor development are described in Chapter 7. These include potentiometric, amperometric, calorimetric, optical, conductimetric, and piezoelectric biosensors. DNA, microbe, and enzyme probes are also explained.

Down-stream processing techniques are treated in Chapter 8. Included in this discussion are mechanical, membrane separation, electrical, thermal, and extraction methods. Chapter 9 presents fermentation techniques including equipment, agitation, oxygen transfer, asepsis, instrumentation and control, and fermenter design.

Scale-up techniques are discussed in Chapter 10, and applications are explained in Chapter 11. The list of glossary presents a description of important terms used in the book. A list of references completes the book.

In conclusion, one might regard this book as a blend of many of the most up-to-date techniques. Each of the techniques may have some limited area where it could stand alone, but for the most part, several techniques are used in conjunction, each one reinforcing and supporting the others.

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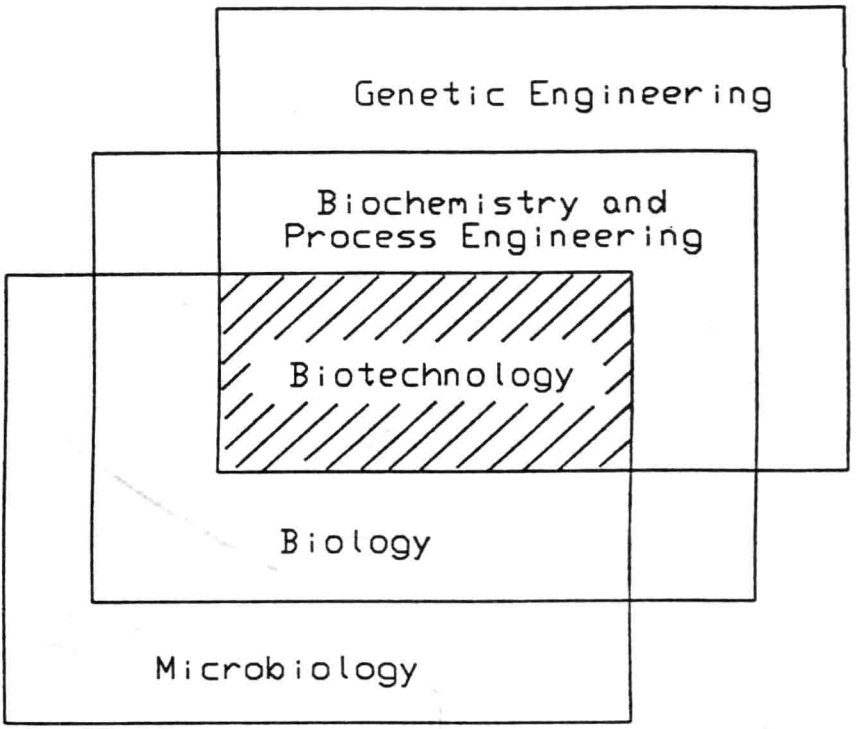

FIGURE 1.1. Scope of biotechnology.

Biotechnology is characterized by five major technological areas (Slotin, 1984):

(1) Recombinant DNA (rDNA)—the insertion of foreign DNA into a new system, where it is cloned and expressed
(2) Enzyme and enzyme technology—the catalytic capabilities of enzymes used either in solution or in immobilized state
(3) Plant cell culture—the *in vitro* culturing of plant cells for required substances and biotransformation
(4) Fused cell techniques—the fusing of two different cells producing hybrids exhibiting characteristics of each of the parents
(5) Process engineering and fermentation technology

1.2 **IMPORTANCE**

Biotechnology is an area of expansion and opportunity involving many sectors of industry, including agriculture, food and feedstuffs. It is moving

toward commercial reality in the food industry. Many common foods and beverage products are based on natural fermentation (bread, wine, pickles, yogurt, cheese, etc.) or are based on the use of enzymes (beer, tenderized meat, and cheese) (Neidleman, 1986). Table 1.1 lists important fermented food products. Beer is the largest product by value in all the biotechnology industry.

Biotechnology in food processing is broadly used in two ways: (1) to design microorganisms that transform inedible biomass into food for human consumption or into feed for animals; and (2) to use biological systems to aid in food processing, either by acting directly on the food itself or by providing materials that can be added to food (Slotin, 1984). Biotechnology can customize raw materials and thus provide food scientists with a powerful tool. For every 1 % increase in tomato solids, the tomato processing industry would save about $80 M per year (Newell and Gordon, 1986). This value was obtained by estimating the savings a tomato processor would realize from reduced raw product volumes, raw material transportation costs and processing energy costs.

The developments in biotechnology are likely to affect the food industry in the following ways (King and Cheetham, 1987):

(1) The modification of food components will provide new and/or improved functional properties. For example, the bio-modification of milk fat in butter-making could result in unsaturation of the fat and subsequent higher polyunsaturated fatty acid levels and improved spreadability of refrigerated butter (Anon, 1985a).

(2) New methods of assaying food constituents, such as immobilized enzyme sensors, will be developed.

TABLE 1.1. *Important food biotechnology products.*

Product	Base Material	Organism
Baked goods	Cereal flour	*Saccharomyces cerevisiae*
Beer	Malt	*Saccharomyces cervisiae*
Brandy/Whisky	Wine/cereals	*Saccharomyces cerevisiae*
Cheese (hard)	Milk	*Streptococcus* and *Lactobacillus*
Cheese (mold)	Milk	*Penicillium camembertii* or *roquefortii*
Coffee	Coffee beans	Lactic acid bacteria
Kefir	Milk	*Candida kefir* and *Lactobacillus kefir*
Sauerkraut	White cabbage	*Lactobacillus plantarum*
Sausage	Beef, mutton, pork	*Lactobacillus* and *Staphylococcus*
Schneidebohnen	French beans	Lactic acid bacteria
Soy sauce	Soybeans	*Aspergillus oryzae*
Vinegar	Malt	*Acetobacter aceti*
Wine	Grape juice	*Saccharomyces cerevisiae*
Yogurt	Milk	*Streptococcus* and *Lactobacillus*

(3) New processes for the production of foods and components, e.g., the use of plant cell cultures for the production of flavors, and the detoxification of certain food products, will be perfected. Specific examples are removal of erucic acid from rapeseed oil, removal of caffeine from coffee, or removal of bitterness from some food products (Kosaric, 1984).

These will improve the cost, quality and acceptability of food products. Table 1.2 lists some biotechnology products used in the food industry.

Enzymes will be engineered to better withstand processes such as heat. For example, rDNA technology applied to microbes producing glucose isomerase could lower the pH optimum to minimize browning reactions (Morris, 1986). The yield and purity of an enzyme can be increased through genetic engineering, and properties such as pH, temperature optima, stability, and resistance to chemicals can be altered (Taylor, 1985). Genetically altered yeast strains, increased productivity, and reduced energy costs have been realized by some major brewers.

The scientific development of complex biotechnology disciplines may be the only avenue left open in today's world of shrinking natural resource supply and growing market demand (Anon, 1985a).

TABLE 1.2. *Some biotechnology products for food processing.*

Product	**Use**	**Organism**
Acetic acid	Pickles	*Acetobacter aceti*
Alginate	Icre cream, meat, pudding	*Acetobacter vinelandii*
Ascorbic acid	Food enrichment	*Gluconobacter oxydans*
Beta-carotene	Food enrichment	*Blakeslea trispora*
Citric acid	Beverages, dairy product	*Aspergillus niger*
Curdlan	Puddings	*Alcaligenes faecalis*
Enzymes	"Various uses"	"Many organisms"
Fumaric acid	Dairy and meat products	*Rhizopus* sp.
Glutamic acid	Flavor enhancer	*Corynebacterium glutamicum*
Inosinic acid	Packaged dishes	*Corynebacterium glutamicum*
Lactic acid	Desserts, juice	*Lactobacillus delbrueckii*
Lysine	Food enrichment	*Corynebacterium glutamicum*
Malic acid	Jam, jelly, beverages	*Aspergillus* sp.
Methionine	Protein enrichment	*Corynebacterium glutamicum*
Nisin	Canned food and meat	*Streptococcus lactis*
Riboflavin	Food enrichment	*Ashbya gossipii*
Single cell protein	Food supplement	*Kluyveromyces fragilis* and *Fusarium graminearum*
Tryptophan	Antioxidant	*Corynebacterium glutamicum*
Xanthan	Beverages, cheese, emulsions	*Xanthomonas campestris*

1.3 ADVANCES AND TRENDS

Developments in molecular biology (e.g., recombinant DNA technology) and process engineering (e.g., continuous reaction using immobilized enzymes) are the basis for the growth of new markets through the introduction of new and higher-quality products, and better process economics for existing products (Joglekar et al., 1983). The biotechnology will also exert a strong impact on the nature of the raw materials for food industry.

The potential for the production of new and unique low-calorie foods is tremendous. Development of low-calorie fats and oils was initiated by inducing the production of shorter-chain fatty acids in commonly used vegetable oils. Efforts are being made to develop better non-nutritive sweetener (Newell and Gordon, 1986). Taste-active proteins act as sweeteners and/or flavor modifiers such as aspartame, thaumatin, monellin, and steviosiole. Thaumatin, a sweetener known under the trade name Talin®, is the sweetest compound in the world (2500 times as sweet as sucrose). Biotechnology is being used to develop specialized yeasts and enhanced enzymes that will aid in the production of low-calorie and high-quality beer.

Xenozymes (engineered enzymes) can be created from natural enzymes via chemical modification, random mutation, or site-specific genetic and protein engineering (Morris, 1986). Chemical modifications are of three types: (1) reactions with ions or small molecules, (2) reactions with water-soluble polymers, and (3) reactions with water-insoluble polymers and matrices, as with immobilized enzymes. Commercial applications of biologically altered and improved fermentation enzymes for both the cheese manufacturing and the brewing industries have been tested, with improved flavors and faster fermentation processes. Efforts are being made to change the structure of the main egg white protein (ovalbumin) and to determine how these structural changes affect the protein's functional properties in baked goods (Anon, 1985a).

Plant tissue culture and recombinant DNA biotechnology can provide both variations and improvements in plants. Plant tissue culture techniques can be used to develop plants with stress tolerance, disease resistance, and nutritional self-sufficiency (Anon, 1985a). Utilization of recombinant DNA technology can provide disease resistance, increased yield, herbicide resistance, efficient fertilizer assimilation, or enhanced oil and protein quality and quantity in crops.

Biotechnology will be the most dynamic area of industrial innovation in future, analogous to the emergence of computers in the 1970s. World markets for biotechnology products are projected to be on the order of hundreds of billions of dollars by the end of the century (Smith, 1985; Joglekar et al., 1983).

1.4 TECHNIQUES AND APPLICATIONS

Major advances have been made in bioreactor designs, in process monitoring techniques, and in computer control of fermentation processes with biosensors. There is an increasing need to design efficient recovery processes, in particular for high value products. Enzymes have long been a part of many biotechnological processes and their catalytic properties are being further utilized with the development of suitable immobilization techniques.

Recombinant DNA techniques involve breaking living cells, extracting DNA, purifying it, and subsequently selectively fragmenting it through highly specific enzymes; the sorting, analysis, selection, and purification of a fragment containing a required gene; chemical bonding to the DNA of a carrier molecule and the introduction of the hybrid DNA into a selected cell for reproduction and cellular synthesis (Smith, 1985).

Computers are used to store and analyze biological sequence data; organic chemistry is used for the synthesis of proteins and polynucleotides; small oligonucleotides can be used as probes to trap larger molecules through hybridization techniques; and peptides are employed to produce monoclonal antibodies for the isolation of proteins (deRosnay, 1985).

Therefore, the following techniques are discussed in this book:

- genetic engineering techniques
- plant cell/tissue culture
- microbial synthesis and production
- protein engineering techniques
- mutagenesis and selection techniques
- immobilization techniques for enzymes and cells
- biosensor techniques
- separation and downstream processing techniques
- fermentation techniques
- reactor design and selection techniques
- scale-up techniques

Applications of these techniques in meat, dairy, cereal, fruits and vegetables, beverages, and oil and fat processing are also briefly discussed.

CHAPTER 2

Genetic Engineering Techniques

2.1 **INTRODUCTION**

Genetic engineering is the methodology that employs recombinant deoxyribonucleic acid (DNA) techniques. This allows the incorporation of the foreign DNA into a host organism where the DNA does not naturally occur, but where it is capable of continued propagation. This technology can alter the hereditary material, the DNA, of a cell so that cells can produce more or different chemicals or perform completely new functions (Anon, 1985b).

At the molecular level, the life can be reduced to the nucleotide sequences in DNA. The basic building blocks of the nucleic acid are the bases, the sugars, and the phosphates (Figure 2.1).

As shown in Figure 2.2, there are five kinds of bases: two purines and three pyrimidines. The two purines that are present in both DNA and RNA (ribonucleic acid) are adenine (A) and guanine (G). The three pyrimidines are cytosine (C), thymine (T), and uracil (U). The thymine is found only in DNA, and uracil appears in RNA. Like purines, the cytosine is present in both DNA and RNA. The sugars are ribose and deoxyribose; RNA contains ribose while DNA contains deoxyribose. Nucleosides are composed of a base and a sugar. The structure of the guanosine (guanine + ribose) is shown in Figure 2.2. The other nucleosides are adenosine (adenine + ribose), thymidine (thymine + deoxyribose), cytidine (cytosine + ribose) and uridine (uracil + ribose). The nucleotide is formed when a phosphate group is attached to a nucleoside. These bases are linked together to form polynucleotides. The phosphate of one nucleotide is linked to the deoxyribose or ribose of another, and so forth. The number of nucleotides in the DNA of different organisms varies greatly from about 5500 in a virus to 10^{10} in human cells.

The two nucleotide chains of DNA are bound to each other by hydrogen bonding between specific base pairs to form a double helix structure (Figure 2.3). However, the majority of RNAs are single-stranded. Various classes of RNA are: mRNA (messenger RNA), tRNA (transfer RNA), and rRNA (ribosomal RNA). These RNAs have specific functions.

The genetic information is present on the DNA in the form of genes. The genes are transcribed in copies of mRNA by the enzyme RNA polymerase.

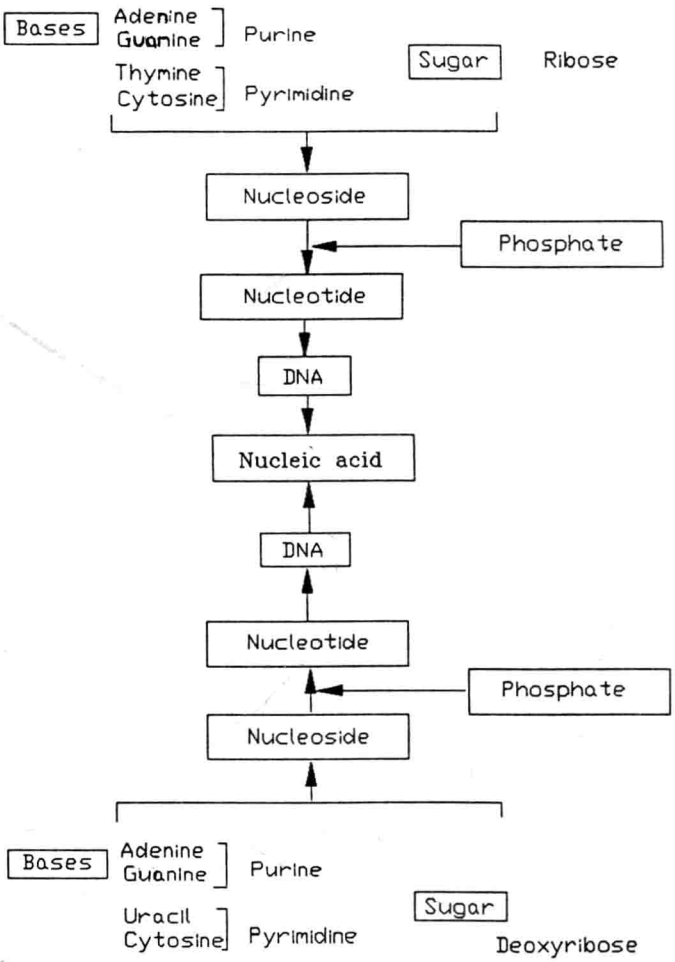

FIGURE 2.1. Formation of nucleic acid.