Tomsk Polytechnic University

L.V. Timoschenko

THE BASICS OF BIOTECHNOLOGY

Textbook

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This textbook consists of ten chapters devoted to the some problems of biotechnologe.

The textbook is prepared at the Organic Chemistry & Organic Synthesis Department of TPU. It is intended for foreign students following the Bachelor's Degree Programme in Biotechnology at the Tomsk Polytechnic University.

Reviewed by: M.S.Yusubov, D.Sc.,
Head of Chemistry Department,
Siberian Medical University

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Chapter 1.

AN INTRODUCTION TO BIOTECHNOLOGY

Biotechnology is a range of biological, chemical and engineering disciplines with varying degrees of application to agricultural, medical, industrial and environmental situations. The assessment of the viability of biotechnology in any country must be made of its biological sciences and their relationships with the productive sectors. Modern biotechnology springs from universities and other research centres that generate the basic knowledge needed to solve practical problems posed by society.

Some selected definitions of biotechnology:

(1) The application of biological organisms, systems or processes to manufacturing and service industries;

(2) A technology using biological phenomena for copying and manufacturing various kinds of useful substance;

(3) Biotechnology is the use of living organisms and their components in agriculture, food and other industrial processes;

(4) The application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services, etc.

The European Federation of Biotechnology (EFB) considers biotechnology as 'the integration of natural sciences and organisms, cells, parts thereof, and molecular analogues for products and services'. The EFB definition is applicable to both 'traditional or old' and 'new or modern' biotechnology. Traditional biotechnology refers to the conventional techniques that have been used for many centuries to produce beer, wine, cheese and many other foods, while 'new' biotechnology embraces all methods of genetic modification by recombinant DNA and cell fusion techniques, together with the modern developments of 'traditional' biotechnological processes.

Biotechnology can draw upon a wide array of relevant fields such as microbiology, biochemistry, molecular biology, cell biology, immunology, protein engineering, enzymology, classified breeding techniques and the full range of bioprocess technologies (Fig.1.1). Biotechnology is not itself a product or range of products like microelectronics: rather it should be regarded as a range of enabling technologies that will find significant application in many industrial sectors. As will be seen in later sections, it is a technology in search of new applications and the main benefits lie in the future. New biotechnological processes will, in many instances, function at low temperature, will consume little energy and will rely mainly on inexpensive substrates for biosynthesis.

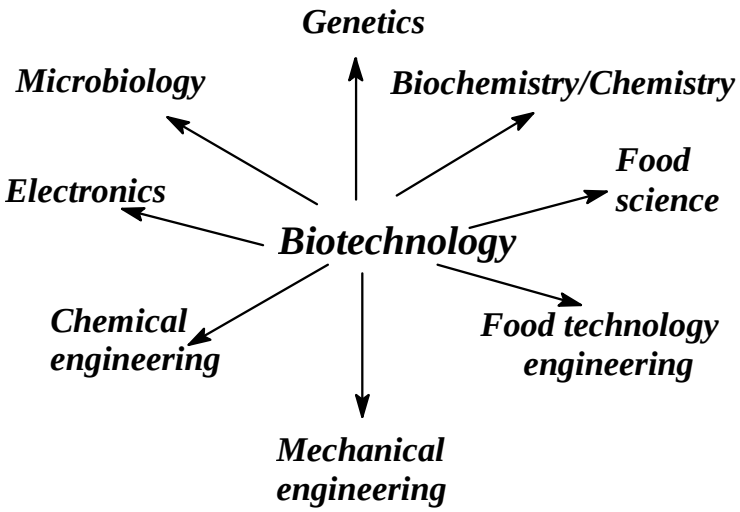


Fig.1.1 The interdisciplinary nature of biotechnology

However, it should be recognized that biotechnology is not something new but represents a developing and expanding series of technologies dating back (in many cases) thousands of years, to when humans first began unwittingly to use microbes to produce foods and beverages such as bread and beer and to modify plants and animals through progressive selection for desired traits. Biotechnology encompasses many traditional processes such as brewing, baking, wine-making, cheese production, the production of oriental foods such as soy sauce and tempeh, and sewage treatment where the use of microorganisms has been developed somewhat empirically over countless years (Table 1.1).

Table 1.1 Historical development of biotechnology

Biotechnological production of foods and beverages
Sumarians and Babylonians were drinking beer by 6000 BC; Egyptians were baking leavened bread by 4000 BC; wine was known in the Near East by the time the book of Genesis was written. Microorganisms first seen in seventeenth century by Antonie van Leeuwenhoek, who developed the simple microscope; fermentative ability of microorganisms demonstrated between 1857 and 1876 by Pasteur – the father of biotechnology; cheese production has ancient origins; so also has mushroom cultivation
Biotechnological processes initially developed under non-sterile conditions
Ethanol, acetic acid, butanol and acetone were produced by the end of the nineteenth century by open microbial fermentation processes; waste-water treatment and municipal composting of solid wastes were the largest fermentation capacity practised throughout the world
Introduction of sterility to biotechnological processes
In the 1940s complicated engineering techniques were introduced to the mass cultivation of microorganisms to exclude contaminating microorganisms. Examples include antibiotics, amino acids, organic acids, enzymes, steroids, polysaccharides, vaccines and monoclonal antibodies
Applied genetics and recombinant DNA technology
Traditional strain improvement of important industrial organisms has long been

practised; recombinant DNA techniques together with protoplast fusion allow new programming of the biological properties of organisms

It is only relatively recently that these processes have been subjected to rigorous scientific scrutiny and analysis; even so it will surely take some time for modern scientifically based practices fully to replace traditional empiricism.

The new biotechnology revolution began in the 1970s and early 1980s, when scientists learned to alter precisely the genetic constitution of living organisms by processes without traditional breeding practices. This 'genetic engineering' has had a profound impact on almost all areas of traditional biotechnology and further permitted breakthroughs in medicine and agriculture, in particular those that would be impossible by traditional breeding approaches. Some of the most exciting advances will be in new pharmaceutical drugs and gene therapies to treat previously incurable diseases, to produce healthier foods, safer pesticides, innovative environmental technologies and new energy sources.

Biotechnology is *a priori* interdisciplinary pursuit. In recent decades a characteristic feature of the development of science and technology has been the increasing resort to multidisciplinary strategies for the solution of various problems.

The term 'multidisciplinary' describes a quantitative extension of approaches to problems that commonly occur within the given area. In contrast, interdisciplinary application occurs when the blending of ideas that occurs during multidisciplinary cooperation leads to the crystallisation of a new disciplinary area with its own concepts and methodologies. In practice, multidisciplinary enterprises are almost invariably mission orientated. However, when true interdisciplinary synthesis occurs the new area will open up a novel spectrum of investigations. Many aspects of biotechnology have arisen through the interaction between various parts of biology and engineering.

Biotechnology can utilize techniques derived from chemistry, microbiology, biochemistry, chemical engineering and computer science (Fig.1.1). It must also aim at achieving a close working cooperation between other related fields such as medicine, nutrition, the pharmaceutical and chemical industries, environmental protection and waste process technology.

The main types of industrial fields involved with biotechnology can be placed in seven categories (Table 1.2).

A key factor in the distinction between biology and biotechnology is their scale of operation. Biologists usually work in the range nanograms to milligrams. Biotechnologists working on the production of vaccines may be satisfied with milligram yields, but many other projects aim at kilograms or tonnes. Thus, one of the main aspects of biotechnology consists of scaling up biological processes.

Many present-day biotechnological processes have their origins in ancient and traditional fermentations such as brewing of beer and the manufacture of bread, cheese, yoghurt, wine and vinegar. However, it was the discovery of antibiotics in 1929 and their subsequent large-scale production in the 1940s that created the

greatest advances in fermentation technology. Since then we have witnessed a phenomenal development in this technology, not only in the production of antibiotics but in many other useful, simple or complex biochemical products, e.g. organic acids, polysaccharides, enzymes, vaccines and hormones (Table 1.2).

Table 1.2 Categories involved in biotechnology

Therapeutics
Pharmaceutical products for the cure or control of human diseases including antibiotics, vaccines, gene therapy
Diagnostics
Clinical testing and diagnosis, food, environmental, agriculture
Agriculture/ foresty /horticulture
Novel crops or animal varieties, pesticides
Food
Wide range of food products, fertilisers, beverages
Environment
Waste treatment, bioremediation, energy production
Chemical intermediates
Reagents including enzymes, DNA/RNA, speciality chemicals
Equipment
Hardware, bioreactors, software and consumables supporting biotechnology

Inherent in the development of fermentation processes is the growing close relationship between the biochemists, microbiologists and chemical engineers. Thus, biotechnology is not a sudden discovery but rather a coming of age of a technology that was initiated several decades ago.

The main dominating reason of biotechnology developing derive from the rapid advances in molecular biology, in particular recombinant DNA technology. By these new techniques (discussed in Chapters 3,8 and 10) it is possible to manipulate directly the heritable material (DNA) of cells between different types of organism, creating new combinations of characters and abilities. The potential of these series of techniques first developed in academic laboratories is now being rapidly exploited in industry.

While in theory the technology is available to transfer a particular gene from any organism into any other organism, microorganism, plant or animal (Chapter 3), in practice there are numerous constraining factors such as which genes are to be cloned, and how they can be selected. The single most limiting factor in the application of genetic engineering is the dearth of basic scientific knowledge of gene structure and function.

The growth in awareness of modern biotechnology parallels the serious world-wide changes in the economic climate arising from the escalation of oil prices since 1973. There is a growing realization that fossil fuels and other non-renewable resources will one day be in limited supply. This will result in the requirement of cheaper and more secure energy sources and chemical feedstocks, which biotechnology could perhaps fulfil. Countries with climatic conditions

suitable for rapid biomass production could well have major economic advantages over less climatically suitable parts of the world.

Another contributory factor to the growing interest in biotechnology has been the current recession in the Western world, in particular the depression of the chemical and engineering sections, in part due to the increased energy costs. Biotechnology has been considered as one important means of restimulating the economy, whether on a local, regional, national or even global basis, using new biotechnological methods and new raw materials. And it is quite feasible that the 1990s will be seen as the era of biotechnology.

New applications followed by that of agriculture and food technology. Exciting new medical treatments and drugs based on biotechnology are appearing with regularity. Prior to 1982 insulin for human diabetics was derived from cow and pigs pancreases. The gene for human insulin was then isolated, and cloned into microorganism, which was then mass-produced by fermentation. This genetically engineering human insulin, identical to the natural human hormone, was the first commercial pharmaceutical product of recombinant DNA technology and now supplies millions of insulin users world wide with a safe, reliable and unlimited source of this vital hormone. Biotechnology has also made it easier to detect and diagnose human, animal and plant diseases. In clinical diagnosis there are now hundreds of specialized kits available for simple home use or for complex laboratory procedures such as blood screening. Biotechnology methods can improve the nutrition, taste and appearance of plants and various food products, enhance resistance to specific viruses and insect pests and produce safer herbicides. For food safety, new probes can rapidly detect and identify specific microbial pathogens in food, e.g. the bacteria *Salmonella* and fungal toxins such as aflatoxin.

Much of modern biotechnology has been developed and utilized by large companies and corporations. However, many small- and medium-size companies are using biotechnology methods too. In many industries traditional technology produces compounds causing environmental damage, whereas biotechnology methods offer a ‘green’ alternative promoting a positive public image and also avoiding new environmental penalties.

Many biotechnological processes have a three-component central core, in which one part is concerned with obtaining the best biological catalyst for a specific function or process, the second part creates (by construction and technical operation), the best possible environment for the catalyst to perform, and the third part is concerned with separation and purification of an essential product or products from a fermentation process.

In the majority of examples, the most effective, stable and convenient form for the catalyst for a biotechnological process is a whole organism (in particular, microorganism), but this does not exclude the use of higher organisms (plant and animal cell cultures).

Microorganisms can be viewed both as primary fixers of photosynthetic energy and as systems for bringing about chemical changes in almost all types of natural and synthetic organic molecules. Furthermore, microorganisms can possess

extremely rapid growth rates in excess far of any of the higher organisms such as plants and animals. Thus, immense quantities can be produced under the right environmental conditions in short time periods.

The main areas of application of biotechnology are shown in Table 1.3.

Table 1.3. World markets for biological products, 1981

Product	Sales ($ millions)
Alcohol beverages	23000
Cheese	14000
Antibiotics	4500
Penicillins	500
Tetracyclines	500
Cephalosporins	450
Diagnostic tests	2000
High fructose syrups	800
Amino acids	750
Baker's yeast	540
Steroids	500
Vitamines	330
Citric acid	210
Enzymes	200
Vaccines	150
Insulin	100
Urokinase	50
Human growth hormone	35
Microbial pesticides	12

Biotechnology will continue to create exciting new opportunities for commercial development and profit in a wide range of industrial sectors including healthcare and medicine, agriculture and forestry, fine and bulk chemicals production, food technology, fuel and energy production, pollution control and resource recovery.

Table 1.3. The main areas of application of biotechnology.

Bioprocess technology
Historically, the most important area of biotechnology, namely brewing, antibiotics, mammalian cell culture, etc; extensive development in progress with new products envisaged, namely polysaccharides, medically important drugs, solvents, protein-enhanced foods.
Enzyme technology
Used for the catalysis of extremely specific reactions; immobilisation of enzymes; to create specific molecular converters (bioreactors). Products formed include L-amino acids, high fructose syrup, semi-synthetic penicillins, starch and cellulose hydrolysis, etc. Enzyme probes for bioassays
Waste technology
Long historical importance but more emphasis now being made to couple these processes with the conservation and recycling of resources; food and fertilisers,

biological fuels

Environmental technology

Great scope exists for the application of biotechnological methods for solving many environmental problems – pollution control, removing toxic wastes; recovery of metals from mining wastes and low-grade ores

Renewable resources technology

The use of renewable energy sources, in particular, lignocellulose to generate new sources of chemical raw materials and ethanol, methane and hydrogen. Total utilization of plant and animal material

Plant and animal agriculture

Genetically engineered plants to improve nutrition, disease resistance, keeping quality, improved yields and stress tolerance. Improve productivity for animal farming. Improve food quality, flavour, taste and microbial safety

Healthcare

New drugs and better treatment for delivering medicines to diseased parts. Improved disease diagnosis, understanding of the human genome

Chapter 2.

SUBSTRATES FOR BIOTECHNOLOGY

It has been estimated that the annual net yield of plant biomass arising from photosynthesis is at least 120 billion tonnes of dry matter on land and around 50 billion tonnes from the world's oceans. Of the land-produced biomass, approximately 50 % occurs in the complex form of lignocellulose.

Biomass agriculture, aquaculture and forestry may hold great economic potential for many national economics, particularly in tropical and subtropical regions. Nowadays photosynthetically derived biomass is used as a source of energy and industrial feedstocks, in particular, in the less industrialized regions such as Latin America, China, India and Africa. In developed nations, biomass derived from agriculture and forestry has been directed largely towards industrial and food uses (Table 2.1).

Table 2.1. Important products derived from biomass.

Fuels
Methane (biogas) especially in the developing world
Pyrolysis products (gas, charcoal)
Ethanol (via cane juice and cellulose fermentation)
Oils (from hydrogenation)
Direct combustion of waste biomass
Feedstocks
Ethanol (potential feedstock for industry)
Synthesis gas (from chemical gasification)
Fertilisers
Compost
Sludge
Feeds
Direct feed supplements
Single cell proteins

Natural raw materials originate mostly from agriculture, food industry and forestry. These are mainly carbohydrates of varying chemical complexity and include sugar, starch, cellulose and lignin. The wide range of by-products obtained from raw materials and used in biotechnological processes is shown in Table 2.2.

Sugar bearing raw materials such as sugar beet, sugar cane and sugar millet are the most suitable and available to serve as feedstocks for biotechnological processing.

Starch-bearing agricultural products include the various types of grain such as maize, rice and wheat, together with potatoes and other root crops such as sweet potatoes and cassava. A slight disadvantage of starch is that it must usually be degraded to monosaccharides or oligosaccharides by digestion or hydrolysis before fermentation.

Table 2.2. A range of by-products that could be used as substrates in biotechnology.

Agriculture	***Forestry***	***Industry***
Straw	Wood waste hydrolysate	Molasses
Bagasse	Sulphite pulp liquor	Distillery wastes
Maize cobs	Bark, sawdust, branches	Whey
Coffee, cocoa and coconut hulls	Paper and cellulose	Industrial waste water from food industries (olive, palm-oil, potato, date, citrus, cassava)
Fruit peels and leaves	Fibres	
Tea wastes		
Oilseed cakes		Wash waters (dairy, canning, confectionery, bakery, soft drinks, sizing, malting, corn steep)
Cotton wastes		
Bran		
Pulp (tomato, coffee, banana, pineapple, citrus, olive)		Fishery effluent and wastes
Animal wastes		Meat by-products
		Municipal garbage

There can be doubt that cellulose, both from agriculture and forestry sources, must contribute a major source of feedstock for biotechnological processes such as fuels and chemicals. However, cellulose is a very complex compound and occurs in nature in close association with lignin. The ability of lignocellulose complexes to withstand the biodegrative forces of nature is witnessed by the longevity of trees, which are composed mainly of lignocellulose.

While biotechnological processes will use many agricultural products such as sugars, starches, oils, etc., as substrates, the vast array of waste products derived from agriculture, and currently not creatively used, will undoubtedly be subjected to detailed examination and future utilization. Agricultural and forestry wastes come in many diverse types: cereal straws, corn husks, wheat bran, sugar cane, bagasse and forestry wastes including trimmings, sawdust, bark, etc. (Table 2.3).

Table 2.3. Biotechnological strategies for utilization organic waste materials.

Upgrading the food waste quality to make it suitable for human consumption
Feeding the food waste directly or after processing to poultry, pigs, fish or other single stomach animals that can utilize it directly
Feeding the food waste to cattle or other ruminants if unsuitable for single stomach animals because of high fibre content, toxins or other reasons
Production of biogas (methane) and other fermentation products if unsuitable for feeding without expensive pretreatments
Selective other purposes such as direct use as fuel, building materials, chemical instruction, etc.

With the development of commercial processes for the production of single cell protein (SCP) and other organic products, a number of chemical and

petrochemical feedstocks have become important for fermentation processes. Thus, natural gas or methane and gas oil have been preferred as raw material because of their easy processing and universal availability. Main commercial interest has been concerned with n-paraffins, methanol and ethanol. Their involvement in various aspects of biotechnology, but particularly in SCP production.

Future biotechnological processes will increasingly make use of organic materials that are renewable in nature or occur as low value wastes that may presently cause environmental pollution. Table 2.4 summarizes the many technical considerations that must be made when approaching the utilization of waste materials.

Table 2.4. Technical considerations for the utilization of waste materials.

Biological availability	*Low* (cellulose) *Moderate* (starch, lactose) *High* (molasses, pulping sugars)
Concentration	*Solid* (milling residues, garbage) *Concentrated* (molasses) *Weak* (lactose, pulping sugars) *Very dilute* (process and plant wash liquors)
Quality	*Clean* (molasses, lactose) *Moderate* (straw) *Dirty* (garbage, feedlot waste)
Location	*Collected* (large installation, small centers) *Collected specialized* (olive, palm oil, date, rubber, fruit, vegetables) *Dispersed* (straw, forestry)
Seasonally	*Prolonged* (palm oil, lactose) *Very short* (vegetable cannery waste)

Chapter 3.

GENETICS AND BIOTECHNOLOGY

All properties of organisms depend on the sum of their gene potential. There are two broad categories of genes: structural and regulatory. Structural genes encode for amino acid sequences of proteins, which, as enzymes, determine the biochemical capabilities of the organism by catalyzing synthetic or catabolic reactions or, alternatively, play more static roles as components of cellular structures. In contrast, the regulatory genes control the expression of the structural genes by determining the rate of production of their protein products in response to intra- or extra cellular signals.

The studies of James Watson and Francis Crick and others in the early 1950s led to the construction of the double helix model depicting the molecular structure of DNA and subsequent hypotheses on its implications for understanding of gene replication. Since then there has been a spectacular unraveling of the complex interactions required to express the coded chemical information of the DNA molecule into cellular and organismal components. Changes in the DNA molecule making up the genetic complement of an organism is the means by which organisms evolve and adapt themselves to new environments. Change in the DNA can occur in two ways:

(1) By mutation, which is a chemical deletion or addition of one or more of the chemical parts of the DNA molecule.

(2) By the interchange of genetic information or DNA between like organisms by sexual reproduction. This is achieved by a process of conjugation in which there is a donor, called male and a recipient, called female.

The manipulation of the genetic material in organisms is now achieved in three ways: organismal, cellular and molecular.

Organismal Genetic manipulation of whole organisms has been realized by sexual reproduction. Active control of sexual reproduction has been practiced in agriculture for centuries. In recent time it has been used with several industrial microorganisms, e.g. yeasts. It involves selection, mutation, sexual crosses, hybridization, etc.

Cellular Cellular manipulations of DNA has been used for over two decades, and involve either cell fusion or the culture of cells and the regeneration of whole plants from these cells (Chapter 10). This is semi-random or directed process, in contrast to organismal manipulation. Successful biotechnological examples of these methods include monoclonal antibodies and the cloning of many important plant species.

Molecular Molecular manipulations of DNA and RNA first occurred nearly two decades ago and heralded a new era of genetic manipulations enabling – a directed control of the changes. This is termed ‘genetic engineering’ or ‘recombinant DNA technology’. In these techniques the experimenter is able to add or delete parts of DNA molecule with a high degree of precision and the product can be easily identified.

Industrial Genetics

Most microorganisms used in biotechnological processes (bacteria, yeast or mould, etc.) were originally isolated from the natural environment and have been modified by the industrial genetics into specialized organisms for specific productivity.

In most industrial genetics the basis for changing the organism's genome has been by mutation using X-rays and mutagenic chemicals. However, such methods normally lead only to the loss of undesired characters or increased production due to loss of control functions. It has rarely led to the appearance of a new function or property. Thus, an organism with a desired feature will be selected from the natural environment, propagated and subjected to a mutational programme, then screened to select the best progeny.

Unfortunately, many of industrial-important microorganisms (in particular, antibiotic-producing microorganisms) do not have a clearly defined sexual cycle; this has meant that the only way to change the genome involves the massive mutational programmes, followed by screening and selection.

Furthermore, there is another constant problem in industrial utilization of microorganisms – strain instability. Undesired spontaneous mutations can often occur at high rate, giving rise to degeneration of the strain's industrial importance. The chance of a high rate of spontaneous mutation is probably greater than the use of special mutagen treatment. Now industry places great emphasis on strain viability and productivity potential of the preserved biological material.

In recent years, industrial genetics has come to depend increasingly on two new ways of manipulation DNA – protoplast and cell fusion, and recombinant DNA technology. These are now important additions to the technical repertoire of the genetics involved with biotechnological industries.

Protoplast and Cell Fusion Technologies

Plants and most microbial cells are characterized by a distinct outer wall that gives the shape characteristic to the cell or organism. Immediately within the cell wall is the plasma membrane retaining all the cellular components such as nuclei, mitochondria, vesicles, etc. For some years now it has been possible to remove the cell wall, releasing spherical membrane-bound structures known as protoplasts. These protoplasts can be maintained in isolation for variable periods of time but they can not be propagated, requiring to regenerate a cell wall before regaining reproductive capacity.

Protoplasts can be obtained from many plants species, bacteria, yeasts and filamentous fungi. Protoplasts from different strains can be fused and so overcome the natural sexual mating barriers. Fusion of protoplasts can be enhanced by treatment with the chemical polyethylene glycol, which, under optimum conditions, can lead to extremely high frequencies of recombinant formation. This can be

increased by ultraviolet irradiation of the protoplast preparations. Protoplast fusion can also occur with human or animal cells.

Protoplast fusion has obvious applications in yield improvement of antibiotics by combining yield-enhancing mutations from different strains or even species. Protoplasts will also be an important part of genetic engineering, in facilitating recombinant DNA transfer.

One of the most exciting and commercially rewarding areas of biotechnology involves a form of mammalian cell fusion leading to the formation of monoclonal antibodies. It is well-known that certain cells (B-lymphocytes) within the body have the ability to secrete antibodies that can inactivate contaminating or foreign molecules (antigens) within the animal system. The antibody has a Y-shaped molecular structure and uses one part of this structure to bind the invading antigen and the other part to trigger the body's response to eliminate the antigen-antibody complex. It has been calculated that mammalian species can generate up to 100 million different antibodies, thereby ensuring the most invading foreign antigens. Antibodies have high binding affinities and specificity against chosen antigen. For the mammalian system it is the major defense against disease-causing organisms and other toxic molecules.

Attempts to cultivate the antibody-producing cells in artificial media were unsuccessful, the cells either dying or creasing to produce the antibodies. However, in 1975 George Köhler and Cesar Milstein successfully demonstrated the production of pure monoclonal antibodies from the fusion product (hybridoma) of B-lymphocytes (antibody-producing cells) and myeloma tumor cells. In 1984 they were awarded the Nobel Prize for Medicine for this outstanding scientific achievement.

The monoclonal antibody technique changes antibody-secreting cells (with limited life span) into cells capable of continuous growth with maintaining their antibody-secreting potential. It is achieved by fusion technique, whereby B-lymphocyte cells are fused to cancer or myeloma cells in a one-to-one ratio, forming hybrides or hybridomas capable of continuous growth and antibody secretion in culture. Single hybrid cells can then be selected and grown as clones or pure cultures of the hybridomas. Such cells continue to secret antibody and the antibody is of one particular specificity as opposed to the mixture of antibodies that occurs in an animal's bloodstream after immunization.

Monoclonal antibody formation is achieved by injecting a mouse or rabbit with an antigen, later removing the spleen and then fusion of individual spleen cells with individual myeloma cells. (Fig. 3.1) Techniques are available to identify the right antibody-secreting cells and then cloning or propagating that cells into large populations for formation of large quantities of the desired antibody.

Monoclonal antibodies have now wide application in many diagnostic techniques: they have been combined into test kits for diagnostic purposes, in healthcare, plant and animal agriculture and in food manufacture.

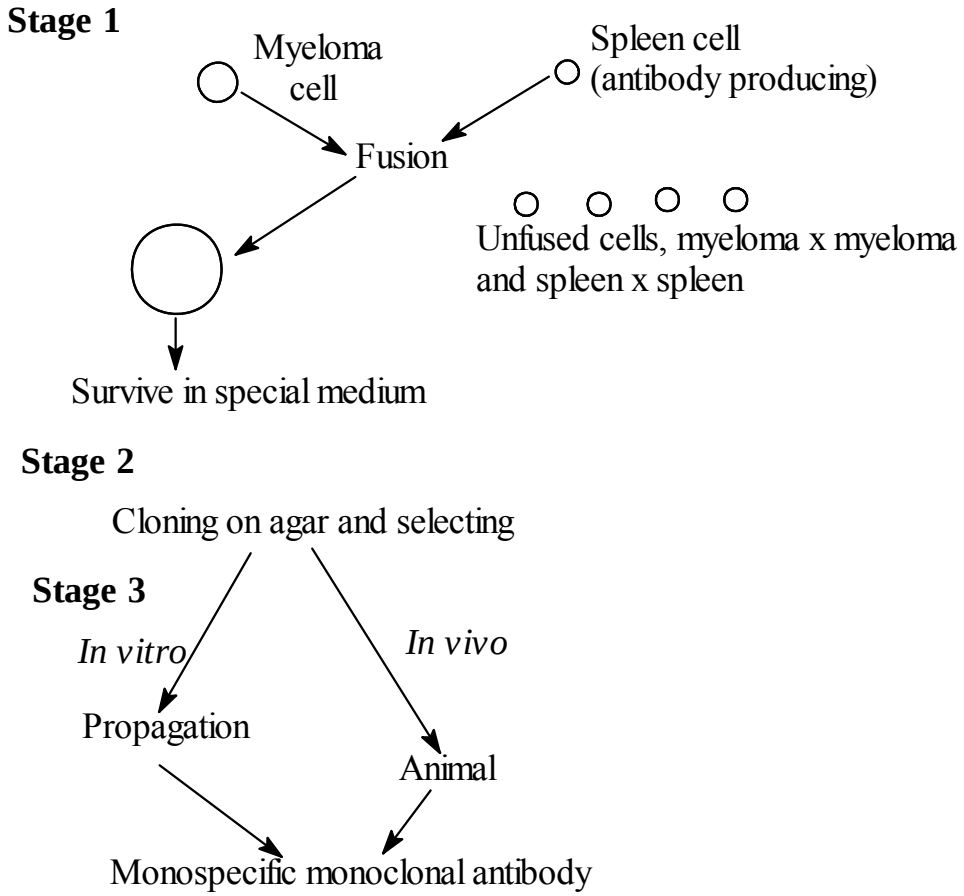


Fig. 3.1 Diagram illustrating the formation of antibody-producing hybridomas by fusion techniques.

Genetic Engineering

Genes comprise the fundamental basis of all life, determine the properties of all living forms of life and are defined segments of DNA. Because DNA structure and composition of all living forms is essentially the same, any technology that can isolate, change or reproduce a gene is likely to have an impact on almost every aspect of society.

Genetic recombination consists of the breakage and rejoining of DNA molecules of the chromosomes and is of fundamental importance to living organisms for the reassortment of genetic material.

Recombinant DNA techniques, often termed ‘genetic engineering’, offer unlimited opportunities for creating new combinations of genes that do not exist under natural conditions. Genetic engineering can be defined as the formation of new combinations of heritable material by the insertion of nucleic acid molecules, produced outside the cell, into any virus, bacterial plasmid or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation. These techniques allow the splicing of DNA molecules of diverse origin, and when combined with techniques of genetic transformation, facilitate the introduction of foreign DNA into other organisms. The foreign DNA is introduced into the genome

of the recipient organism host in such a way that the total genome of the host is unchanged except the single manipulated gene.

Thus, DNA can be isolated from cells of plants, animals or microorganisms (the donors) and can be fragmented into groups of one or more genes. Such fragments can then be coupled to another piece of DNA (the vector) and passed into the host cell, becoming part of the genetic complement of the new host. The host cell can then be propagated in mass to form novel genetic properties and abilities which impossibly reach by ways of selective breeding or mutation.

This is the most significant new technology in modern biotechnology. In industrial microbiology it will permit the production such as human and animal proteins and enzymes; in medicine there will be better vaccines and hormones; in agriculture improved plants and animals will achieve greater productivity, quality of products and disease resistance; in food production we can expect improved quality, flavour and taste; and in environmental aspects there will be a wide range of benefits such as pollution control.

The basic molecular requirements for the *in vitro* transfer and expression of foreign DNA in a host cell are the following:

The vector or carrier system Two broad categories of vector molecules have been developed as vehicles for gene transfer, namely plasmids (small units of DNA distinct from chromosomes) and bacteriophages (or bacterial viruses). Vector molecules normally exist within a cell in an independent or extrachromosomal form. Vector molecules should be capable of entering the host cell and replicating within it. Ideally, the vector should be small, easily prepared and must contain at least one site. Plasmids have been found in a wide range of organisms, e.g. bacteria, yeasts and mould fungi; they have been studied mostly in Gram-negative bacteria.

Splicing genes The most significant advances towards the construction of hybrid DNA molecules *in vitro* have come from the discovery site-specific restriction endonuclease enzymes produce specific DNA fragments that can be joined to any similarly treated DNA molecule using another enzyme, DNA ligase. Restriction enzymes are present in a wide range of bacteria and can distinguish between DNA from their own cells and foreign DNA by recognizing certain sequences of nucleotides. There are techniques available for breaking open a length of DNA into shorter fragments that contain a number of genes determined by the enzyme used. Such DNA fragments can then be separated from each other on the basis of differing molecular weights and can be joined together in a number of ways, provided that the ends are complementary.

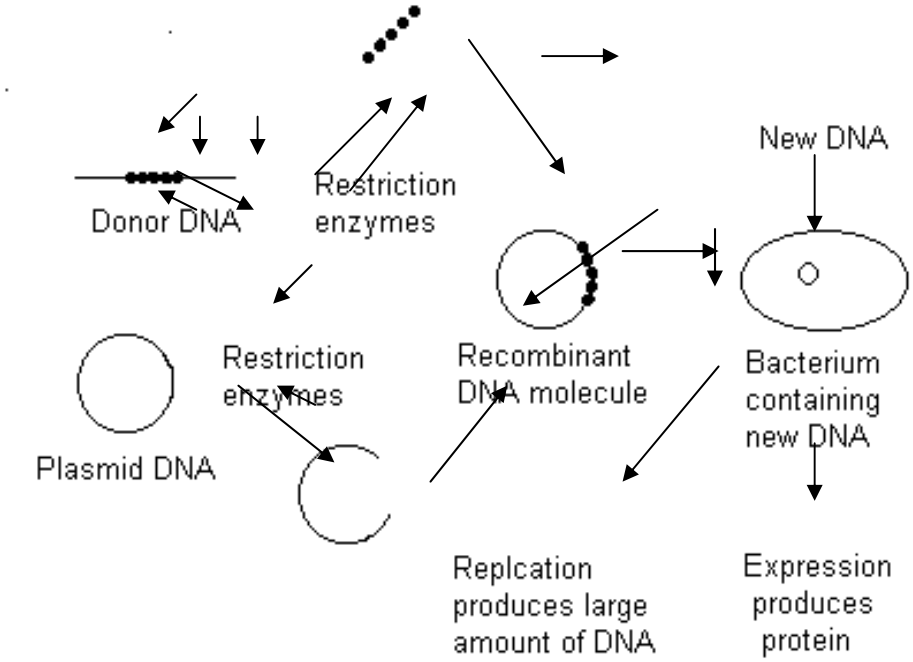
Introduction of vector DNA recombinants The new recombinant DNA can be introduced into the host cell by transformation (the direct uptake of DNA by a cell from its environment) or transduction (DNA transferred from one organism to another by way of a carrier or vector system) and the new DNA will be cloned with the propagation of the host cell using different methods.

The strategies involved in genetic engineering are outlined in Table 3.1 and Fig. 3.2.

Table 3.1. Strategies involved in genetic engineering

Formation of DNA fragments
Extracted DNA can be cut into small sequences by specific enzymes, restriction endonucleases found in many species of bacteria
Splicing of DNA into vectors
The small sequences of DNA can be joined or spliced into the vector DNA molecules by an enzyme, DNA ligase, creating an artificial DNA molecule
Introduction of vectors into host cells
The vectors are either viruses or plasmids, are replicons and can exist in an extrachromosomal state; transfer is normally by transduction or transformation
Selection of newly acquired DNA
Selection and ultimate characterisation of the recombinant clone

Fig.3.2 Recombinant DNA: the technique of recombining genes from one species with those from another.



The Polymerase Chain Reaction

The greatest development in modern biology during the last 10 years has been the invention of the polymerize chain reaction (PCR). This is basically a technique that allows the selective amplification of any fragment of DNA. The inventor of PCR, Kary Mullis, shared the Nobel Prize for Chemistry in 1993.

The PCR process relies on the sequence of 'base-pairs' along the length of the two strands that make the complete DNA molecule. In DNA there are four deoxynucleotides derived from the four bases adenosine (A), thymidine (T),

guanine (G) and cytidine (C). The strands or polymers that comprise the DNA molecule are held to each other by hydrogen bonds between the base-pairs. In this arrangement A binds only to T while G binds only to C and this unique system folds the entire molecule into the now well-recognized double helix structure.

PCR involves three processing steps: denaturation, annealing and then extension by DNA polymerase. In step 1, the double-stranded DNA is heated (95-98^{o}C) and separated into two complementary single strands. In step 2 (60^{o}C), the synthetic oligonucleotide primers (chemically synthesised short-chain nucleotides), are added and bind to the single strands in places where the strand's DNA complements their own. In step 3 (37^{o}C), the primers are extended by DNA polymerase in the presence of all four deoxynucleoside triphosphates resulting in the synthesis of new DNA strands complementary to the template strands. The completion of the three steps comprises a cycle and the real power of PCR is that with 25-30 cycles this experimental synthesis leads to massive amplification of DNA, which can then be used for analytical purposes.

The applications of PCR increase almost daily and include molecular biology and genetic engineering, forensic validation, plant and animal breeding and environmental monitoring.

Chapter 4.

BIOPROCESS/FERMENTATION TECHNOLOGY

The very beginnings of fermentation technology or, as it is now better recognized, bioprocess technology were derived in part from the use of microorganisms for the production of food such as cheeses, yogurts, sauerkraut, fermented pickles and sausages, soy sauce, and other oriental products, and beverages as beers, wines and derived spirits (Table 4.1). In many cases, the present day production processes for such products are similar.

Table 4.1. Fermentation products in various industrial branches

Branch	***Products***
Chemical	
Organic (bulk)	Ethanol, acetone, butanol, organic acids (citric, itaconic)
Organic (fine)	Enzyme, perfumeries, polymers (polysaccharides)
Inorganic	Metal beneficiation, bioaccumulation and leaching (Cu, U)
Pharmaceutical	Antibiotics, diagnostic agents (enzymes, monoclonal antibodies), enzyme inhibitors, steroids, vaccines
Energy	Ethanol (gasohol), methane (biogas), biomass
Food	Dairy products (cheeses, yogurts, fish and meat products), Beverages (alcoholic, tea and coffee), baker's yeast, Food additives (antioxidants, colours, flavours, stabilizers) Mushroom products, novel foods (soy sauce, tempeh, miso), Amino acids, vitamins, starch products, glucose and high fructose syrups, functional modifications of proteins, pectins
Agriculture	Animal feedstuffs (SCP), veterinary vaccines, microbial pesticides, ensilage and composting processes, plant cell and tissue culture (vegetative propagation, genetic improvement)

Although the traditional forms of bioprocess technology relate to food and beverages, new products are increasingly being derived from microbial fermentations, namely:
(1) To produce essental primary metabolites such as acetic and lactic acids, glycerol, amino acids, vitamins, etc.
(2) To produce secondary metabolites (metabolites that do not have an obvious role in the metabolism of the producer organism) such as penicillin, cephalosporin, streptomycin, etc.
(3) To produce many forms of industrially useful enzymes, e.g. exocellular enzymes and intracellular enzymes, etc.

The product formation stages in bioprocess technology are very similar no matter what organism is selected, what medium are used and what product is formed. All biotechnological processes are performed within containment systems or bioreactors. Large numbers of cells are involved in these processes and the bioreactor ensures their close involvement with correct medium and conditions for growth and product formation. Examples of the diverse product categories produced industrially in bioreactors are given in Table 4.2.

Table 4.2. Examples of products in different categories produced in industrial bioreactors

Category	***Example***
Cell mass	Baker's yeast, single cell protein
Cell components	Intracellular proteins
Biosynthetic products	Antibiotics, vitamins, amino and organic acids
Catabolic products	
Bioconversion	High fructose corn syrup, 6-aminopenicillanic acid
Waste treatment	Activated sludge, anaerobic digestion

Principles of Microbial Growth

The growth of organisms depends on the availability and transport of necessary nutrients to the cell and subsequent uptake and on environmental parameters such as temperature, pH and aeration.

The quantity of biomass or specific cellular component in a bioreactor can be determined gravimetrically (by dry weight, DNA or protein) or numerically (by number of cells). Doubling time means the period of time required for the doubling in the weight of biomass. Average doubling times increase with increasing cell size and complexity, e.g.: bacteria 0,25-1h; yeast 1-2h; mould fungi 2-6,5h; plant cells 20-70h; and animal cells 15-48 h.

In normal practice an organism seldom has ideal conditions for unlimited growth – growth often depends on a limiting factor, e.g. an essential nutrient. As

the concentration of this factor drops, so will the growth potential of the organism decrease.

In biotechnological processes there are three main ways of growing microorganisms in the bioreactor, namely batch, semi-continuous or continuous.

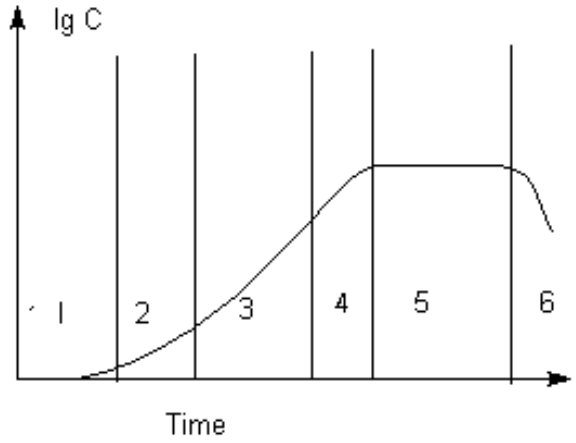


Fig. 4.1. Growth characteristics in a batch culture of a microorganism. 1 lag phase; 2 transient acceleration; 3 exponential phase; 4 deceleration phase; 5 stationary phase; 5. death phase.

In a batch culture the microorganisms are inoculated into a fixed volume of medium and as growth takes place nutrients are consumed and products of growth (biomass, metabolites) accumulate. The nutrient environment within the bioreactor is continuously changing and thus, in turn, enforcing changes to cell metabolism. Cell multiplication ceases because of exhaustion or limitation of nutrient(s) and accumulation of toxic excreted products.

The complex nature of batch growth of microorganisms is shown in Fig. 4.1.

The initial **lag phase** is a time of no apparent growth, when the cells are adapted to the environmental conditions. Then there is a **transient acceleration phase** when the inoculum begins to grow and an **exponential phase** then quickly is followed. In the exponential phase microbial growth proceeds at the maximum possible rate for that organism with excess of nutrients and absence of growth inhibitors. After the nutrient conditions change growth rate decreases, entering the **deceleration phase** and then the **stationary phase** is followed, when overall growth does not depend on nutrient concentration. The final phase is the **death phase** when growth rate has ceased. Most biotechnological processes are stopped before this stage because of decreasing metabolism and cell lysis.

In industrial usage, batch cultivation has been operated to optimize organism or biomass production and then to allow the organism to perform specific biochemical transformations such as end-product formation (e.g. amino acids, enzymes) or decomposition of substances (sewage treatment, etc). Many important products such as antibiotics are formed during the stationary phase of the growth cycle in batch cultivation.

In contrast to batch conditions the practice of continuous cultivation gives near-balanced growth with little fluctuation of nutrients, metabolites, cell numbers or biomass. This practice depends on fresh medium entering a batch system at the exponential phase of growth with a corresponding withdrawal of medium and cells. Continuous methods of cultivation permit organisms to grow under unchanging conditions in which growth occurs at a constant rate and in a constant environment. Factors such as pH and the concentrations of nutrients and metabolic products can be held near constant. In industrial practice continuously operated systems are of limited use and include only single cell protein and ethanol and some forms of waste-water treatment processes.

The full range of cultivation methods for microorganisms is shown in Table 4.3.

Table 4.3. Characteristics of cultivation methods

Type of culture	***Operation characteristics***	***Application***
Solid	Simple, cheap, selection of colonies from single cell possible; process control limited	Maintenance of strains, genetic studies; production of enzymes; composting
Film	Various types of bioreactors	Waste-water treatment, monolayer culture (animal cells); bacterial leaching; vinegar production
Submerged homogeneous distribution of cells; batch	'Spontaneous' reaction, various types of reactors, agitation by stirred, air, liquid process control for physical parameters, less for chemical and biological parameters	Standard type of cultivation antibiotics, solvents, acids, etc.
Fed-batch	Simple method for control of regulatory effects, e.g. glucose repression	Production of baker's yeast
Continuous one-stage homogeneous	Proper control of reaction; excellent for kinetic and regulatory studies; high cost for experiment; problems of aseptic operation; the need for highly trained operators	Few cases of application in industrial scale; production of single cell protein; waste-water treatment

The Bioreactor/Fermenter

Bioreactors using in biotechnological processes range from simple stirred or non-stirred open containers to complex, aseptic systems involving varying levels of advanced computer inputs. Bioreactors are of two distinct types (Fig. 4.2). In the first type they are non-aseptic systems where it is impossible to operate with

entirely pure cultures; in the second type they are aseptic systems allowing to produce antibiotics, vitamins, etc.

In all forms of fermentation the main aim is to ensure that all parts of the system are subject to the same conditions. Within the bioreactor the microorganisms are suspended in the aqueous nutrient medium containing the necessary substrates for growth of the organism and required product formation. All nutrients, including oxygen, must be diffused into each cell, and waste products, such as heat, CO_2 and waste metabolites must be removed.

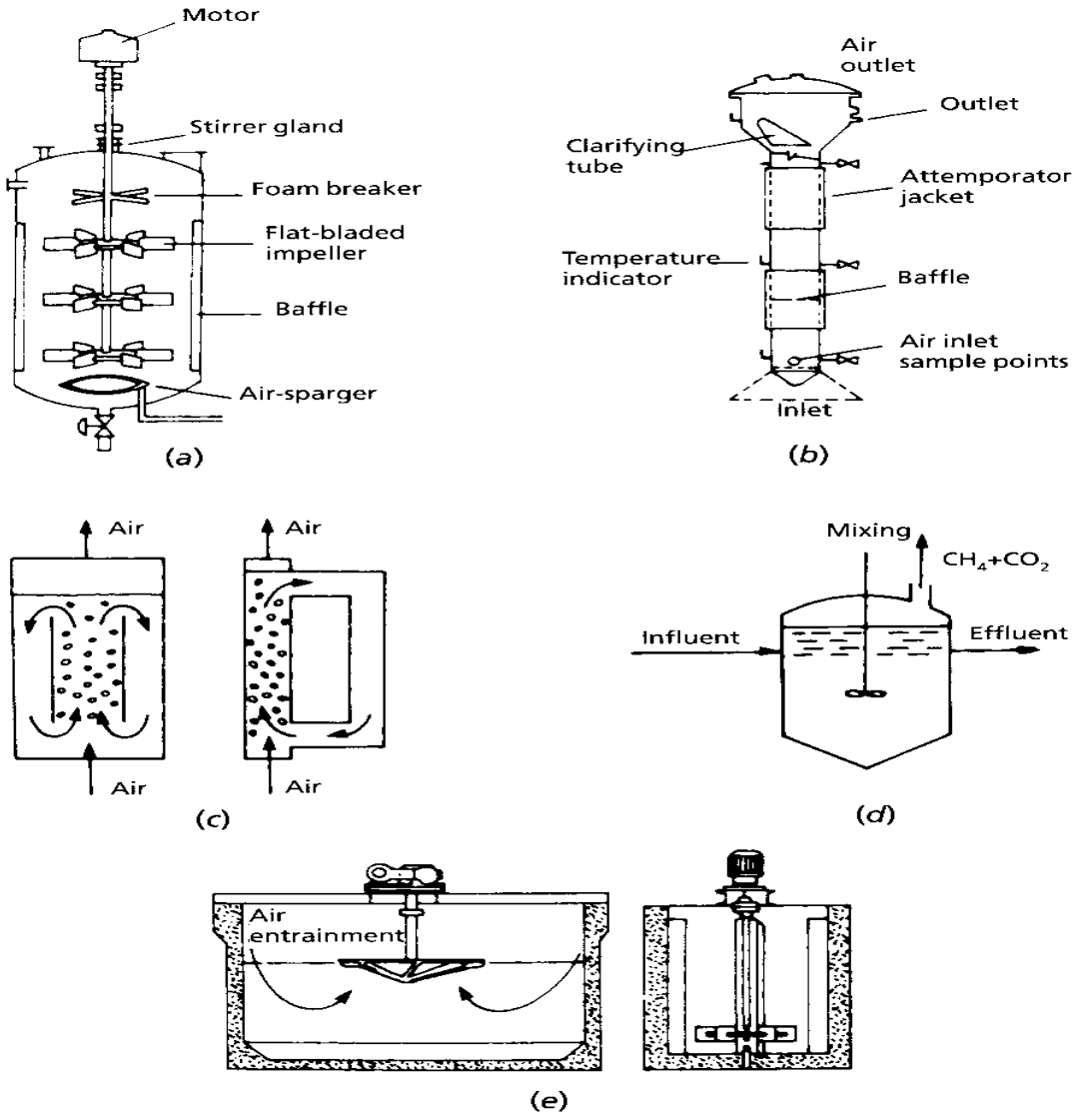


Fig. 4.2. Various forms of bioreactor. (*a*) Continuous stirred tank bioreactor. (*b*) Tower bioreactor. (*c*) Loop (recycle) bioreactor. (*d*) Anaerobic digester or bioreactor. (*e*) Activated sludge bioreactor.

The concentration of the nutrients must be held within a definite range, since low values will limit the rate of organism metabolism whereas excessive concentrations can be toxic.

Fermentation reactions are multiphase, involving a gas phase (containing N_2, O_2 and CO_2), one or more liquid phases (aqueous medium and liquid substrate) and solid phase (the microorganisms and possibly solid substrates). All phases must be kept in close contact to achieve rapid mass and heat transfer.

To achieve optimization of the bioreactor system it must be necessary adhered to the following guidelines:

(1) The bioreactor should be designed to exclude entrance of contaminating organisms;

(2) The culture volume should remain constant, i.e. no leakage or evaporation;

(3) The dissolved oxygen level must be maintained above critical levels of aeration and culture agitation for aerobic organisms;

(4) Environmental parameters such as temperature, pH, etc. must be controlled; and the culture volume must be well mixed.

The standard of materials used in the construction of sophisticated fermenters is also important (Table 4.4).

Table 4.4. Standards of materials used in fermenter design

All materials contacting with the solutions or organism culture in the bioreactor must be corrosion resistant
The materials must be non-toxic so that do not inhibit culture growth
The materials of bioreactor must withstand repeated sterilization with high pressure steam
The bioreactor stirrer system, entry ports and end plates must not be deformed or broken under mechanical stress
Transparent materials should be used wherever possible to visual inspection of the medium and culture

Media Design for Fermentation Processes

Water is at the centre of all biotechnological processes and in most cases is the dominant component of the media in which microorganisms grow. The quality of water is highly relevant because it affects microbial growth and the production of specific bioproducts. The basic nutritional requirements of microorganisms are an energy or carbon source, an available nitrogen source, inorganic elements and, for some cell types, specific growth factors. In most biotechnological processes carbon and nitrogen sources are more often derived from relatively complex mixtures of cheap natural products or by-products (Table 4.5).

Availability and type of nutrient can exert strong physiological control over fermentation. Raw material input to a fermentation will be dependent on the cost of the material.

Sterilization practices for biotechnological media must achieve maximum kill of contaminating microorganisms, with minimum temperature damage to medium components.

The media preparation is one of the important parts of the overall bioprocess leading to high efficiency of growth and concomitant rich product formation.

Table 4.5. Sources of carbohydrate and nitrogen for industrial media

Sources of carbohydrate	Form	Sources of nitrogen (% nitrogen by weight)
Glucose	Glucose monohydrate, Hydrolysed starch	Barley (1,5-2,0), beet molasses (1,5-2.0), corn-steep liquor (4,5),
Lactose	Pure lactose, whey powder	groundnut meal (8,0), oat flour (1,5-2,0), rye flour (1,5-2,0),
Starch	Barley, groundnut meal, oat flour, soya bean meal, rye flour	soya bean meal (8,0), whey powder (4,5)
Sucrose	Beet molasses, cane molasses, crude and pure sugar	

Solid Substrate Fermentation

There are many biotechnological processes that involve the growth of microorganisms on solid substrates in the absence or near absence of free water (Table 4.6). The most regularly used solid substrates are cereal grains, legume seeds, wheat bran, lignocellulose materials such as straws, sawdust, and a wide range of plant and animal materials. Most of these compounds are polymeric molecules, insoluble or sparingly soluble in water, but are mostly cheap, easily obtainable and represent a concentrated source of nutrients for microbial growth.

Table 4.6. Some examples of solid substrate fermentations

Example	Substrate	Microorganism(s) involved
Mushroom production (European and oriental)	Straw, manure	*Agaricus bisporus* *Lentinula edodes*
Sauerkraut	Cabbage	Lactic acid bacteria
Soy sause	Soya beans and wheat	*Aspergillus oryzae*
Tempeh	Soya beans	*Rhizopus oligosporus*
Ontjom	Peanut press cake	*Neurospora sitophila*
Cheeses	Milk curd	*Penicillium roquefortii*
Leaching of metals	Low grade ores	*Thiobacillus sp.*
Organic acids	Cane sugar, molasses	*Aspergillus niger*
Enzymes	Wheat bran, etc.	*Aspergillus niger*

Composting	Mixed organic materials	Fungi, bacteria, actinomycetes
Sewage treatment	Components of sewage	Bacteria, fungi and protozoa

Many of these fermentations have great antiquity and in many instances there are records dating back hundreds of years. In the Orient there is a wide array of food fermentations including soy sause and tempeh as well as many large industrial enzyme processes. In the West, the fermentation processes have centred on the production of silage, mushroom cultivation, cheese and sauerkraut production, and the composting of plant and animal wastes.

The microbial components of solid substrate fermentations can occur as single pure cultures, mixed identifiable cultures or totally mixed indigenous microorganisms.

Bioreactors designs for solid substrate fermentations are more simple than for liquid cultivations. They are classified into fermentations (a) without agitation, (b) with occasional agitation, and (c) with continuous agitation.

Technology of Mammalian and Plant Culture

Many potentially important organic compounds can be produced from animal or human cell cultures. The culture of mammalian cells and tissues is a widely used technique in modern cell biology and biotechnology. The range of cell types now grown in culture includes cells derived from bone, cartilage, liver, lung, breast, skin, bladder, kidney, neurons, pituitary cells and many types of cancers. There is increasing use of animal cell cultivation for industrial level production of high value products such as vaccines (for polio, mumps, measles, rabies, etc.), interferons, hormones, insulin, plasminogen and various antibodies. The main problems that occur in mass cultivation of mammalian cells include their extreme sensitivity to water impurities, the high cost and the need to avoid contamination by more rapidly growing microorganisms.

Freshly isolated cultures from mammalian systems are termed **primary cultures**. At this stage they are heterogeneous but closely representative of the parent cell types and in the expression of tissue-specific properties. After transferring primary cultures onto fresh media, the cell line will transform to become a **continuous cell line**. Such cell lines show many alterations from the primary cultures, including changes in cytomorphology, increase in chromosome variation and increase of growth rate.

Animal cells can be grown either in an unattached suspension culture or attached to a solid surface. Cells derived from a human malignancy can grow in either state, lymphoblastoid cells can grow in suspension culture, while primary or normal diploid cells grow only when they are attached to a solid surface. Most future commercial development with animal cells will be dominated by the cultivation of attach-required cell types.

The use of plant cell culture techniques for micropropagation of certain plants is discussed in Chapter 10. In our days large-scale production of suspension cell cultures of many plant species has been achieved and yields of products typical of the whole plant have been impressive, e.g. nicotine, alkaloids and ginseng.

Downstream Processing

It is not enough to grow the required cells in a bioreactor: extraction and purification of the desired end-product (the so-called downstream processing) from the bioprocess is necessary. Downstream processing of biotechnological processes represents a major part of the overall end-product cost.

Downstream processing is primarily concerned with initial separation of the bioreactor broth into a liquid phase and a solid phase and subsequent concentration and purification of the product. Processing usually involves more than one stage (Table 4.7).

Table 4.7. Downstream processing operations

Separation
Filtration
Centrifugation
Flotation
Disruption
Concentration
Solubilisation
Extraction
Thermal processing
Membrane filtration
Precipitation
Purification
Crystallisation
Chromatography
Modification
Drying

Final products of the downstream purification stages should be stable for commercial application. Stability is best achieved by using some methods of drying: spray-drying, fluidised-bed drying or freeze-drying. The choice of method depends on the product and cost. Products sold in the dry form include organic acids, amino acids, antibiotics, polysaccharides, enzymes, single cell protein and many others. Many products cannot be supplied easily in a dried form and must be sold in liquid preparations.

The role of downstream processing will continue to be one of the most challenging and demanding parts of many biotechnological processes. Purity and stability are the hallmarks of most high value biotechnological products.

Chapter 5.

ENZYME TECHNOLOGY

Enzymes are complex proteins molecules present in living cells, where they act as catalysts in bringing about chemical changes in substances. Without enzymes there can be no life. Although enzymes are formed only in living cells, many can be separated from the cells and can continue to function *in vitro*. This unique ability of enzymes to perform their specific transformations in isolation has led to an ever-increasing use of enzymes in industrial processes, collectively termed enzyme technology (Table 5.1)

Table 5.1. Approximate annual world production of some industrial enzymes

Enzyme	Tonnes pure enzymes
Bacillus protease	550
Bacillus amylase	350
Glucose isomerase	60
Microbial rennet	25
Fungal protease	15

The activity of an enzyme is due to its catalytic nature. An enzyme carries out its activity without being consumed in the reaction, while the reaction occurs at a very much higher rate when the enzyme is present. Enzymes are highly specific and function only on certain types of compound, **the substrates**. The catalytic function of the enzymes is due not only to its primary molecular structure but also to the intricate folding configuration whole enzyme molecule. It is this configuration that endows the protein with the specific catalytic function. Disturb the configuration by, for example, a change in pH, or temperature, and the activity can be lost. For some enzymes there is an obligatory need for additional factors, termed **cofactors**, that can be metal ions, nucleotides and other. Because of their specificity enzymes can differentiate from chemicals with closely related structures and can catalyse reactions at relatively low temperatures (0 – 100^{o}C) and the pH range 2 – 14. In industrial application this can result in high quality products, fewer by-products and simpler purification procedures. Furthermore, enzymes are non-toxic and biodegradable and can be produced from microorganisms in large amounts without the need for special chemical resistant equipment.

Enzyme technology embraces production, isolation, purification, use in soluble form and finally the immobilization and use of enzymes in a wide range of bioreactor systems. Enzyme technology will undoubtedly contribute to the solution of some of the most vital problems with which modern-day society is confronted, e.g. food production, energy shortage and preservation and improvement of the environment. For the future, enzyme technology and genetic engineering will be two very closely related areas of study dealing with application of genes and their products.

The Application of Enzymes

For thousands of years processes such as brewing, bread-making and the production of cheeses have involved the unrecognized use of enzymes. The Greek epic poems *The Odyssey* and *The Iliad*, dating from around 700 BC both refer to the use of what we now recognize as enzymes in cheese-making.

In the West the industrial understanding of enzymes revolved around yeast and malt , where traditional baking and brewing industries were rapidly expanding. Much of the early development of biochemistry was centered around yeast fermentation and processes for conversion of starch to sugar. The year 1896 saw the true beginning of modern microbial enzyme technology with the first marketing in the West of takadiastase, a rather crude mixture of hydrolytic enzymes prepared by growing the fungus *Aspergillus oryzae* on wheat bran.

In Europe leather has always been an important commodity and originally the process by which hides were softened before tanning, termed 'bating' was the most unpleasant requiring the use of dog faeces and pigeon dropping. However, at the turn of this century, Otto Röhm, a distinguished German chemist, determined that the active components in dog faeces and pigeon dropping were proteases – enzymes that degrade proteins. He was able to demonstrate that extracts from animal organs that produced similar enzymes could be used instead of the faeces and from 1905, pig and cow pancreases were to provide socially acceptable and reliable source of these enzymes.

Until the mid-1950s rapid development in enzyme technology occurred, using, in particular, microbial enzyme sources. The reasons for this are varied but depended largely on the following:

(1) Most enzymes of potential industrial importance could be produced from some microorganism;

(2) A major development in cultivation practices with microorganisms was primarily connected with the World War II penicillin production processes and this newly knowledge was readily applied to the large-scale cultivation of other microorganisms and subsequently microbial enzyme production.

The further development of enzymes as additives was largely to provide enhancement of traditional processes rather than to open up new possibilities. Even now most bulk production of crude enzymes is concerned largely with enzymes that hydrolyze the glucosidic links of carbohydrates such as starch and pectins and with the proteases that hydrolyze the peptide links of proteins.

Cell-free enzymes have many advantages over chemical processes where a number of sequential reactions are involved. In fermentation processes the use of microbial cells as catalysts can have a number of limitations:

(1) The conditions for growth of the organisms may not be the same for product formation.

(2) Wasteful side-reactions may occur.

(3) The isolation and purification of the desired product from the fermentation liquor may be difficult.

Many, if not all, of these limitations may be alleviated by the use of purified enzymes and possibly by the further of enzymes in an immobilised form.

There is now a rapid proliferation of uses and potential uses for more highly purified enzyme preparations in industrial processing, clinical medicine and laboratory practice. The range of pure enzymes now available commercially is rapidly increasing. The use of such highly refined preparations is rather expensive, which can be as much as hundreds of dollars per gram. In contrast, the crude enzymes, which may have less than 1% by weight active material, may cost some dollars per kilogram. In many operations, such as clarification wines and juices, chill proofing of beer and improving bread doughs, the crude enzymes is likely to add very little to the cost of the product. Most of the enzymes used in industry are extracellular enzymes, i.e. enzymes that are normally excreted by the microorganism and are analogous to the digestive enzymes of human beings and animals. Thus, when microorganisms produce enzymes to split large external molecules into an assimilable form the enzymes are usually excreted into the fermentation media. In this way the fermentation broth from the cultivation of certain microorganisms, e.g. bacteria, yeasts of filamentous fungi, then becomes a major source of proteases, amylases and lipases, etc. Most industrial enzymes are hydrolases and are capable of acting without complex cofactors; they are readily separated from microorganisms without rupturing the cell walls and are water soluble.

Some intracellular enzymes are now being produced industrially and include glucose oxidase for food preservation, asparaginase for cancer therapy and penicillin acylase for antibiotic conversion.

The sales of industrial enzymes were relatively small until about 1965 when enzymes in detergents came into general use. There was a massive increase in the use of enzymes in detergents between 1966 and 1969 but this was to collapse between 1969 and 1970 when allergic symptons were discovered in workers handling enzymes at the factory level. There was much press hysteria and enzymes were mostly taken out of detergents. However, careful studies found no adverse environmental effects from the use of enzymes nor any effects on domestic users. And once again the application of enzymes in detergents has achieved good levels and there is a constant growth in the detergent industry where enzymes can improve washing results.

In our days the further growth of world enzyme markets will be polarised around: (a) high volume, industrial grade enzyme production; and (b) low volume, high purity enzyme products for analytical, diagnostic or therapeutic applications.

Among the new areas of opportunity for enzyme technology is the utilization of woody materials in biotechnological processes. Many research works are now being directed to discover new and efficient enzyme systems that can attack the complex molecular configurations of lignocellulose and make available the

component molecules. This could well be the most future area of expansion in enzyme technology.

Genetic Engineering and Protein Engineering of Enzymes

Recombinant DNA technology has allowed the transfer of useful-enzyme genes from one organism to another. Thus, when an enzyme has been identified as a good candidate enzyme for industrial use, the relevant gene can be cloned into a more suitable production host microorganism (Fig. 5.1) an industrial fermentation carried out. In this way it becomes possible to produce industrial enzymes of very high quality and purity.

A recent example of this technology is the detergent enzyme Lipolase, which has improved removal of fat stains in fabrics. The enzyme was first identified in the fungus *Humicola languinosa* at levels inappropriate for commercial production. The gene DNA fragment for the enzyme was cloned into the production fungus *Aspergillus oryzae* and commercial levels of enzyme achieved. The enzyme has proved to be efficient under many wash conditions. The enzyme is also very stable at a variety of temperatures and pH conditions relevant to washing.

The modification of enzymes to improve or alter their catalytic properties has been carried out for several decades. In the past, this was achieved by random mutational programmes but in recent years advanced technology has brought about major changes in the field. Table 5.2 gives some main objectives for the preparation of modified enzymes.

Table 5.2. Objectives for the preparation of modified enzymes

To enhance the activity of the enzyme
To improve the stability
To permit the enzyme to function in a changed environment
To change pH or temperature optimum
To change the specificity of an enzyme so that it catalyses the conversion of a different substrate
To enhance the efficiency of a process

Protein engineering or ‘molecular surgery’ has been used to alter the performance of enzyme molecules. Protein engineering of enzymes involves the creation of a three-dimensional graphical model of the purified enzyme obtained from X-ray crystallographic data. Changes to the enzyme structure can then be considered that might result in increased stability to, for example, pH and temperature, and the requisite molecular changes made in gene coding for the enzyme.

Two main ways of research carry out in order to alter the performance of enzymes. In one approach, mutagenesis of clone-gene product, amino acid residues at defined position in the structure of enzyme can be replaced by other suitable coded amino acid residues. The altered gene is then transformed into a suitable host organism and the mutant enzyme subsequently produced with the requisite changes

in position. This process is known as site-directed mutagenesis. The second method used involves the isolation of the natural enzyme and modifications to its structure out by chemical or enzymic means – sometimes termed as 'chemical' mutation. A recent successful example of protein engineering is that of the enzyme phospholipase, which was modified to resist higher concentrations of acid. This enzyme is widely used as a food emulsifier.

Clearly, genetic engineering and protein engineering will have huge impacts on the enzyme industry in its many forms. Genetic engineering will ensure better product economy, production of enzymes from rare microorganisms, faster development programmes, etc.

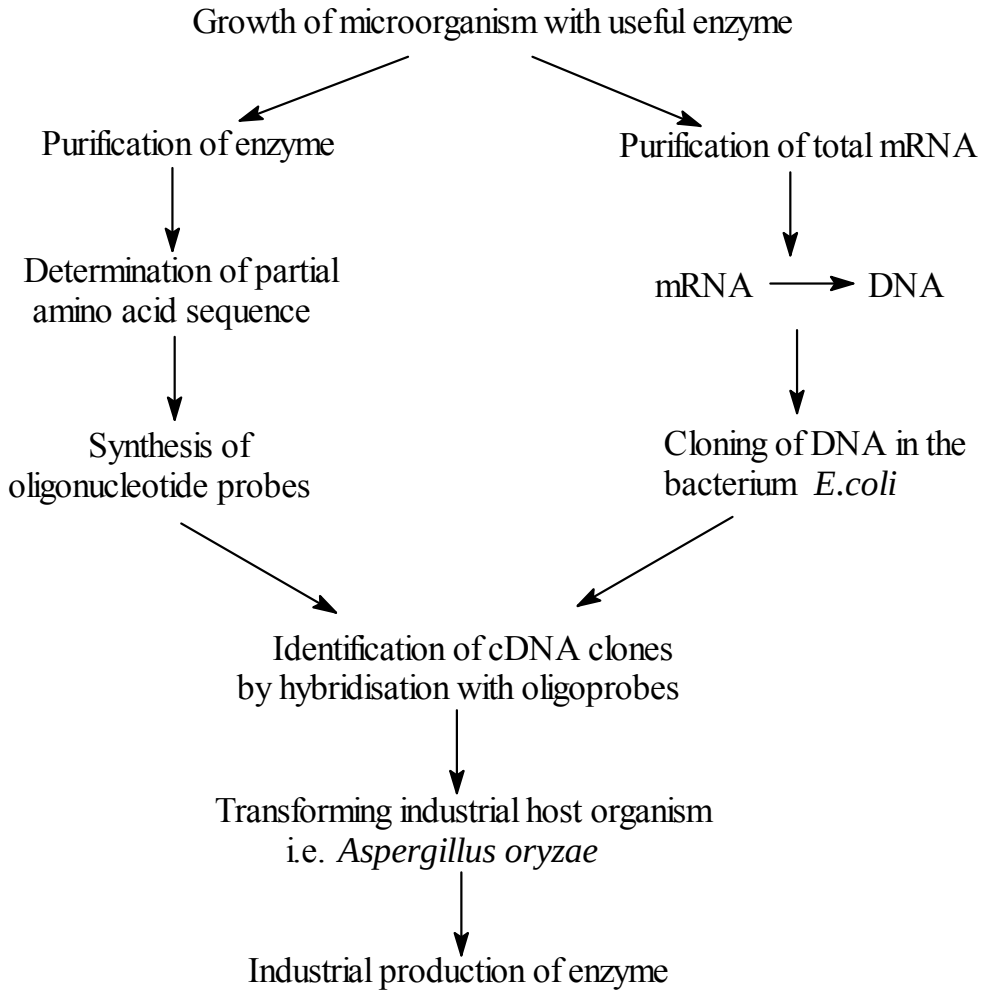


Fig. 5.1 Cloning strategy for enzymes.

The Technology of Enzyme Production

Although many useful enzymes have been derived from plant and animal sources, most future developments in enzyme technology will relay on enzymes of microbial origin.

The use of microorganisms as source material for enzyme production has developed for several important reasons.

(1) There is normally a high specific activity per unit dry weight of product.

(2) In microbes a wide spectrum of enzyme characteristics, such as pH range and high temperature resistance, is available for selection.

(3) Industrial genetics has greatly increased the possibilities for optimising enzyme yield and type through various ways and mostly by using of gene transfer technology and protein engineering.

The raw materials for industrial enzyme fermentations have been limited to substances that are available in large quantities at low cost and are nutritionally safe. Some of most commonly used substrates are starch hydrolysate, molasses, corn steep liquor, whey and many cereals.

Industrial enzyme production from microorganisms relies on either liquid conditions or solid substrate fermentation as described in Chapter 4.

Solid substrate methods of producing fungal enzymes have long-standing historical applications, particularly in Japan and other Far East countries. This method uses moist wheat or rice brain with added nutrient salts as substrates. The growing environment usually comprises rectangular or circular trays held in constant-temperature rooms. Commercial enzymes produced in this way include fungal amylases, proteases, pectinases and cellulases.

Industrial methods using liquid systems relies on bioreactors similar to those used in antibiotic production processes (Fig.5.2). The choice of fermentation medium is important since it supplies the energy needs as well as carbon and nitrogen sources.

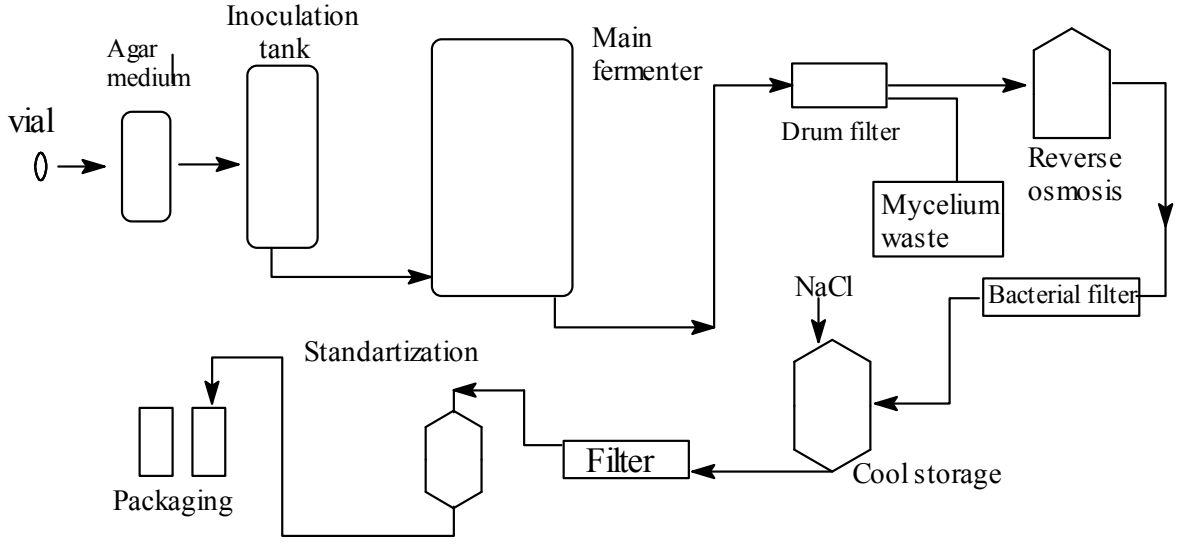


Fig. 5.2. The stages in the production of a liquid enzyme preparation

On the completion of the fermentation the enzyme may be present within the microorganism or excreted into the liquid or solid medium. commercial enzyme preparations for sale will be in either a solid or a liquid form, crude or highly purified. The concentration and purification of an enzyme is shown in Fig. 5.3. The final cost of an enzyme depends on downstream processing that is required to achieve a saleable product.

Immobilized Enzymes

The use of enzymes in a soluble or free form is very wasteful because the enzyme generally cannot be recovered at the end of the reaction. A new and valuable area of enzyme technology is the immobilization of enzymes on insoluble polymers, such as membranes and particles, acting as carriers for enzyme activity. The enzymes are physically confined during a continuous catalytic process and may be recovered from the reaction mixture and used over and over again, thus improving the economy of the process. Some enzymes that are rapidly inactivated by heat when in cell-free form become heat stable by attachment to inert polymeric supports. Whole microbial cells can also be immobilized inside polyacrylamide beads and used for a wide range of catalytic functions.

Present applications of immobilized catalysts are connected with the production of L-amino acids, organic acids and fructose syrup. In the near future these immobilized enzymes will be used as an alternative to existing processes using non-immobilized biocatalysts.

For enzyme immobilization are used both physical and chemical methods. Physically, enzymes may be adsorbed on an insoluble matrix, entrapped within a gel, or encapsulated within a microcapsule or behind a semi-permeable membrane (Fig. 5.4). Chemically, enzymes may be covalently attached to solid supports or cross-linked.

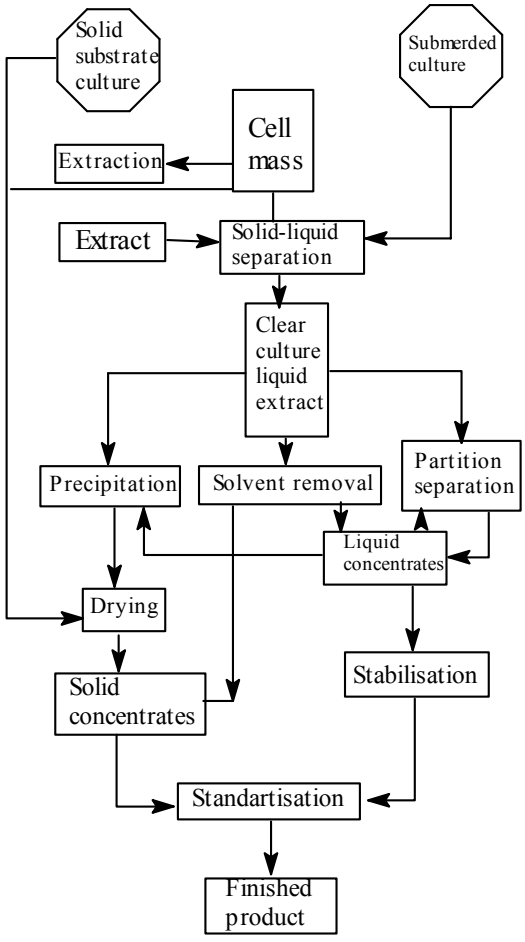


Fig. 5.3. The extraction and purification of an enzyme

A large number of chemical reactions have been used for the covalent binding of enzymes of their non-essential functional groups to inorganic carriers such as ceramics, glass, iron, zirconium and titanium, to natural polymers such as sepharose and cellulose, and to synthetic polymers such as nylon, polyacrylamide and other vinyl polymers and copolymers possessing reactive chemical groups.

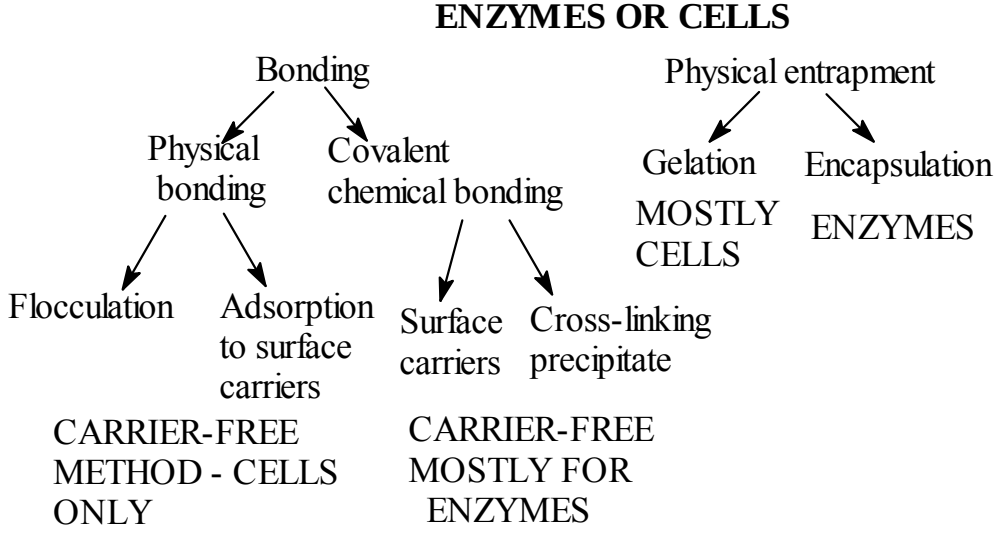


Fig 5.4. Techniques of enzyme/cell immobilisation.

The entrapment of enzymes in gel matrices is achieved by carrying out the polymerization or coagulation reactions in the presence of the enzyme.

Immobilization of whole cells is achieved by the same methods as for cell-free enzymes.

The greatest potential for immobilized cell systems is a replacement of complex fermentations such as secondary product formation (i.e. semi-synthetic antibiotics) in continuous chemical processes. In Europe, immobilized penicillin acylase is used to prepare 6-amino penicillanic acid (6-APA) from naturally produced penicillin G or V:

Penicillin G or V 6-APA

This compound is an important intermediate in the synthesis of semi-synthetic penicillins. Two types of penicillin are produced by industrial fermentation: penicillin G (phenylacetyl-6-APA) and penicillin V (phenoxyacetyl-6-APA), each containing a nucleus of 6-APA and a side-chain. The antibiotic activity of the

penicillin molecule is governed by the side-chain and, if removed and replaced with another, can considerably alter the antibiotics properties. Many pharmaceutical companies use immobilized enzyme processes for the production of 6-APA on an industrial scale. At least 3500 tones of 6-APA are produced each year, requiring the production of about 30 tones of the enzyme.

Immobilized glucose isomerase is used in the USA, Japan and Europe for the industrial production of high fructose syrups by partial isomerization of glucose derived from starch. Thousands of tones of high fructose syrup are produced by this enzyme process, which is widely used of all the immobilized enzyme systems. The industrial and commercial success of this process is due to the following: glucose derived from starch is relatively cheap; fructose is sweeter than glucose; the high fructose syrup contains approximately equivalent amounts of glucose and fructose, and from a nutritional aspect is similar to sucrose.

Another important use of immobilized enzymes is aminoacylase production of amino acids. Aminoacylase columns are used in Japan to produce thousands of kilograms of L-methionine, L-phenylalanine, L-tryphophan and L-valine.

Looking into the future, it seems reasonable to expect that the production and application of enzymes will continue to expect.

Chapter 6.

BIOLOGICAL FUEL GENERATION

The total economically recoverable world reserves of the three main fossil fuels, namely coal, natural gas and oil are respectively less than 1000, 35 and 16 years. Modern industry is almost totally dependent on these limited supplies. Approximately 93 % of fossil fuel is consumed for energy production and only 7 % being used for the production of solvents, plastic and other organic chemicals. The continual depletion of global fossil fuel has to seek alternative sources of energy. These have included the harnessing of hydro, tidal, wave and wind power, the capture of solar and geothermal energies supplies and nuclear power. With all of these systems there is no yet definitive answer on both the economic and energetic outlay necessary for successful using.

That is why, there is now a growing appreciation of biological energy systems, which may soon bring the economic profit. Biomass such as forest, agricultural and animal residues and industrial and domestic organic wastes can now be converted by physico-chemical and (or) fermentation processes to fuel and petrochemical substitutes. As fossil fuel resources are depleted, conversion of organic residues to liquid fuels become a more economically attractive consideration. Photosynthetically derived material is best suited to modern technology.

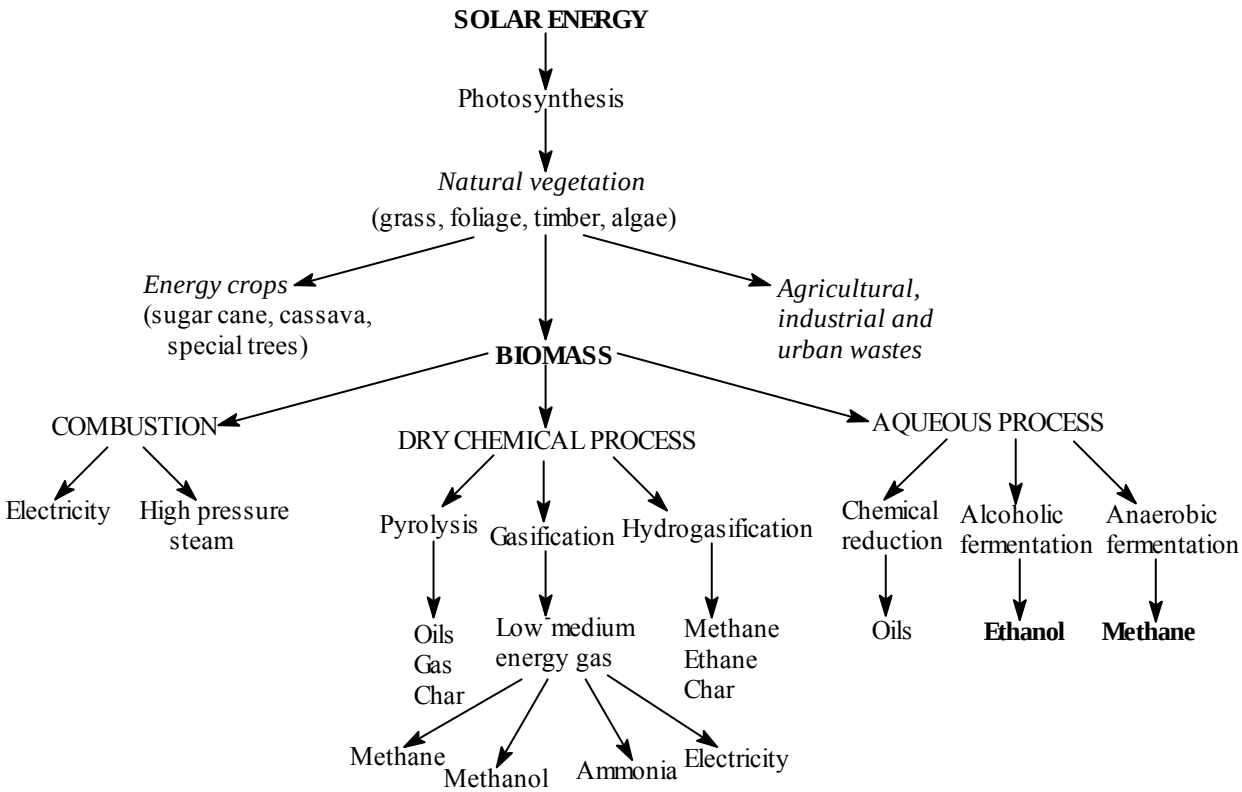


Fig. 6.1.Options for the conversion of biomass to energy

Photosynthetic organisms, both terrestrial and marine, can be considered as continuous solar energy converters and constantly renewable. Plant photosynthesis fixes about 2×10^{11} tonnes of carbon with an energy content of 2×10^{21} joules, which represents about 10 times the world's annual energy use and 200 times our food energy consumption. The magnitude and role of photosynthesis has gone unappreciated because we use a small proportion of fixed carbon. The actual efficiency of solar energy capture by green plants can be as much as 3-4 %, the more effective photosynthetic plants such as maize, sorghum and especially sugar cane being the most productive.

Biomass can be considered as a renewable energy source, and can be converted into either direct energy or energy-carrier compounds by direct combustion, anaerobic digestion systems, destructive distillation, gasification, chemical hydrolysis and biochemical hydrolysis.

There are three main directions that can be followed to achieve biomass supplies (Fig.6.1):

(1) Cultivation of the so-called energy crops
(2) Harvesting of natural vegetation
(3) Utilisation of agricultural and other organic wastes

The conversion of the resulting biomass to usable fuels can be accomplished by biological or chemical methods or by a combination of both. The two main end-products are methane or ethanol, except these products other products depending on initial biomass are formed, e.g. solid fuels, hydrogen, methanol and longer-chain hydrocarbons.

The technical processing of the biomass depends on many factors, including moisture level and chemical complexity. Materials with a high water content usually undergo aqueous processing, which avoids the need for substrate drying. Alcoholic fermentation to ethanol, anaerobic digestion to methane and chemical reduction to oily hydrocarbons are possible. Low moisture level materials such as wood, straw and bagasse can be: burnt to give heat; subjected to thermochemical processes as gasification and pyrolysis to produce energy-rich compounds such as gaseous oil, char and eventually methanol and ammonia; or treated by alkaline or biological hydrolysis to produce chemical feedstocks for use in further biological energy conversions.

Ethanol from Biomass

The production of alcohol by fermentation of sugars and starch is considered to be one of the first microbial processes.

$$C_6H_{12}O_6 \longrightarrow 2\,CH_3CH_2OH + 2CO_2$$

At present, industrial alcohol production is largely synthetic (non-microbial), deriving from petrochemical processes; petrochemical ethanol is made by the hydration of ethylene, and the decline of microbial production of alcohol dates from the large-scale production of ethylene from the 1940s. A large change in the economics of alcohol production resulted from the considerable increases in the

world prices of crude oil in the 1970s, but the price of suitable cheap carbohydrates has risen far less.

Table 6.1 Potential raw materials for fuel ethanol production

Starch containing	Cellulosics	Sugar-containing	Other
Cereal grains	Wood	Sucrose and invert sugar	Jerusalem artichoke
Corn	Sawdust	Sweet sorghum	
Grain sorghum	Waste paper	Molasses	
Wheat	Forest residue	Sugar beet	
Barley	Agricultural residues	Fodder beet	
Milling products		Sugar cane	
Wheat flour	Municipal solid wastes	Lactose	
Wheat millfeeds		Whey	Raisins
Starchy roots	Intensive livestock Production wastes	Glucose	Bananas
Mandioca		Sulphite wastes	
Potatoes			

Since alcohol can be used as a partial or complete substitute for motor fuel and can also be converted into ethylene and related compounds, its production from local and renewable resources still seems an attractive alternative strategy. Nowhere has this been more actively pursued than in Brazil. Vast biotechnological processes convert sugar cane and cassava into ethanol by yeast fermentation. Brazil's success in pioneering this production of 'green petrol' has created worldwide interest, particularly among Third World nations with the climate and land to grow their own fuel crops but with limited currency to buy oil. Even developed nations such as Australia, the USA, Sweden and France began to produce biological alcohol production utilising either large agricultural surpluses or forestry wastes.

Potential raw materials for fuel ethanol production is presented in Table 6.1.

A flow diagram for the production of ethanol from diverse substrates is shown in Fig. 6.1.

The Brazil programme is based on batch fermentation systems. At present the standards of these fermentations are modest and leave much room for improvement. Continuous methods of production offer many advantages but are studied and operated only in developed nations. Improvements in continuous fermentations have utilised many approaches, including retention of the yeast cells in the bioreactor by separation and recycling and by continuous evaporation of the fermentation broth. Application of these biotechnological improvements to ethanol production will make these processes economically attractive as a substitute for fossil fuel.

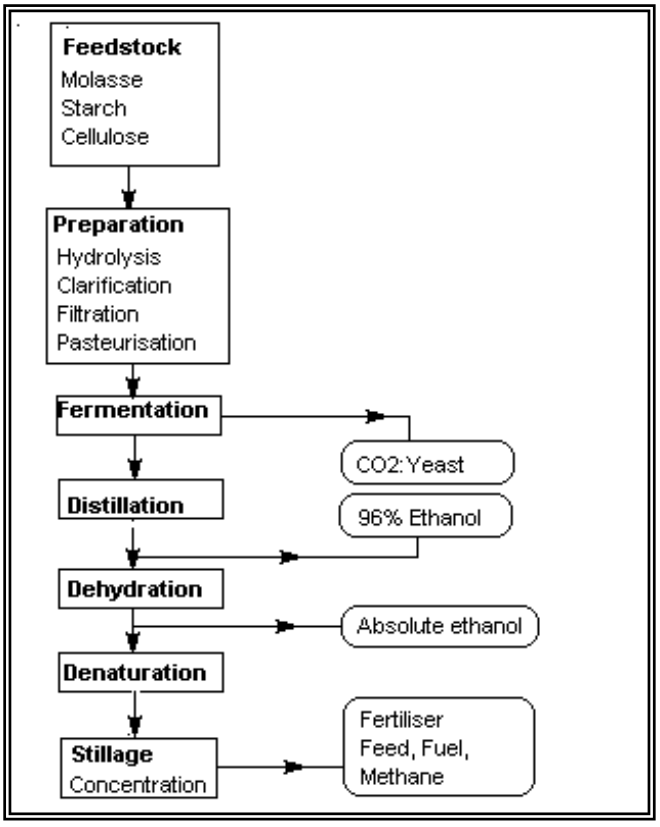


Fig. 6.1. Flow diagram for the production of ethanol.

Methane from biomass

Methane gas can be used for the generation of mechanic, electrical and heat energy and now is used as a fuel source for domestic and industrial purposes through gas pipelines or can be converted to methanol and used as fuel in combustion engines. Such natural gas sources were originally derived from biomass in ancient times.

Methane gas also exists in the atmosphere and is derived mainly from microbial action in natural wetlands, rice paddies and enteric fermentation in animal contributing about 20 %, 20 % and 15 % respectively, to the total methane flux. After CO_2, methane is the next most important greenhouse gas and is expected to contribute 18 % of future warming.

The microbiology of methane production is complex, involving mixtures of anaerobic microorganisms (Fig. 6.2). In principle, anaerobic fermentation of complex organic mixtures proceeds through three main biochemical phases, each of which requires specific microbial parameters. The initial stage requires the solubilisation of complex molecules such as cellulose, fats and proteins. The result soluble products of this stage are then converted to organic acids; in the final phase of microbial activity, these acids (primarily acetic) are decomposed by the methanogenic bacteria to methane and CO_2.

There are several possible ways by which methane can be produced in an economy: from sewage, from agricultural and urban wastes, and in biogas reactors.

When methane is produced by the fermentation of animal dung the gaseous products are usually referred to as **biogas**. Biogas is a flammable mixture of 50 – 80 % methane, 15 – 45 % CO_2, 5 % water and some trace gases. Biogas is produced via biomethanogenation and is a self-regulation symbiotic microbial process operating under anaerobic conditions and functions best at temperatures around 30 ^{o} C. Production of biogas by such methods has importance in India, China and Pakistan. The system of biogas production in the Far East ranges from small peasant systems to quite large plants producing large volumes of gas.

Under ideal conditions, 10 kg of dry organic matter can produce 3 m^3 of biogas, which will provide 3 h of cooking, 3 h of lighting or 24 h of refrigeration with suitable equipment.

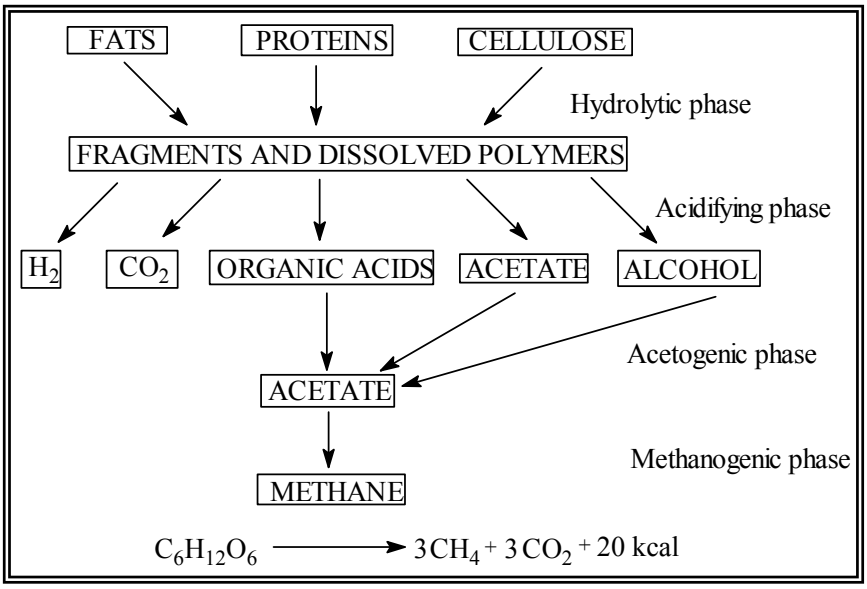


Fig. 6.2. The microbiology of methane generation

Methane generated from organic materials by anaerobic fermentation offers a valuable source of energy that could be put directly to many uses. Furthermore, the associated by-products may be useful forms of fertilisers for agriculture. However, methane as an energy source may well have economic value at local small-scale production levels, but there are some economic arguments against large-scale methane-production by microbial processes:

(1) Methane production by gasification of coal is commercially more attractive;

(2) Microbial production of methane is more expensive than natural gas;

(3) Costs of storage, transportation and distribution of gaseous fuels are not economically worth while nowadays;

(4) Methane cannot be used in automobiles and it is difficult and expensive to convert it to liquid state.

However, anaerobic digestion of municipal, industrial and agricultural wastes can have positive environmental value, since it can combine waste removal and stabilisation with net biogas formation. The solid or liquid residues can further be

used as fertiliser, soil conditioner or animal feed. Biogas production will continue to have high priority in alternative energy research.

Chapter 7.

SINGLE CELL PROTEIN

A major problem facing the world, in particular the developing nations, is the explosive rate of population growth. At least 25 % of the world's population constantly suffer from hunger and malnutrition. However, productivity is increasing throughout the world in all branches of agriculture. World supply of grain per head has outpaced population growth, but there are still major imbalances in the availability of cereals. The extent of the protein problem also varies from country to country. The shift from grain to meat diets in developed and developing countries is leading to a much higher per capita grain consumption, since it takes 3 to 10 kg of grain to produce 1 kg of meat by animal rearing and fattening programmes.

The search for sources of protein is pursued. New agricultural practices are widespread, high protein cereals have been developed, the cultivation of soya beans and groundnuts is ever expanding, protein may be extracted from liquid wastes by ultrafiltration, and now the use of microbes as protein producers has gained wide experimental success. This field of study has become known as **single cell protein** or SCP production.

During the last two decades there has been a growing interest in using microbes for food production, in particular for feeding domesticated food-producing animals such as poultry. The use of SCP derived from low value waste materials for animal feed would improve human nutrition by taking protein-rich vegetable foods out of the human versus animal competition and making them more available for human consumption in the producer countries, which are often developing countries.

SCP may be used as a protein supplement for human and animals. With human it can serve as a protein supplement, as a food additive to improve flavour, fat binding, and more recently as a replacement for animal protein in the diet. In animal feeding it can serve as a replacement for such traditional protein supplements as fishmeal and soya meal. The high protein levels, bland odour and taste of SCP, together with ease of storage, confer considerable potential to SCP in food . Its high protein content makes its use attractive in aquaculture, e.g. farming of shrimps, prawns, trout, salmon etc.

Microorganisms produce protein much more efficiently than any farm animal (Table 7.1).

Table 7.1. The time required to double the mass of various organisms

Organism	***Time***
Bacteria and yeasts	20 –120 min.
Moulds and algae	2– 6 h
Grass and some plants	1– 2 weeks
Chickens	2–4 weeks
Pigs	4–6 weeks
Cattle (young)	

Humans (young)	1–2 months 3–6 months

The protein-producing capacity of a 250 kg cow and 250 g of microorganisms are compared. Whereas the cow will put on 200 g of protein per day, the microbes could produce 25 tonnes in the same time under ideal growing conditions. The advantages of using microbes for SCP production are outlined in Table 7.2.

Table 7.2. The advantages of using microbes for SCP production

Microorganisms can grow at remarkably rapid rates; Microorganisms are more easily modified genetically than plants and animals; they are more amenable to large-scale screening programmes to select for higher growth rate and can be easily prone to gene transfer technology
Microorganisms have relatively high protein content
Microorganisms can be grown in vast number in relatively small continuous fermentation processes, using small land area, and growth is also independent of climate
Microorganisms can grow on a wide range of raw materials, in particular low value wastes

A unique aspect of the SCP field is the extent to which it has been influenced by factors other than technological or economic. In particular, a tremendous amount of attention has been given to the problems of safety, nutritional value and acceptability of the product.

The nature of the raw materials used in SCP processes represents the main safety hazard, e.g. the possibility of carcinogenic hydrocarbons in the gas oil or n-paraffins, of heavy metals or other contaminants in the mineral salts, solvent residues after extraction, and of toxin production by certain fungi. The process organism must be non-pathogenic and non-toxigenic. Rigorous sanitation and quality control procedures must be maintained throughout the process to avoid contamination by pathogenic or toxigenic microorganisms. Toxicological testing of the final product must include short-term acute toxicity testing with several different laboratory animal species, followed by extensive and detailed long-term studies, for two years or more, in both rodent and non-rodent species.

The critical parameters of SCP processes are interdependent. The choice of substrate will reflect local political and economic factors and the availability of alternative outlets. The choice of organism will partly determine the process technology and the nature of the product. Acceptability of the product will depend in part on the substrate. Because of the large volumes that will be involved in SCP processes, continuous culture techniques will be preferable for economic reasons.

SCP Derived from High Energy Sources

Materials with a high commercial value as energy sources or derivatives of such chemicals, e.g. gas oil, methanol, ethanol, methane and n-alkanes have found wide commercial interest.

Methane as an SCP source has been extensively researched but is now considered to present too many technical difficulties to application. In contrast, methanol offers great economic SCP interest. A large-scale (75 000 litres) fermentation plant for producing the methanol-utilising bacterium *Methylophilus methyltrophus* by ICI, UK. Hoechst (West Germany) and Mitsubishi (Japan) work on a similar process, using yeast strains instead of bacteria. The ICI SPC proteins (named Pruteen) is used for animal feeding. Methanol as a carbon source for SCP has many advantages over n-paraffines, methane gas and even carbohydrates, because it is independent of seasonal fluctuations. Methanol is non-toxic and dissolves in the aqueous phase in all concentrations.

The vast range of studies carried out in the 1960-70s on potential use of methanol as substrate for SCP production. Nowadays, the aerobic process for Pruteen production is the world's largest continuous bioprocess system.

Ethanol is a particularly suitable source if the SCP is intended for human consumtion. In the near future the comparative status of ethanol will depend on local factors (agricultural, economical and political).

The use of n-alkanes as a substrate for SCP has been extensively studied in many countries and represents a very complex biotechnological process. However, most of these processes have now ceased because of suspected health hazards resulting from the presence of carcinogenes in the SCP. And recently the massive technology developed in this field in Japan and other Eastern countries has been turned to the study of alcohol based SCP and SCP from wastes.

SCP from Wastes

The materials that make up wastes are straw, bagasse, citric acid, olive and date wastes, whey, molasses, animal manures and sewage. The amount of these wastes can be locally very high. Thus, the utilisation of such materials in SCP processes serve two functions – reduction in pollution and creation of feed proteins. Each waste material must be assessed for its suitability for conversion to SCP. In particular, the level of available technology is important. When a waste is available in large quantities and over a prolonged time then a suitable method of utilisation can be planned (Table 7.3).

Table 7.3. Advantages for using widely available organic wastes for SCP production

Reduces environmental pollution
Most organic wastes are available at low cost in most countries
The wastes are upgraded in energy and protein level
Many of the wastes such as cellulose and whey already form accepted parts of animal diets and will avoid the acceptability problems of other unusual wastes, e.g. human wastes and fossil fuels

SCP processes utilising waste substrates have been carried out on a commercial scale using various yeast organisms in sophisticated bioreactor systems. Substrates used and producer organisms include molasses (*Saccharomyces cerevisiae*) and cheese whey (*Kluyveromyces fragilis*), while the Symba process developed in Sweden utilises starchy wastes by combining two yeasts, *Endomycopsis fibuligira* and *Candida utilis.*

The feed value of the yeast produced by the Symba process has been evaluated in vast feeding experiments on different types of animal, including pigs, chickens and calves. The animals grew well and no adverse effects were recorded. The Symba process can be separated into three phases:

Phase 1. Waste water (from, for example, a potato processing plant), containing starch is fed through a heat exchanger and sterilised by stream injection.

Phase 2. Sterilised starch solution is fed through two bioreactors with the starch hydrolysing yeast *Endomycopsis fibuligira*. The hydrolysed starch then passed into a large bioreactor with *Candida utilis* as the growing organism.

Phase 3. The harvest stream from the *Candida* bioreactor is passed through vibro-screening and hydrocycloning equipment, then centrifuged. The samples collected can be spray-dried and the dried material sifted and bagged.

Cellulose from agriculture and forestry sources and from wastes must constitute the future major feedstock for many biotechnological processes including SCP. Cellulose, in its more natural association with lignin, is the most prevalent organic material available for biotechnological conversion. Lignin is the earth's second most abundant natural biopolymer found in plants. However, a limited number of fungi and bacteria have the necessary enzymic activity for lignin and cellulose degradation. The fungi *Phanerochaete chrysosporium* is one of the most studied lignin and cellulose degrades and have great potential for future biotechnological applications. Nowadays many research efforts are being directed to discover new and effective enzyme systems for degradation of lignin and cellulose, and to understand breakdown mechanisms for lignin and cellulose biodegradation. As a result of these studies scientists will receive the knowledge for establishing the technology for bioconversion of plant residues and waste lignins to useful materials, and for the protection of the environment from lignin-derived pollutants.

SCP from Algae

There has been some interest in the use of algae as SCP because they grow well in open ponds and need only CO_2 as a carbon source and sunlight as a energy source for photosynthesis. Algae such as *Chlorella* and *Senedesmus* have long been used as food in Japan, *Spirulina* is widely used in Africa and Mexico. *Spirulina maxima* is commercially produced in Mexico as a by-product of a large solar evaporator used for production of soda lime. Up to 2 tonnes per day are produced as used as animal feed. *Chlorella* is used as a protein and vitamin supplement in some Japanese yoghurts, ice-cream and breads. In some parts of the world algae are

used in ponds or lagoons to aid in the removal of organic pollution and the resultant biomass are harvested, dried and added to animal feed.

The worldwide, large-scale development of SCP processes has contributed to the advancement of present-day technology. Research and development into SCP processes has involved work in the fields of microbiology, biochemistry, genetics, chemical and process engineering, food technology, agriculture, animal nutrition, ecology, toxicology, medicine and veterinary science and economics.

The future of SCP will heavily depend on reducing production costs and improving quality. This may be achieved with lower feedstock costs, improved fermentation and downstream processing and improvement in the producer organisms as a result of conventional applied genetics together with recombinant DNA technologies.

However, the main limitations of SCP products for human use are sociological rather than technical, and in most cases the major nutritional role of microbial biomass (SCP) will be in animal feed supplements.

Chapter 8.

BIOTECHNOLOGY AND MEDICINE

Until this century people lived in constant association with death: infant mortality was 25 %, childhood mortality was 25 %, and various epidemics could regularly strike and kill at all ages. Fewer than 2 % of population lived beyond 65 years. And only when scientists realised that the ever-present diseases had specific causes that with research it became possible to seek out specific therapies for treatment: enter the antiseptics, vaccines, antibiotics, etc.

Nowadays in advanced societies, infectious diseases are no longer the main threat of life but rather it is the chronic diseases (cancer, Alzheimer's disease, etc.) that plague our increasingly aging population. It is now believed that the solution to these chronic diseases could come through genetic medicine and that modern biotechnology will play a major role.

The impact of pharmaceuticals on human health care is an area where biotechnological innovations are likely to have the earliest commercial realisation. The long-standing awareness within the health-related industries of biological and biochemical innovations has led to these industries being heavily involved in biotechnological research, particularly molecular biology. However, the considerable time required to develop a modern pharmaceutical product must not be underestimated, and long periods of toxicological testing are necessary before the national regulatory bodies will grant approval for marketing. The cost of achieving this approval can be many millions of dollars, and the product must have a high sales potential to warrant this investment. Many potential worth-while products will not appear on the market because it is not in the financial interest of the producing companies to meet such vast costs of gaining official approval.

New medical treatments based on biotechnology are appearing daily in the marketplace. These include therapeutic products (hormones, regulatory proteins, antibiotics), prenatal diagnosis of genetic diseases, vaccines, immunodiagnostic and DNA probes for disease identification and genetic therapy.

Pharmaceuticals and Biopharmaceuticals

The vast bulk of pharmaceutical drugs presently on sale are synthetic chemicals derived either directly by chemical synthesis or by chemically modifying molecules derived from biological sources. Biopharmaceuticals are recombinant protein drugs, recombinant vaccines and monoclonal antibodies. Biopharmaceuticals are becoming increasingly relevant in biological applications but are still only a small part of the pharmaceutical industry. However, the techniques of molecular biology and genetic engineering will become a dominating factor of drug discovery, design and development. Biotechnology will also accelerate screening, speed bioassays and the production of new drugs, and also explain how drugs act in the human system.

Antibiotics

The discovery in 1928 by Alexander Fleming that a fungus called *Penicillium notatum* could produce a compound selectively able to inactivate a wide range of bacteria, without unduly influencing the host, began scientific studies that altered the relationship of humans to the controlling influence of bacterial diseases. From these studies appeared the fungal antibiotics penicillin and cephalosporin, and the actinomycete antibiotics streptomycin, auremycin, tetracyclines and many others. Many bacterial diseases have largely been brought under control by the use of antibiotics. Pneumonia, tuberculosis, cholera and leprosy have been relegated to minor diseases.

Antibiotics are antimicrobal compounds produced by living microorganisms, and are used therapeutically and sometimes prophylactically in the control of infectious diseases. Over 4000 antibiotics have been isolated but only about 50 have achieved wide usage (Table 8.1). The other antibiotic compounds failed to achieve commercial importance for reasons such as toxicity to humans and animals, ineffectiveness or high production costs.

Table 8.1. Some economically important antibiotics

Antibiotic compound	Producer microorganism	Activity spectrum
Actinomycin D	*Streptomyces* sp.	Antitumour
Bacitracin	*Bacillus* sp.	Antibacterial
Bleomycin	*Streptomyces* sp.	Anticancer
Cephalosporin	*Acremonium* sp.	Antibacterial
Chloramphenicol	*Cephalosporium* sp.	Antibacterial
Daunorubicin	*Streptomyces* sp.	Antiprotozoal
Fumagillin	*Aspergillus* sp.	Amoebicidal
Griseofulvin	*Penicillium* sp.	Antifungal
Mitomycin	*Streptomyces* sp.	Antitumour
Natamycin	*Streptomyces* sp.	Food preservative
Nisin	*Streptococcus* sp.	Food preservative
Penicillin G	*Penicilium* sp.	Antibacterial
Rifamicin	*Nocardia* sp.	Antituberculosis
Streptomycin	*Streptomyces* sp.	Antibacterial
Tetracycline	*Streptomyces* sp.	Antibacterial, antiamoebic

Antibiotics are extensively used in human and veterinary medicine from about 1945. Antibiotics can also be used to control plant diseases and as insecticides.

Antibiotics that effect a wide range of microorganisms are termed **broad spectrum**, for example, chloramphenicol and the tetracyclines, which can control such unrelated organisms as *Rickettsia, Chamydia* and *Mycoplasma* species. In contrast, streptomicin and penicillin are examples of **narrow-spectrum** antibiotics, being effective against only a few bacterial species.

The production of antibiotics has undoubtedly been a highly profitable part of the pharmaceutical industries in the industrial world. The world market for

antibiotics is over $US 10 billion per year and is the most valuable segment of the total pharmaceutical market (about $US 200 billion).

In 1992, the cephalosporins (products derived from cephalosporin C and penicillin G or V) were one of the largest business sectors in the world pharmaceutical market sales at $US 8,3 billion. The present processes are highly efficient and have been achieved with knowledge about the genetics of the producing organisms. However, new techniques such as protoplast fusion and gene transfer technologies are leading to development of new strains with higher productivity, improved stability and possibility. These improvements have resulted in decreases of production cost. At present all antibiotic fermentations involve centrally stirred tank reactors (Chapter 4) run under aerobic batch conditions.

Most studies on antibiotics have been concerned with diseases prevalent in developed countries. Many diseases in developing natures, including many tropical diseases, have received little attention from the major pharmaceutical industries. The main reason lies in economics of developing new drugs for countries with limited financial resources. One can hope that the advances in biotechnology will make it possible to develop the antibiotics necessary to solve the massive specific disease problems of the developing nations. Biotechnology may make possible to economically produce **orphan drugs** – drugs with specific needs but small profit return.

The application of antibiotics in animal feeds and food preservation is now very important. Addition of small amounts of certain antibiotics (e.g. bacitracin, chlortetracycline, procain, penicillin) in the feed of livestock and poultry leads to the production of animals that are healthier, grew more rapidly and achieve marketable weight faster. However, the usage of medically important antibiotics in animal feed leads to the increase of spread of drug-resistant microorganisms and transfer of antibiotic residues into human food. That is why, there are a massive effort to produce antibiotics of low therapeutic potency in humans specifically for animal feed, which will replace the medically used antibiotics.

The combination of new and traditional technology in the pharmaceutical industry holds huge potential for improving microorganisms used in antibiotic production and the isolation of new antibiotic products. The high values of antibiotics and the low cost of raw materials is a further incentive to research.

Vaccines and Monoclonal Antibodies

When a foreign substance (e.g. a microorganism) enters an animal system a chain of molecular reactions is started and which could result in the inactivation and exclusion of the invading organism. This molecular response can remain in the animal systems for many years, giving complete or partial immunity against that type of microorganism. The foreign molecule is the **antigen** (Chapter 3), which can reveal a counteracting response, the **antibody**, from the host system.

In general, antigens are proteins, or proteins combined with other substances such as sugars, though polysaccharides and other complex molecules may also act

as antigens. In the disease process, antigens usually reside on the surface of the invading microorganism and trigger the body's defences against it.

Antibodies are made by special cells of the body and it is now known that individual animal species, including humans, can produce enormous number of different antibodies. The antibody-producing cells recognise the sharp of particular determinant groups of the antigen and produce specific antibodies to neutralise and eliminate the foreign substance. Thus, the mammalian system can bind and inactivate almost any foreign molecule. However, if a particular antigen cannot be neutralised, then the invading microorganism rapidly multiply and create imbalance, ills and dies in the host.

The ability of vaccines to stimulate the natural antibodies has long been known. Vaccines are preparations of dead microorganisms, or living attenuated microorganisms that can be given to humans or animals to stimulate their immunity to infection. In this way they mimic infectious agents without pathogenic properties and elicit in the body protective immune responses.

Vaccines have been developed against many microbial diseases. However, the success of antimicrobial effect varies between types of vaccines. Thus, a vaccine for poliomyelitis has almost eliminated this disease on a world scale, while vaccines against typhoid and cholera are still unsatisfactory. A massive effort is now in progress to develop a vaccine against human immune deficiency virus (HIV), which is responsible for acquired immunodeficiency syndrome (AIDS).

In practice antibodies have been obtained from immunnised animals, but this is usually time-consuming operation. At the end of the various extraction and purification stages the antibodies are usually weakly specific and available in small batches. Furthermore, such systems normally produce mixtures of different antibodies (polyclonal antibodies).

Nowadays, vaccines are being extensively developed by recombinant DNA technology (against the influenza virus, polio virus, herpes virus, etc.). Extensive studies are also in progress with certain bacterial vaccines and vaccines for parasitic diseases (e.g. malaria).

A significant new development in medical biotechnology has been the ability to produce monoclonal antibodies. A major advance of this technique is that when antibody-producing cells are stabilised the secreted antibodies will always be the same from the particular cell line.

Monoclonal antibodies are now finding wide applications in diagnosis requiring highly specific reagents for the detection and measurement of soluble proteins in blood transfusions, haematology, histology, microbiology and clinical chemistry and other, non-medical areas (Table 8.2).

Table 8.2. Monoclonal antibodies markets

Cancer diagnosis and therapy
Diagnosis of pregnancy
Prevention of immune rejection of organ implants
Purification of industrial products
Detection of trace molecules in food, agriculture and industry

Monoclonal antibodies may also be used in the treatment of tumours to carry cytotoxic drugs directly to the tumour site.

On a commercial scale monoclonal antibodies are being produced in 100 litre airlift fermenters, by encapsulation in 100 litre fermenters, and in perfusion chambers using lymph from live cattle. Commercially, monoclonal antibodies have been one of the most rewarding areas of new biotechnology.

Biopharmaceuticals

The vast majority of pharmaceutical products are compounds derived either from synthetic chemical processes, from naturally sources (plants, microorganisms), or combinations of both. Such compounds are used to regulate essential bodily functions or to combat disease-causing microorganisms. Limited quantities of some of these have historically been derived from organs of animals and from blood. Now genetic engineering helps to produce some of these molecules in unlimited quantities. In practice this involves inserting the necessary human-derived gene into suitable host microorganism that will further produce the therapeutic protein (biopharmaceutical) in quantities related to the scale of operation. Table 8.3 indicates some of the main biopharmaceuticals approved for marketing world between 1982 and mid-1992.

Table 8.3. Biopharmaceuticals approved for marketing between 1982 and 1992

product	Broad approved medical use
Insulin	Diabetes
Human growth hormone	Growth deficiency
α-Interferon	Cancer, viral infections
anti-T-cell	Organ transplantation
Hepatitis B vaccine	Hepatitis B prevention
Tissue plasminogen activator	Cardiovascular disease
Erythropoietin	Anaemia
Interleukin-2	Cancer

The first human gene sequences encoding important therapeutic proteins, cloned into microorganisms, were insulin, human growth hormone (somatostatin) and the interferons.

Insulin

There are millions of people in the world who need regular intakes of insulin to overcome the lethal effects of diabetes. Insulin extracted from pigs and cattle has long been the source of worldwide usage, and some unfortunate side-effects have occurred due to additional contaminating compounds presented in the animal insulin. Recombinant human insulin has not such problems and now has the largest market share of sales.

Somatostatin

The growth hormone, somatostatin, has been extremely difficult to isolate from animals; half a million sheep brains were required to yield 0,005 g of pure

somatostatin. By cloning the human gene for somatostatin into a bacterium, the same amount of hormone can be produced from 9 litres of a transgenic bacterial fermentation. One child of 5000 suffers from hypopituitary dwarfism resulting from growth hormone deficiency and easy availability of this biopharmaceutical will be of immense benefit to these child sufferers. The annual world market is estimated at $US 100 million.

Interferons

In 1957 two British researchers discovered substances produced within the body that could act against viruses by making cells resistant to virus attack. Most vertebrate animals can produce these substances, known as **interferons**, and many animal viruses can induce their *in* vitro synthesis and become sensitive to them. However, only small amounts of interferon are produced within cells, and it is very difficult to extract and separate them from other cellular proteins.

Human interferons are glycoproteins (proteins with attached sugar molecules) and they control many types of viral infection, including cancer. They attack the cancer cells by inhibiting their growth, and they can also stimulate the body's natural immune defences against the cancer cell.

There are many different types of interferon characteristic of individual species of animals; mouse interferon will respond to mouse cells but not human cells, and vice versa. Furthermore, different tissues from the same species produce different interferons. Thus, interferon for human studies must be derived from human cells (primarily, using leukocytes from blood).

Two sources of interferon are currently available. The first is from human diploid fibroblasts growing attached to a suitable surface. The second source is from bacteria in which the gene for human fibroblast interferon has been inserted into a plasmid in such a manner that interferon is synthesised and then extracted and purified.

Lymphokines

Lymphokines are proteins produced by lymphocytes (part of the body's immune system) and are important to immune reactions. They have a capability of restoring the capacity of the immune system to fight infectious diseases or cancer. Interleukin-2 at present offers the greatest potential and is now produced by genetic engineering.

At present, all biopharmaceuticals are produced by way of genetically engineered mammalian cell or microbial fermentations. However, with the development of transgenic animals (Chapter 10) it has become possible to produce certain human proteins of biopharmaceutical potential, including tissue plasminogen activator, blood clotting factors, etc. In the lactating glands of several animal species, such as mouse, cow and pig. These products can then be more easily extracted and purified.

An American company can now produce human haemoglobin in the blood of transgenic pigs that could therefore serve as a human blood substitute. This transgenic haemoglobin is free from human pathogens such as HIV and it does not need matching before transfusion because it is not composed of red blood cells.

Gene Therapy

The most important area of genetic engineering of humans is gene therapy. This is the treatment of disease by the transfer and expression of genetic material in a patient's cells in order to restore normal cellular function.

The main application of gene therapy has been directed at correcting single-gene defects (mutations), such as haemophilia, that have been observed in families by their Mendelian pattern of inheritance. It is considered that many hundreds of such diseases could be treated by this process and the next decade should see significant technical progress.

Gene therapy is a complex series of events relying on new biotechnological techniques. Therapy requires a full understanding of the mechanism by which the defective gene exerts its effect on the individual, an ability to switch off the defective gene and to substitute a healthy gene copy (Fig. 8.1). Gene therapy involves the delivery of DNA vectors to specific target cells within the body, uptake (commonly by endocytosis), transcription and translation of sequences within the DNA vector, and production of a therapeutic gene product.

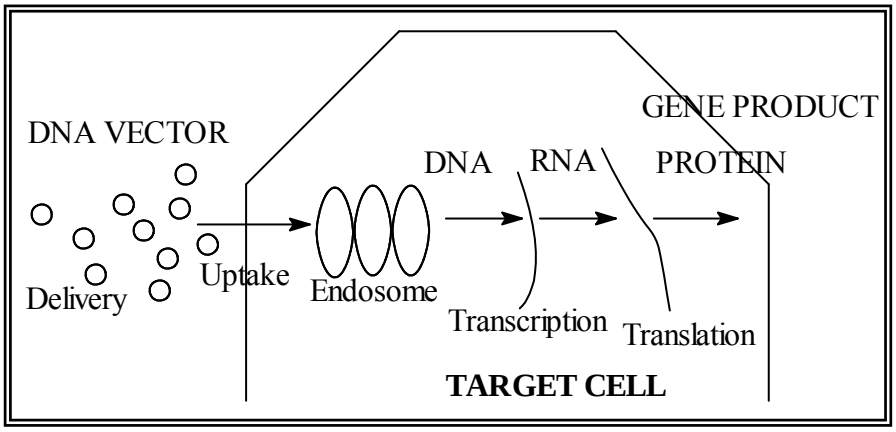


Fig. 8.1. Functional steps in gene transfer and expression.

Chapter 9.

ENVIRONMENTAL BIOTECHNOLOGY

Waste generation is a side-effect of consumption and production activities and tends to rise with the level of economic advance. Wastes arise from domestic and industrial activity, e.g. sewage, waste-waters, agriculture and food wastes from processing, wood wastes and ever-increasing range of toxic industrial chemical products and by-products. The large-scale production and application of synthetic chemicals and their subsequent pollution of the environment is now a serious problem of most countries in the world. The list of high awareness pollutants includes most pesticides, halogenated aliphatics, aromatics, polychlorinated biphenyls, polycyclic aromatic hydrocarbons and nitrozamines.

While many of these compounds are used directly by people in agriculture and health, others may be derived from a spectrum of industrial processes used to make a variety of useful products. Some are associated with the petroleum industry and others are solvents. Such toxic and hazardous chemicals are entering a variety of environments. These synthetic compounds are found at very high concentrations at factory sites and industrial spillages where they can exert deleterious effects, whereas others occur at low levels in natural environments but because of their toxicity, e.g. the pesticide dioxin, constitute a serious health hazard.

In many parts of the world underground water sources are demonstrating dangerous levels of contamination. Chemical pollutants can remain in water-bearing rocks for decades and their removal could be lengthy, unbelievably complicated and restrictively costly.

Microbial Ecology (Environmental Biotechnology)

Microbial ecology is the science that studies the interrelationships between microorganisms and their living (biotic) and non-living (abiotic) environments. The increasing scientific and public awareness of microbial ecology since the 1960s derives from the recognition of the central role of microorganisms in maintaining good environmental quality. The microbes in their multivarious forms have an ability to transform inorganic and organic materials.

Biodegradation is defined as decomposition of substances by microbial activities. Microorganisms found in soil and water can utilise any organic substances encountered as sources of energy and carbon by enzymically breaking them down into simple molecules that can be absorbed and utilised. Under suitable environmental conditions all natural organic compounds could be degraded (Fig. 9.1).

Environmental biotechnology studies the application of biological processes in waste treatment and management. Many successful biotechnological processes now have been developed for water, gas, soil and solid waste treatments.

Organic chemicals which cannot easily be degraded by microorganisms are termed **recalcitrant**. Xenobiotics are synthetic compounds not formed by natural biosynthetic processes and in many cases are recalcitrant. A xenobiotic compound is a foreign substance in our ecosystem and may often have toxic effects. All environmental biotechnological processes make use of metabolic (degradative and anabolic) activities of microbes in our ecosystem nature.

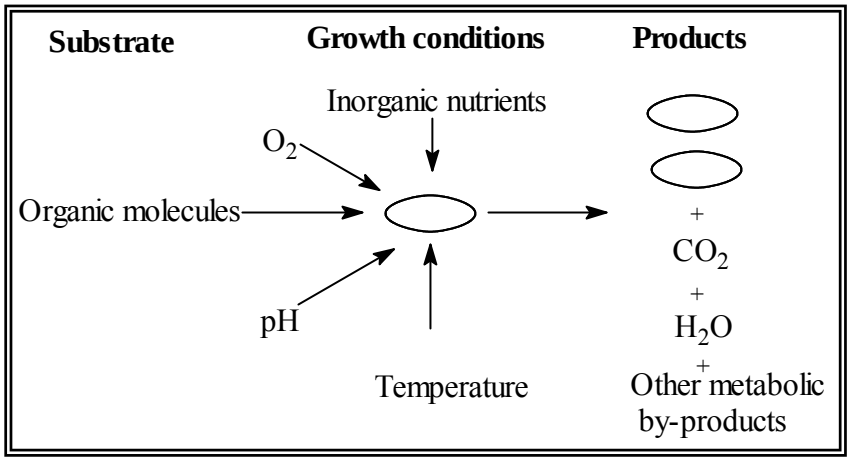


Fig. 9.1. Natural microbial biodegradation of organic molecules.

Waste-Water and Sewage Treatment

Growth in human populations has generally been matched by a concomitant formation of a wide range of waste products, many of which cause serious environmental pollution if they are allowed to accumulate in the ecosystem. The recycling of human, animal and vegetable wastes has been practised for centuries, providing in many cases valuable fertilisers or fuel. However, it was also a source of disease to human and animals by residual pathogenicity of enteric bacteria. In urban communities efficient water collection and specific treatment processes have been developed because it not allows to discharge high volumes of waste into natural land and waters.

Mainly by empirical means a variety of biological treatment systems have been developed beginning from cesspits, septic tanks and sewage farms to gravel beds, percolating filters and activated sludge processes coupled with anaerobic digestion. The primary aim of all of these bioreactors is to reduce the amount of biologically oxidisable organic compounds and to produce a final effluent that can be discharged into the natural environment without any adverse effects.

Such bioreactor systems rely on the metabolic activity of mixed microbial populations (microbial ecology). The systems in which they perform their biological functions can be likened to other industrial bioreactors (e.g. antibiotic production), large-scale plants, (e.g. aeration tanks) which can be very complex, requiring the skills of engineer and the microbiologist for successful operation. The fundamental feature of these bioreactors is that they contain a range of microorganisms with the overall metabolic capacity to degrade most organic

compounds. The development of these systems was an early example of biotechnology.

The biological disposal of organic wastes is achieved in many ways throughout the world. A widely used practice for sewage treatment is shown in Fig. 9.2. This complex but highly successful system involves a series of three stages of primary and secondary processing followed by microbial digestion. A tertiary stage involving chemical precipitation may be included. The primary stage is to remove coarse particles, leaving the dissolved organic materials to be oxidised by microorganisms in aerated open bioreactor. The secondary stage requires considerable energy input to drive the mechanic aerators that mix the whole system, ensuring constant contact of microorganisms with substrates and air. The microorganisms multiply and form a biomass or **sludge** that can be removed and dumped, or passed to an anaerobic digester (bioreactor), which reduces the volume of solids, the odour and the number of pathogenic microorganisms. A further useful feature is the generation of methane or biogas, which can be used as fuel.

Another important means of degrading dilute organic liquid wastes is the **percolating** bioreactor. In this system the liquid flows over a series of surfaces, which may be stones, gravel, plastic sheets, etc., on which attached microbes remove organic matter for their growth. Excessive microbial growth can be a problem, creating the loss of biological activity. Such techniques are widely used in water purification systems.

A biotechnological innovation in waste-water treatment is the deep-shaft fermentation system. The deep shaft is a hole in the ground (up to 150 m in depth), divided to allow the cycling and mixing of waste-water, air and microorganisms (Fig. 9.3). It is the most economical in use, and produces much less sludge than other systems.

Microbiological treatment will be a major field of biotechnological interest in the future. Integrated systems will be developed for treating complex wastes. The role of the biocatalysts or microbes will be assessed.

A comparison of several widely used treatment processes for liquid wastes is shown in Table 9.1.

Table 9.1. Comparison of aerobic biological treatment processes for liquid wastes

Process	Advantages	Disadvantages
Aerated lagoons	High BOD* removal efficiency Low operating costs Low operator skills required	Can create smells Solids carry over Considerable land requirements Sensitive to cold weather
Activated sludge	High BOD removal efficiency Moderate ground requirements	High energy consumptions Requires disposal of excess sludge Sensitive to sudden high inputs
Tricking filters		Moderate BOD removal

		Disposal of excess sludge necessary
Rotating biological contactors	Low operator costs Moderate space requirements Resistant to sudden high inputs High BOD removal efficiency, compact Moderate energy input	Possible odour formation Requires skilled operators Disposal of excess sludge required

* BOD, biological oxygen demand; the amount of dissolved oxygen required by aerobic microorganisms to stabilise organic matter in waste-water or sludge.

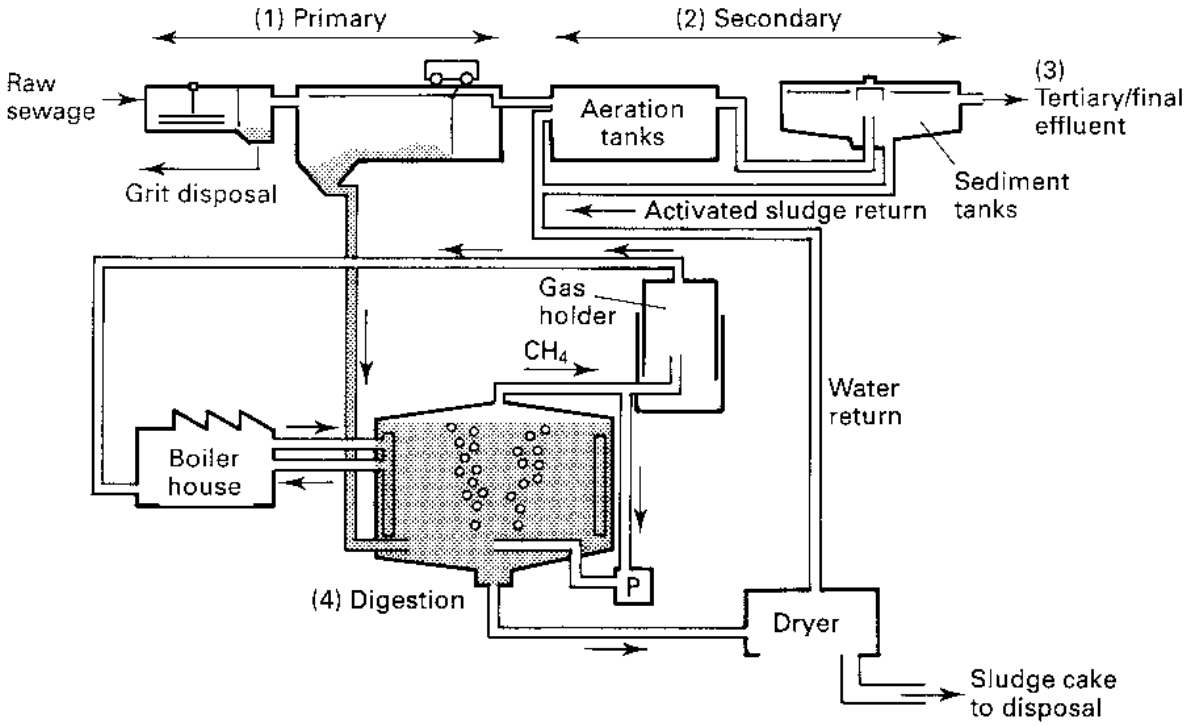


Fig. 9.2. Stage of sewage treatment in a complex incorporating anaerobic digestion.

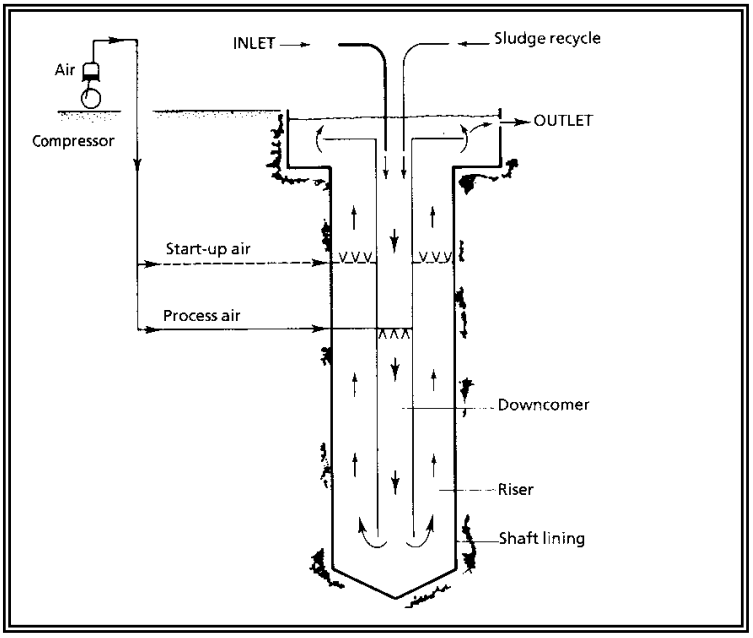


Fig.9.3. Diagram illustrating the principle of the deep-shaft fermentation system used in waste-water treatment.

Landfill Technologies

One of the well-used system for utilization of solid wastes, such as paper, food wastes, sewage wastes, wastes from large-scale poultry and pig farms, etc., is the low cost anaerobic landfill technology. In this procedure solid wastes are deposited in low lying, low value sites and each day's waste deposit is compressed and covered by a layer of soil. The complete filling of such sites can take months or years depending on the size of site and flow of wastes. These constructed landfill sites (Fig. 9.4) can be used to generate methane gas for commercial use.

Landfill sites must be air- and watertight to protect the environment. Regular monitoring is necessary to detect contamination of groundwater, surface water and air.

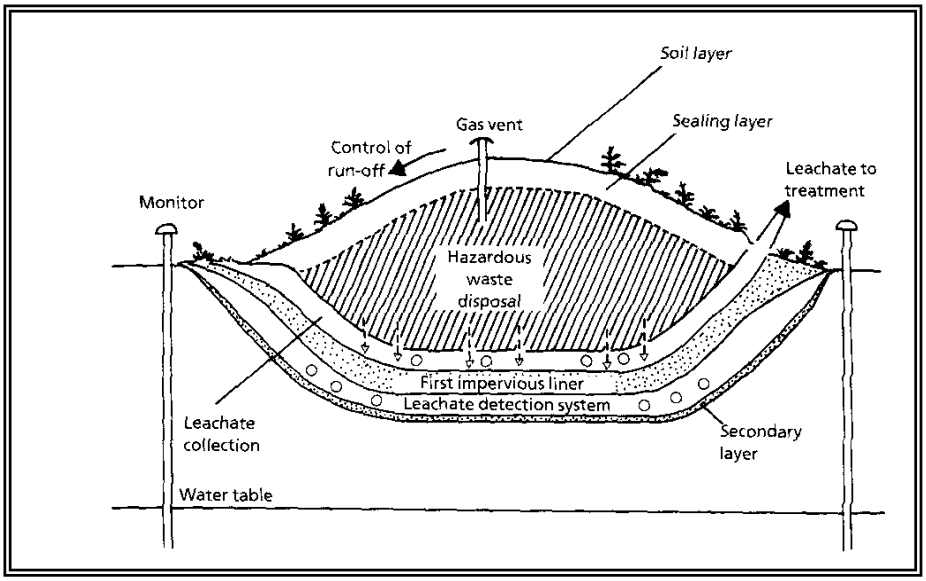


Fig.9.4. Diagram of landfill site conforming to new regulations

In the past, landfill sites were the storage vessels where the waste was sealed from surrounding environment. Nowadays, new sites are managed as bioreactor vessels where stabilisation enhancement systems are operated during the working life. The practice of landfilling will continue to have an important role in the overall management of solid wastes.

Composting

Composting is an aerobic microbially driven process which converts solid organic wastes into stable, sanitary humus-like material reduced in bulk and can be returned to the environment. In large-scale operations using largely domestic solid organic wastes, the final product is mostly used for soil improvement but in more specialised operations using specific organic raw substrates (straw, animal manures, etc.), the final product becomes the substrate for the worldwide commercial production of the mushroom *Agaricus bisporus*.

Composting has only recently become a serious waste management technology. The primary aim of a composting operation is to obtain a final compost with a desired product quality.

Composting is carried out in a packed bed of solid organic particles in which the indigenous microbes grow and reproduce. Free access to air is an essential requirement. The starting materials are arranged in static piles, aerated piles or in rotating bioreactors. Preliminary shredding or grinning of these wastes may be required. The basic biological reactions of the composting process is the oxidation of the mixed organic substrates with oxygen to produce CO_2, water and other organic by-products (Fig. 9.5). after the composting process is completed, the final product often needs for some time period to stabilise.

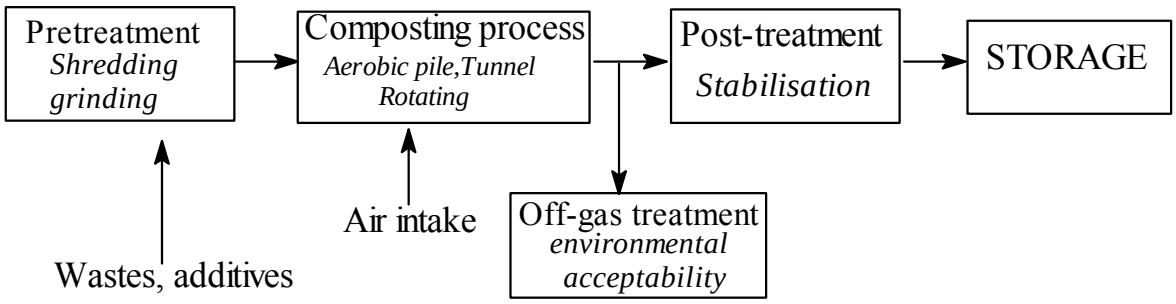


Fig. 9.5. Diagram showing composting plant process.

Successful composting requires optimisation of the growth conditions for the microorganisms. It is a mixed culture fermentation and an outstanding example of microbial ecology in action. Compost process should be regulated to prevent

temperatures in excess of 55 ^{o} C. Moisture level of the organic substrates should be between 45 and 60 %.

For large-scale commercial composting the **aerated pile** system is carried out in closed buildings. In these systems forced aeration with constant turning is used to create good composting conditions.

Tunnel composting is performed in closed plastic tunnels 30 – 50 m long and 4 – 6 m in width and height. Such tunnel systems have been in operation for many years for the composting of sewage sludge and domestic wastes, and for specialised substrate preparation for mushroom production.

Rotating drum systems have been used for composting domestic wastes worldwide. They are especially useful for wettish organic wastes. Small drum systems have been widely accepted for small quantities of garden waste.

Composting is the one of the principal strategies for solid organic waste treatment and recycling back into the environment. For future expansion of composting and recycling, three criteria will be required:

(1) Suitable quality and quantity of substrates must be available.

(2) There must be markets for the end-products.

(3) Process must be environmentally sound and demonstrate economic viability.

Bioremediation

Large areas of the earth's surfaces and the oceans have already been contaminated with oil-derived compounds and toxic chemicals. More than 2 million tonnes of oil are entered the sea each year. Most oils have a relatively low toxicity to the environment in general, but can have catastrophic effects on bird and animal life associated with water. The contamination of soil usually results from a range of activities related to our industrial society.

For the environmental management of such contaminants biotechnology develops the systems that involve biological catalysts to degrade, detoxify or accumulate contaminating chemicals.

There are three main approaches to be assessed in dealing with contaminated sites: a) identification, b) assessment of the nature and degree of the hazard, and c) the choice of remedial action. Up to the present, a considerable number of remedial activities have centred on physical and chemical methods of separation and (or) removal of the pollutants, but now great attention is devoted to the biological methods of remediation, variously termed **bioremediation, biorestoration** or **biotreatment.**

The basic principles of bioremediation are: optimise the environmental conditions so that microbial biodegradation can occur rapidly and completely. Microbes that are present in soil and water environments are potential candidates for the biological transformations. Microbial populations in natural environments exist in a dynamic equilibrium that can be altered by modifying environmental conditions such as nutrient availability. The metabolic effect of microorganisms on

pollutants can take many forms and not always to the environmental advantage of the ecosystems (Table 9.2).

Table 9.2. The effect of microbes on chemical pollutants

Category	Chemical change
Degradation	Complex compound is transformed into simple products, sometimes mineralisation
Conjugation	Formation of complex or addition reactions to more complex compound
Detoxification	Conversion to non-toxic compounds
Activation	Compound is converted into more toxic compound

The application of bioremediation to environmental clean-up has been in two ways:

1. Promotion of microbial growth *in situ* is achieved by the addition of nutrients. When the indigenous microbial population has been exposed to specific polluting compounds for prolonged periods, subpopulations will have developed a limited metabolic ability to utilise the pollutant. However, if essential amount of nutrients such nitrogen and phosphorus is added, microbial growth will normally occur with a increase in pollutant breakdown. This method was successfully applied to the Gulf of Alaska in 1989-90 to clean up the oil spillage from the oil tanker Exxon Valdez. Over $US 3 million were spent on that bioremediation and has been the largest application of this technology.
2. The alternative approach to direct nutrient supplementation and *in situ* microbial growth stimulation has been to remove microbial samples from the polluted site, enrich the useful microbes, scale-up from the mixture by bioreactor cultivation, and re-inoculate large quantities of these microbes into the contaminated site.

A further possibility in bioremediation is to genetically engineered microorganisms to be able to degrade organic pollutants that at present they are unable to attack. To date no such microorganisms has left the laboratory and been tested in the field.

Bioremediation is a new technology and will require time for full development and application. Some of the relative strengths and weaknesses of bioremediation for the treatment of oil spillages are shown in Table 9.3.

Table 9.3. Strength and weaknesses of bioremediation of oils

Strengths	Weaknesses
Relatively simple techniques	Can be slow when compared with physical clean-up methods
Relatively low cost	Applicable only for compounds suitably biodegradable
Technology can be unobtrusive and non-disruptive	Requires public explanation because it is a new technology
Results in easily dispersed by-products	Involves addition of synthetic chemicals, nutrients and dispersants - possible source of environmental contamination

Microbes and the Geological Environment

Microbes are recognised as important catalytic agents in certain geological processes, e.g. mineral formation, mineral degradation, sedimentation, weathering and geochemical cycling. One of the most examples of microbial involvement with minerals is the production of acid mine waters. This occurs from microbial pyrite oxidation when bituminous coal seams are exposed to air and moisture during mining. The huge volumes of sulphuric acid are produced in this way.

Also, microbes are used to extract commercially important elements by solubilisation (**bioleaching**). For example, metals such as cobalt, copper, zinc, lead or uranium can be more easily separated from low-grade ores using microbial agents.

The biological reactions in extractive metal leaching are usually concerned with the oxidation of mineral sulphides. Many bacteria, fungi, yeasts, algae and even protozoa are able to carry out these reactions. Many minerals exist in close association with other substances such as sulphur, e.g. iron sulphide, which must be oxidized to free metal. A widely used bacterium *Thiobaccillus ferrooxidans* can oxidize both sulphur and iron, the sulphur in the ore wastes being converted by the bacteria to sulphuric acid and simultaneously, the oxidation of iron sulphide to iron sulphate is enhanced.

The commercial process involves the repeated washing of crushed ore with a bioleaching solution containing live microorganisms and some nutrients (phosphate and ammonia). The leach liquor collected from the heaps contains the essential metal that can easily be separated (downstream processing) from the sulphuric acid.

In the USA almost 10 % of total copper is obtained by this method. Large-scale bioleaching of uranium ores is widely practised in Canada, India, the USA and the countries of former Soviet Union. Bacterial leaching possibles to recover uranium from low-grade ore (0,01 to 0,5 % U_3O_8) which would be uneconomic by any other known process.

Another important potential application for bacterial bioleaching is the removal of the sulphur-containing pyrite from high sulphur coal. High sulphur coal have a little use because of sulphur dioxide pollution which occurs with burning. Thus, the bacterial removal of pyrite from high sulphur coal could have huge economic and environmental significance.

Microorganisms can also be used as metal bioaccumulators from dilute solutions. The microorganisms, bacteria, yeasts and moulds can actively take the metals by various ways and such processes have a potential use in extracting rare metals from dilute solution. In a similar way, microorganisms are being used to extract toxic metals from industrial effluents, reducing environmental poisoning.

Chapter 10.
BIOTECHNOLOGY IN THE AGRICULTURAL AND FORESTRY INDUSTRIES

Many aspects of modern biotechnology are now being applied to agriculture. Genetic engineering is creating a revolution in agriculture allowing an ever-increasing range of plants and animals. There will be increased stability in the marketplace and much less wastage. Agricultural biotechnology will allow higher quality standards with lower costs of production.

Plant Biotechnology

Plants are the primary source of food for human. Since early times human beings have tried to improve the quality and productivity of important plants. This was done by selection and traditional breeding procedures – a slow and difficult process. Traditional breeding programmes involving sexual crosses will continue to dominate the approach to improve the agronomic characteristics of food crops but will rise by new techniques including micropropagation, protoplast fusion and genetic engineering.

From 1939 it became possible to isolate small numbers of cells from certain plants and to keep them alive in artificial cultivation. The cultivation of these tissues required the presence of plant hormone that allowed the cells to propagate in unorganised manner. These individuals or groups of cells were treated like microbial suspensions and were able to grow under aerated and shaken conditions, initially in flasks and further in large traditional bioreactors. (By this method it is possible to produce important secondary metabolites with high commercial value.

The second major advance in plant cell culture was to achieve the complete reversal of this process by causing these individual plant cells to develop from individual cells to tissues, to organs, and finally to entire plants. In this way it has become possible to clone plant cells.

Rapid, large-scale clonal propagation of many plant species including trees is now feasible. Small tissue explants of many species can be removed from the parent plant and artificially maintained and increased in number. Outstanding examples of this technology have recently been demonstrated by cloning of oil palms and coffee plants from callus tissues. This area of micropropagation not only allows mass production of identical clones of plant but also has the following uses:
(1) Elimination of viruses and other pathogens.
(2) Storage of essential germ plasm instead of seeds.
(3) Production of haploids of ovary culture (gametoclonal variants), useful for cereals.

It is now possible to take plant cells and subject them to the mutation, strain selection and process development. Thus, the genetic diversity of plants may be altered without normal sexual process of fertilisation: by production of haploid,

triploid and tetraploid cells; by the use of protoplast fusion between different species and genera; and by transformation, i.e. transferring DNA from one plant cell (or even another type of organism) into the cells of another. The technique of recombinant DNA technology is now available to the plant technology (Fig. 10.1).

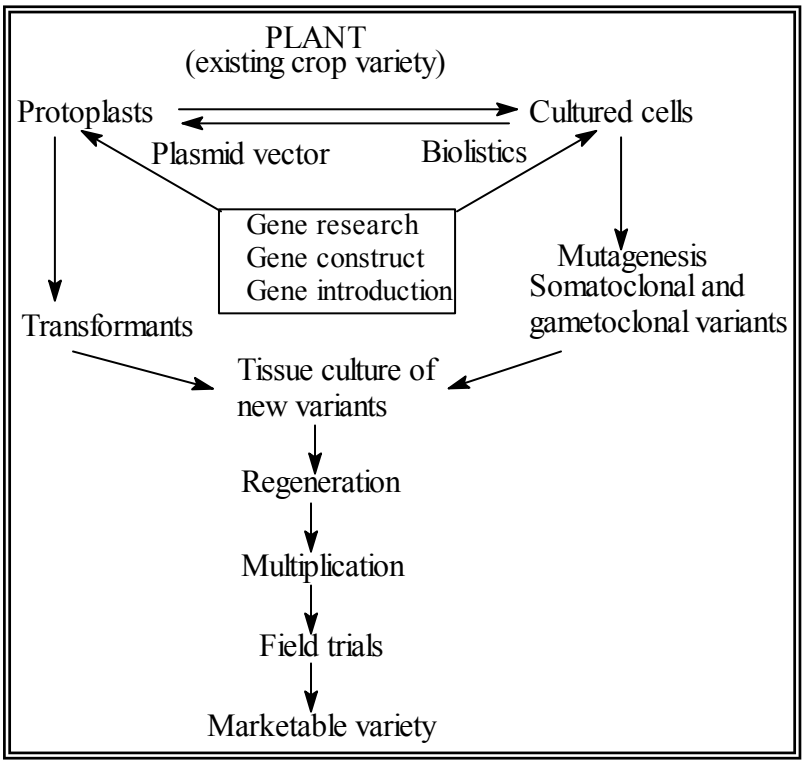


Fig. 10.1. Experimental approaches used to create new plant varieties by biotechnology.

Protoplasts can be produced from most plant cells by digesting away the cell wall and maintaining the protoplasts in a suitable osmotic medium. Many types of protoplast can be induced to reform cell walls and to divide to form cell colonies. Some plants, including potato, pepper, tobacco and tomato, can be fully regenerated from protoplasts. Full regeneration for important cereal crops is not yet possible.

With tissue culture of callus of large structures, regeneration leads mainly to uniformity of plants. In contrast, regeneration from single plant cells or protoplasts can be accompanied by minor extensive changes in the final plant phenotype. This has been termed **somaclonal variation** and is widely used for crop improvement.

Furthermore, genetic engineering methods allow the introduction of single-gene characteristics into plant cells and this should extend later to multiple genes and indeed to whole plant. The main improvements will include:

(1) Improved resistance to specific herbicides.

(2) Improved resistance to insect pests and microbial diseases.

(3) Improved post-harvest characteristics.

Improved resistance to specific herbicides

The application of selective herbicides for killing of plant weed gives a growth advantage to commercial crop plants. The annual world herbicide market is approximately $US 6 billion. However, there is increasing opposition to the

continued use of such chemical compounds for environmental and human health. Herbicide-tolerant crop plants have now been produced by genetically engineering plants genomes resistant to specific herbicides. This way of producing is more effective, less costly and more environmental safely. This will permit reduced overall herbicide use, and a return to herbicides with low mammalian toxicity and rapidly degradable by soil microorganisms.

Improved resistance to insects and microbial diseases

Gene transfer into crop plants to pass insect or microbial resistance is a major new area of research into plant protection. Genes from *Bacillus thuringiensis* have been introduced into several crops, including tomato and cotton, and field-testing has demonstrated impressive results against many insects.

Microbial diseases, in particular fungal and viral, remain one of the major factors limiting crop worldwide productivity. The global estimate of losses due to plant diseases in 1987 was approximately $US 90 billion. Much improved resistance to viruses by the integration of genes for viral coat proteins has now been achieved in several crop plants, particularly rice.

However, the necessary widespread use of insecticides, fungicides and pesticides for crop protection undoubtedly has damaging effects on the environment.

Forestry

Throughout the world there is an escalation in demand for wood-derived products and many countries are now in deficit. This will be further compounded by increased pollution such as 'acid rain' and the huge losses because of indiscriminate felling. Forests have not had the extensive research unlike other cultivated tree crops such as rubber, coffee and citrus.

However, biotechnology will play an important role in achieving an increase in production and bringing improvements to the quality of the trees. Tissue culture technology such as micropropagation, somatic embryogenesis (induction of single cells to develop into embryo-like structures from which a shoot and root develop), selection of somaclonal and gametoclonal variants and gene transfer are being developed to improve forests

Trees have long generation time and, as a consequence, genetic improvements will be slow. Loblolly pine has been transformed by *Agrobacterium tumefaciens* and this may allow gene transfer techniques similar to those used with other plant species.

Biological Control

The use of chemical pesticides has led to important improvements in the production levels of agriculture and forestry. The pesticide market is dominated by synthetic chemicals and will remain so. However, consumers are becoming increasingly concerned about food quality and possible carry-over of pesticide

residues into food products. Biotechnologists are now actively examining alternatives to chemical pesticides as a means of controlling agricultural insects and diseases. An obvious approach would be to use naturally occurring biological means of controlling these problems. It is well-known that all organisms have their own specific diseases and predators. Biological control will use the microorganisms to control diseases. Insect predators can also be used for control purposes.

The most successful biocontrol agent is *bacillus thuringiensis,* a spore-forming bacterium containing crystalline protein inclusions. The proteins are highly toxic to some insects but very specific. The commercial application of these biopesticides is usually as formulations of spores and are applied at 4 – 6 g/ ha $^{-1}$.

In recent years the toxin genes have been isolated and recombinant DNA-based products produced and approved. These genes have also been incorporated into various plant species and expressed in the plant tissue. While the *B. Thuringiensis* toxines dominate the market there are many examples of viral and fungal biopesticides that can be applied for these purposes.

The commercial potential for these products will be determined by their efficacy, cost-benefits ratios when compared to synthetic chemicals, ease of use and spectrum of activity (Table 10.1). The comparisons between chemical and microbial pesticides are shown in Table 10.2.

Table 10.1. Requirements for a microorganism to be a successful biological control agent.

It must have a substantial market-size and consumer demand
It must be equal to or better than chemical pesticides in performance and persistence
The product must be safe, with low mammalian toxicity
It should remain on storage
Mass production by bioprocess technology should be cheap
It should be applied without recourse to major changes in agricultural practices

Table 10.2. Chemical versus microbial pesticides

	Chemical pesticide	Microbial pesticide
Product use		
Speed of action	Usually rapid	Can be slow
Killing efficacy	Often 100 %	Usually 90-95 %
Spectrum of effect	Generally broad	Generally narrow
Resistance of target organism	Often developed	Not yet seen
Product safety	Lengthy and costly	Low cost
Toxicological testing	Many well-known examples	None yet shown
Environmental hazards residues	Interval to harvest usually required	Crop may be harvested immediately

Animal Biotechnology

Animal agriculture in the form of cattle, pigs, sheep, poultry and fish represents a major aspect of food worldwide production. In the developed world animal production is highly intensified and technologically driven. Animal production reflect on quality of feed, availability and need of growth hormones, pesticides, antibiotics and vaccines, good animal husbandry and increasing selective breeding, molecular biology, embryo manipulation and gene transfer.

Genetic engineering for transgenic animals

Selective breeding is a slow process and, especially with large animals, can take many years to establish desired phenotypic changes. However, the advent of recombinant DNA technology and its application to animal breeding programmes could increase the speed and range of selective breeding. The first example of the transfer of foreign gene into animal by recombinant DNA technology was the insertion and expression into mouse genome of a rat gene for growth hormone. The subsequent progeny were much larger than the parents. This 'Super Mouse' was the first example of a transgenic animal, i.e. an animal that has acquired novel genetic material by artificial means rather than by normal route of sexual reproduction

Subsequently, there has been external speculation on the economic potential of transgenic farm animals and this should become a highly profitable worldwide industry with great benefit to humans. However, as discussed in Chapter 13, it is the most controversial of all areas of modern biotechnology. Some of the main opportunities where this new technology can be used with animal breeding programmes are listed in Table 10.3.

Table 10.3. Anticipated changes involving transgenic animals

Efficiency of meat production	Wool quality and quantity
Improved quality of meat	Disease resistance in animals
Milk quality and quantity	Production of low cost pharmaceuticals and biologicals
Egg production	

How can novel DNA be incorporated into animal genomes and then inherited into the offspring? At present time the most successful method for gene transfer is by microinjection into pronucleus of fertilised eggs. The eggs are then surgically transferred into surrogate mothers (Table 10.4).

Table 10.4. Sequences necessary to establish transgenic animals

Identification and construction of foreign gene
Microinjection of DNA directly into pronucleus of a single fertilised egg
Implantation of these engineered cells into surrogate mothers
Bringing the developing embryo to term
Proving that the foreign DNA has been incorporated into the DNA of at least some of the offspring
Demonstrating that the gene is regulated well enough to function in its new environment

Transgenic pigs, sheep and cattle have now been obtained, although the frequency of success is only 1 % compared with 2-5 % with mice. However, successful fish transgenics can be as high as 70 %.

A novel and commercial use of transgenic animals is the production of human proteins (biopharmaceuticals) in transgenic lactating animals. Transgenic constructs that allow the mammary glands of lactating animals to secrete high value human proteins are now possible and will be the first commercial use of transgenic animals for product formation. The animals will in fact become bioreactors producing pharmaceutical products. Gene constructs for human coagulation factor IX (blood-clotting agent) have been successfully inserted into the sheep genome and while expression levels are still low, factor IX is present and the trait is heritable. The potential of transgenic animals to secrete a wide range of commercially valuable healthcare products is almost unlimited and should be realised in the future.

Diagnostics in Agriculture

In traditional analytical methods applied to plant and animal agriculture the primary aim is to isolate or separate out the analysed component from the complex chemical milieu of the sample. Such methods require an operator of considerable chemical analytical experience and are expensive. However, new methods based on biotechnology-derived techniques are improving many aspects of agricultural analysis, not only by being able to equal the sensitivity, but also by being able to carry out many determinations *in situ* without the need for complex isolation procedures. Furthermore, these methods are usually cheaper and more rapid. Often they can be performed by unskilled operators (Table 10.5).

Table 10.5. Criteria required for successful rapid methods

They should:
Be fast, accurate and reliable
Be simple to operate and have low costs
Have readily available and stable reagents
Have minimal labour requirements
Have a high degree of sensitivity and specificity

Nowadays, three new biotechnology-derived rapid methods are known, namely **immunoassay, DNA probe** and **biosensor**. Primarily, these methods were developed with the human healthcare market, with huge sales anticipated in hospitals, surgeries and for home care and monitoring and further they began to apply to plant and veterinary diseases.

Immunoassay method uses monoclonal antibodies and is widely recognised for its commercial success in clinical and veterinary diagnostics. Nucleic acid probe technology is based on the principle of hybridisation of complementary sequences of DNA or of DNA and RNA. The respective nucleotide strand must be exact, corresponding sequences of nucleotides for exact hybridisation occur; thus a given strand can hybridise only with its complementary strand. This high level of

specificity is directed to identify microorganisms in complex mixture – the DNA probe or hybridisation assay.

Using these diagnostic methods it is now possible to detect microbial diseases in animals at very low levels of infection in body fluids or tissues and to be able to isolate an animal before it becomes infectious. One of the largest areas of application for diagnostic methods is in measuring fertility hormones in animal blood or milk, e.g. progesterone, oestrogen sulphate and equine gonadotropin. Illegal use of growth hormones and antibiotics can also be monitored.

Immunochemical technology using monoclonal antibodies is now widely used for the analysis of pesticide residues in foods, together with toxic microbial products such as mycotoxines. Specific plant diseases can now be detected in a crop at very early stages. These methods now allow more efficient crop breeding and international trade.

Chapter 11.

FOOD AND BEVERAGE BIOTECHNOLOGY

Food production is the largest worldwide industry.

The food chain has its origins in production of agriculture, with the planting of the seed or the rearing of animals, and concludes with the utilization of the food products by the consumer. Apart from fruits and vegetables, most food raw materials, e.g. cereals and meats, require some degree of processing. The link between the products of the farm and the consumer is the food processing industry, whereby relatively bulky, perishable, raw agricultural products are transformed into shelf-stable, convenient and palatable foods and beverages.

Food biotechnology is concerned with the integration of both modern biological knowledge and techniques and current bioengineering principles in food processing and preservation. Modern biotechnological techniques have considerable importance in the food market, namely cost, preservation, taste, consistency, colour, safety and, above all, health aspects.

The food and beverage industries are very different from the pharmaceutical industry; their products are cost and marketing driven rather than technology driven. Research and development in most of the food and beverage industries is usually less than 1 % of sales. Most food and drink products are high volume and low cost items.

The impact of biotechnology on the food and beverage industries can be anticipated in two directions:

(1) Agronomic Increased plant and animals yields, extended growth range and environments from which the farmers will mainly benefit.

(2) Non-agronomic Improving plants and microorganisms to provide benefits to the food producer, retailer or consumer (Table 11.1)

New developments in biochemical engineering could also be of advantage to those industries using mechanical (e.g. grinding), physical (e.g. membrane separation, cooking) and chemical (e.g. hydrolysis, salting) methods.

Food and Beverage Fermentations

Fermented foods and beverages have a significant role in all societies and result from the action of microorganisms or enzymes on a wide range of agricultural materials with associated desirable biochemical changes giving significant organoleptic improvements to the final product. As a result of the fermentation process the product is usually more nutritious, more digestible, has improved flavour and is toxicologically and microbiologically safer.

Fermented foods and beverages derived from plant and animal materials are an accepted and essential part of the diet in almost all parts of the world, involving a wide diversity of raw materials as substrates, using technology from the most primitive to the most advanced, and achieving an astounding range of sensory and textural qualities in the final products. Fermented foods include breads, cheeses,

Table 11.1. Biotechnology at all levels of the food production

Food chain	Potential biotechnological impact
BIOLOGICAL LIVING RAW MATERIALS FOOD RAW MATERIALS FOOD INGREDIENTS	*Agronomic*: increased yield, extend geographical and environmental range, all year growing *Non-agronomic*: increased benefit to processor by lowering the costs of manufacturing operations, keep fresh longer, improve texture and taste, phytoproduction of flavours, colours and other more natural additives, using tissue culture, single cell protein
FOOD PRODUCTS AT THE FACTORY GATE	Improving processing and reducing product manufacture costs, e.g. starter cultures, enzyme treatments, genetic engineering of microorganisms, detoxification of food 'toxins', upgrading of waste materials, analytical applications and modification of fatty acids, carbohydrates and proteins
FOOD PRODUCTS AT THE POINT OF CONSUMPTION	Improving distribution and product quality by inhibiting physical, chemical and microbiological deterioration, introducing less harsh processes and new preservation regimes
PRODUCTS CONSUMED	Ensuring products meet the consumer's expectations of texture, flavour, nutrition, preservation, wholesomeness, and being more natural

Table 11.3. Substrates for selected alcoholic beverages

Substrates	Beverage	Country	Saccharifying agent
Starch (barley + other cereals)	Ale	Belgium, Germany, Canada, Australia	Barley malt
	Lager	World wide	Barley malt
Barley, rye, rice, beet	Kvass	Russia	Barley and rye malt
	Bouza	Russia (Crimea)	
Millet	Thumba	India	Millet malt
	Arrack	India, SE Asia	
	Pachwai	India	*Mucor* sp.
Rice	Sake	Japan	*Aspergillus oryzae*
	Sonti	India	
	Ancu	Taiwan	*Rhizopus* sp.
Rice (red)	Hung-Chu	China	
	Kaffir beer	Malawi	Sorghum malt
			Aspergillus sp.
Sorghum			*Mucor rouxii*
	Merissa	Sudan	*Bacillus* spp.
Sweet potato	Awamori	Japan	
Agave (sap)	Pulque	Mexico	
Apple (juice)	Cider	UK, France, N.America	
Grape (juice)	Wine	Temperate	Not required since sugar
Honey	Mead	UK	is present in the substrate
Pear (juice)	Perry	UK,France	
Palmyra (juice)	Toddy	India, SE Asia	
Palm flower-stalk juice	Tuwak	Indonesia	

yoghurts, sauerkraut, soy sauce, mushrooms, etc., while fermented beverages include alcoholic beers, wines, sake, brandy, whisky and non-alcoholic tea, coffee and cocoa.

Climate and available raw materials have influenced the types of food and beverage fermentation that developed in different geographic regions. While a very important reason for the development of such fermentations was to preserve the basic organic components from spoilage, of equal or greater importance were the resulting changes in organoleptic, physical and nutritional characteristics of the bland starting materials, resulting in products of enhanced flavour, improved vitamin content and, in some vegetable products, a meat-like texture and flavour. For most of these fermentations the procedures were developed in ignorance of the role of microorganisms. The original artisans controlled and directed microbial activity by empirical methods but most often achieved consistent end-products. The Egyptians and Babylonians produced alcoholic beverages from barley, sour-dough bread from rye occurred in Europe in 800 BC, and accounts of fermented dairy products are found in early Sanskrit and Christian works, and only in relatively recent time the microbial nature of most of these fermentations has been recognised.

Fermented foods can be divided into nine groups, namely beverages, cereal products, dairy products, fish products, fruit and vegetable products, legumes, meat products, starch crop products and miscellaneous products. The relative importance of these fermentations in geographical areas is shown in Table 11.2.

Table 11.2. Production of classes of fermented food according to geographical region

World production rate	Region	Importance	
		Major	Minor
High	Europe	Dairy, beverage, cereals, meat	Legumes, starch crops
	North America	Beverages, dairy, meat	Fish, legumes, starch crops
	South Africa	Starch crops, cereals, beverages	Dairy
Medium	South America	Beverages, dairy	Legumes
	Middle East	Dairy	Legumes, meat
	India	Cereal, legumes	Meat
	East Asia	Fish, legumes	Dairy
Low	North Africa	Dairy	Legumes

Alcoholic beverages

Alcoholic beverages occur in many different forms and tastes. The types of beverage produced in any country almost entirely reflects the crops grown. Thus, the cool regions of Europe, Scandinavia, Poland and Russia produce and consume

beers and lagers from barley whereas the countries with warm climate (e.g. Spain, Greece, Italy, etc.) produce wines from grapes. Alcohol beverages and potable spirit industries represent one of the most economically stable sectors.

The starting material usually comprises either sugary materials (fruit juices, plant sap, honey) or starchy materials (grain or roots) which need to be hydrolysed to simple sugars before the fermentation (Table 11.3). when these are incubated with suitable microorganisms and allowed to ferment, the end-product is a liquid containing from a few percent up to 16 % or more of alcohol with an acid pH. The alcoholic beverages can be drunk fresh but in normal practice a period of storage or aging is required to improve organoleptic properties. Further distillation increases the alcohol strength and produces spirits of many types, e.g. whisky, brandy, vodka, gin, rum, which can contain between 40 % and 50 % ethanol (Table 11.4). Cordials and liqueurs are sweetened alcohol distillates derived from fruits, flowers, etc.

Table 11.4. Alcohol production from sugars or starch-containing raw materials

Sugar	Product	Starch	Product
Molasses	Rum Cognac	Barley	Whisky
Agave	Tequila	Maize and rye	Bourbon whiskey
Pear	Pear brandy	Potatoes, rye and	Vodka
Cherry	Kirsch	wheat	Chinese brandies
Plums	Slivovitz	Rice	

The most often used fermenting organism is the yeast *Saccharomyces cerevisiae.* This organism can assimilate and utilise simple sugars such as glucose and fructose and metabolise them to ethanol.

The process of wine and beer production represents the major worldwide biotechnological industries.

Wines Historically, wine is a Middle Eastern and European drink.

Most commercial wines use the wine grape *Vitis vinifera*. Red wine is formed when black grapes are crushed and fermented whole. In contrast, if the skins are removed from black grapes or when white grapes are used, white wine is the final product. Rose wine results from some limited contact with the skins of black grapes, dry wine is the end-product of complete sugar utilisation, sweet wine retains some residual sugar, etc.

The grapes, containing 15 – 25 % sugar, are crushed mechanically or by treading of feet. The juice (termed **must**) is the substrate for the biotechnological stage of the production. The must contains many contaminating yeasts and bacteria, so sulphur dioxide is usually added to abolish this natural fermentation capacity. In large-scale wine production the must is sterilised, inoculated with yeast and subjected to controlled fermentation in suitable tanks or bioreactors. The dryness or sweetness of the wine will depend on the degree of sugar conversion, glycerol levels, levels of bacterial contamination, etc.

Fermentation conditions such as time and temperature will depend on the type of wine. After fermentation, the wines are run into storage vats or takers where

the temperature drops quickly, precipitates form and subtle chemical changes take place. Many wines undergo a secondary bacterial or malolactic fermentation, converting residual malic acid to lactic acid. The final alcoholic content of wines ranges between 10% and 16 %.

Fortified wines, such as sherry, port and vermouth, are wines to which additional alcohol is added after fermentation, raising the alcohol level to about 20 %.

Beers Beers and ales are produced from starchy cereals such as barley. Additional carbohydrate sources, known as adjuncts, are usually added in varying proportions. In practice, there are five major steps in the manufacture of beers from grains: malting, mashing, fermentation, maturation and finishing.

Malting: dried barley is soaked in water and then spread out on the malthouse floor, where the seeds germinate with the formation of starch-degrading (amylase) and protein-degrading (protease) enzymes. The germinated seeds are then killed by kilning (slow heating to 80 ^{o} C) while still retaining most of the enzyme activity (**malt**).

Mashing: In this stage the malt is mixed with hot water (55-65 ^{o} C), and the starches and proteins break down to produce dextrins, maltose and other sugars, minerals and other growth factors (the **wort**). This is the medium for the beer fermentation. Hops may be added prior to the fermentation to give characteristic flavour and some antiseptic properties.

Fermentation: The wort is transferred to open bioreactor systems and inoculated with pure strains of yeast. A top-fermenting *Saccharomyces cerevisiae* is used at 20 – 28 ^{o} C to produce beers, ales or stouts, a bottom-fermenting yeast *Saccharomyces uvarum* ferments the wort at a lower temperature (10 – 15 ^{o} C) to produce lager.

Maturation and finishing: Beer is usually matured in casks at 0 ^{o} C for several weeks to improve flavour, settle out the yeasts and remove haze. Bottled or canned beers are usually pasteurised at 60 ^{o} C for 20 minutes. The alcohol content of beer is usually between 4 % and 9 %, with ales it is somewhat higher.

Traditional applied genetics, together with protoplast fusion and recombinant DNA technology, are constantly improving the yeast strains used in these fermentations. A new commercial brewing yeast has been developed and approved using recombinant DNA techniques.

Coffee, tea and cocoa

In Asia, India, Africa and South America non-alcoholic beverages are derived from coffee, tea and cocoa plants. These beverages have gained worldwide approval and high commercial value. Tea is derived from the enzymic activity released after the crushing of the leaves, while for coffee and cocoa the pulp surrounding the beans is removed by a natural fermentation with bacteria, yeast and fungi, which is very important for full flavour and aroma development. The dried products, namely tea leaves and coffee and cocoa beans, are shipped throughout the world and the final beverage is formed by the addition of water. It is little known

about the exact microbial contribution to these fermentation processes. The processes are still empirical, without science.

Dairy products

Dairy products, such as fermented milk, butter and cheeses, have been known for centuries. Such fermentations are related to areas with lactating animals, cows, goats and sheep. It is now known that these fermentations result from the activity of a group of organisms called lactic acid bacteria. In the past, these fermentations arose directly from the natural occurrence of lactic acid bacteria. Nowadays, an inoculum (a pure starting culture) of selected bacteria is added to the milk to be fermented.

The lactic acid bacteria can have many beneficial effects in the foods in which they grow:

(1) They have an inhibitory effect on many undesirable bacteria; in this way they preserve the milk.

(2) They produce highly acceptable texture and flavour modifications in the milk.

(3) They have health effects on intestinal microflora.

One of the largest activities of the dairy industry is cheese production. Cheese is made by separating the casein of milk from the liquid or **whey**. Over 900 types of cheese are recognised.

Cheese production from milk is a dehydration process in which the milk protein (casein) and fat are concentrated 6- to 12-fold. The common steps in most cheese production are:

(1) Acidification of the milk by the conversion of sugar lactose into lactic acid by the lactic bacteria.

(2) Coagulation of the casein by a combination of proteolysis and acidification

Proteolysis is started by the rennet (chymosin enzyme) (animal or fungal origin) and the coagulated casein in a gel form containing any fat. (Fig. 11.1) The separated curd is cut into blocks, drained and pressed into shapes, matured and made into cheese. The details of cheese production are very complicated and involve many strains of bacteria and in some cases filamentous fungi, special milks and related additives.

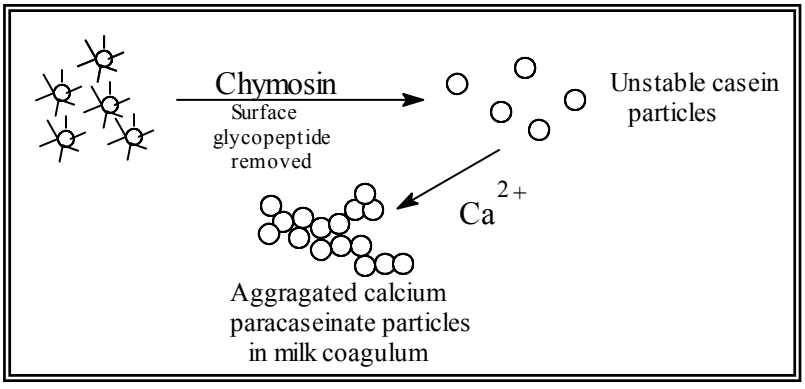


Fig. 11.1. Mode of action of chymosin (rennet).

An important recent biotechnological innovation in cheese production has been the use of recombinant DNA techniques for chymosin production and

commercial use. At present there are six sources of commercial rennet: three from animals (veal calves, adult cows and pigs) and three fungal sources. The fungal sources are almost identical in function to the animal chymosin, but they give yield reduction and poor flavour in comparison with animal chymosin.

Within the last decade, genetically modified microorganisms have been produced that can yield chymosin identical to the animal chymosin. The production of recombinant chymosin by genetically modified microorganisms is shown in Fig.11.2.

The second major group of dairy products are the yoghurts. Traditionally, yoghurt is fermented whole milk; the process uses a mixed culture of *Lactobaccillus bulgaricus* and *Streptococcus thermophilus*. The characteristic flavour compound, acetaldehyde, generates the fresh acid taste by the conversion of lactose to lactic acid. Both bacteria produce extracellular polymers that give the characteristic viscosity of the product. Incubation is at 30 or 45 ^{o} C. Set yoghurt is packed into the container after inoculation and allowed to ferment in the container. Frozen yoghurt is gaining increasing popularity as an alternative for ice cream.

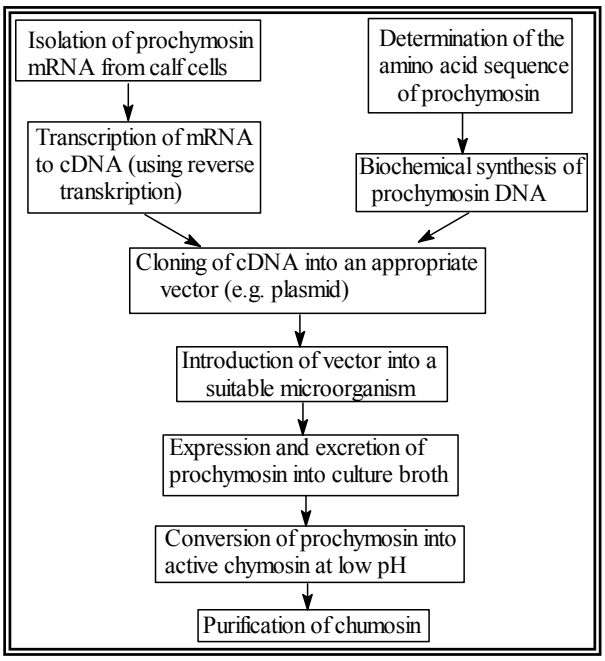


Fig. 11.2. Production of calf chymosin by genetically modified microorganisms

Vegetable fermentations

In various ways fruits and vegetables can be preserved using salt and acid, the acid being derived from bacteria in the form of lactic bacteria. The most Western interest are the fermentation preservation of cabbage to give sauerkraut and the pickling of cucumbers and olives.

In sauerkraut production shredded cabbage is packed anaerobically with salt, the salt reduces the water activity and promotes the leakage of sugars from the cabbage leaves (Fig. 11.3). Then, lactic acid bacteria releases lactic acid, lowering the pH and preventing the growth of putrefying bacteria. Process is carried out at low temperature, 2,25 % salt concentration and anaerobic conditions.

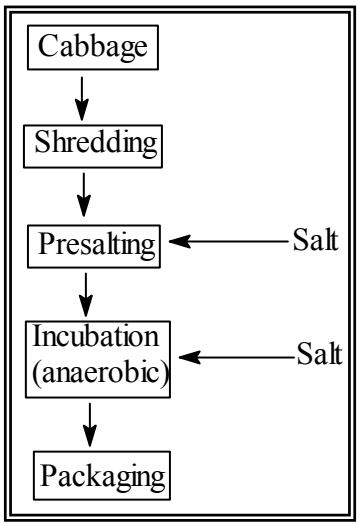


Fig. 11.3. The production of sauerkraut.

In cucumber and olive fermentations, the fermentations are carried out at much higher salt concentrations (5-8%) and the microbial sequences are similar to those of sauerkraut fermentation.

Cereal products

In almost all parts of the world cereals are produced and are the main class of food; a considerable proportion of these cereals are fermented into solid foods or into alcoholic beverages.

Bread is the principal fermented cereal product and has been known since Roman times. In Europe wheat and rye are two widely used cereal flours and are usually mixed with water or milk, salt, fat, sugar and other ingredients with the yeast *Saccharomyces cerevisiae*. After the fermentation proceeds the dough rises due to the formation CO_2. The expansion and stretching of the dough is due to the unique elastic protein gluten.

Overall the fermentation achieves three primary objectives: leavening, flavour development and texture changes in the dough. At the end of fermentation process the risen dough is baked in an oven, giving a final product free from living microorganisms and an extended shelf-life.

Modern applied genetics improves the quality of the yeast organism, leading to improved activity, better flavour and improved texture of the product. A genetically engineered *Saccharomyces cerevisiae* with improved fermentation properties has been produced and has passed all regulatory requirements for safety. However, it has not put into commercial operation yet.

Enzymes and Food Processing

In recent years, one of the main applications of modern biotechnology to food production has been the use of enzymes. Enzymes are an essential part of most food and beverage fermentations and while most of the enzymes will be

derived from the specific microorganisms, there is the opportunity to improve the processes by the addition of exogenous enzymes (Table 11.5)

Table 11.5. Use of enzymes in food processing.

Industry	Enzymes
Brewing	α-Amylase, β-amylase, protease, papain, amyloglucosidase, xylanase
Dairy	Animal or microbial chymosins, lactase, lipase, lysozyme
Baking	α-Amylase, xylanase, protease, phospholipases A and D, lipoxygenase
Fruit and vegetable processes	pectin esterase, polygalacturanase, pectin lyase, hemicellulases
Starch and sugar	α-Amylase, β-amylase, glucoamylase, xylanase, pullulanase, isomerase, oligoamylases

The role of exogenous enzymes to facilitate or even replace mechanic processes. A major development in the using of enzymes in the food industry involves the near elimination of water from enzyme reaction media. Thus, hydrolytic enzymes can be reversed so that with no metabolic energy input the same enzymes, degrading biomolecules, can now synthesise them. A wide range of food-related compounds have now been produced by this novel approach and include polyglycerol esters (emulsifiers), chiral flavour esters and oligopeptides, etc. Enzymes are also used in the design of novel foods. In Japan, for example, there has been considerable research into oligosaccharides for insulin-low calorie response, designer fats and special food fibre ingredients.

Miscellaneous Microbially Derived Food Products

These products are derived from microbial fermentations and are used as ingredients in food production.

Vinegar Vinegar is an aqueous solution containing about 4 % by volume acetic asid and small amounts of esters, sugars, alcohol and salts. It is usually derived from wine, malt or apple cider. The fermenting bacteria are species of *Acetobacter.* It is widely used as flavouring compound in liquid foods such as sauces and ketchups.

Organic acids Citric acid is widely used in fruit drinks, confectionery, jams, etc. Over 100 000 tonnes of citric acid are manufactured by fermentation processes involving the fungus *Aspergillus niger* and molasses as substrate.

Amino acids and vitamins Amino acids are widely used in the food and beverage industries as flavour enhancers or nutritional additives. World production are about 600 000 tonnes per year. Glutaminic acid and lysine are two amino acids produced by fermentation process involving the bacteria *Corynebacterium glutamicum* and *Brevibacterium flavum*. Extensive mutant selection has produced microorganisms that further produce these primary metabolites.

Polysaccharides Extracellular microbial polysaccharides are produced by many microorganisms and have been used in foods to enhance thickening and to form gels. They stabilise food structure and improve palatability. The bacteria species used mainly are *Pseudomonas* (xanthan gums) and *Leuconostoc mesenteroides* (dextrans). Species of *Acetobacter* can produce cellulose which forms the basis of certain oriental foods.

Flavours The best-known flavour is monosodium glutamate, now made by fermentation using natural or engineered microorganisms. Enzymic degradation of yeast RNA produce nucleotide derivatives that are powerful flavour enhancers for meat. The world market for food flavours is about $US 2 billion.

Chapter 12.

SAFETY IN BIOTECHNOLOGY

For centuries, society has safely used the products and processes of biotechnology. These processes have involved microorganisms of known pathogenic potential and, with the exception of vaccines production, all microorganisms used are non-pathogenic to human and other animals. However, for all biotechnology processes, safety is of paramount importance. Table 12.1 lists the main areas of consideration for safety aspects specific to biotechnology.

Table 12.1. Safety considerations in biotechnology

Pathogenicity: potential ability of living organisms and viruses to infect humans, animals and plants and to cause disease
Toxicity and allergy associated with microbial production
Increasing the environmental pool of antibiotic-resistant microorganisms
Problems associated with the disposal of spent microbial biomass and the purification of effluents from biotechnological processes
Safety aspects associated with contamination, infection or mutation of process strains
Safety aspects associated with the industrial use of microorganisms containing *in vitro* recombinant DNA

Problems of Organism Pathogenicity

Most microorganisms used by industry are harmless and are used directly for the production of human or animal food. Only a small number of potentially dangerous microorganisms have been used in the manufacture of vaccines or diagnostic reagents, e.g. *Bordella pertussis* (whooping cough), *Mycobacterium tuberculosis* (tuberculosis) and some others.

A classification of the degree of potential hazard of microorganisms has been drawn up by the European Federation of Biotechnology (Table 12.2). Group E contains those microorganisms that present risks only to the environment, particularly, to animals and plants.

Table 12.2. Classification of microorganisms according to pathogenicity

Class 1
Microorganisms that have never been identified as causative agents of disease in human and that offer no threat to the environment
Class 2
Microorganisms that may cause human disease and might offer a hazard to laboratory workers. They are unlike to spread in the environment. Prophylactics is available and treatment is effective
Class 3
Microorganisms that offer a severe threat of the health of laboratory workers but a small risk to the population at large. Prophylactics is available and treatment is effective

Class 4
Microorganisms that cause severe illness in human beings and offer a serious hazard to laboratory workers and to people at large. In general effective prophylactics is not available and no effective treatment is known

Class E
This group contains microorganisms that offer a more severe threat to the environment than to people. They may be responsible for heavy economic losses. National and international lists and regulations concerning these microorganisms are already in existence in contexts other than biotechnology (e.g. for phytosanitary purposes)

Problems of Biologically Active Biotechnology Products

Vaccines and antibiotics are examples of biologically active products and care must be taken to prevent their indiscriminate dispersal. Contaminants in otherwise safe processes may produce toxic molecules that could become incorporated into final products, leading to food poisoning. Allergic reactions to produce formulations must also be guarded against. Use of antibiotics in agriculture could lead to carry-over into human foods, resulting in possible development of antibiotic resistance in human disease organisms. Many countries now restrict the use of antibiotics in agriculture.

When properly practised, biotechnology is safe, and the benefits deriving from biotechnological innovations will lead to major improvements in the health and well-being of the world's population. The potential risks of biotechnology are manageable, and regulations have been constructed for the management.

CONCLUSION

Historically, the applied use of biological organisms (in particular, microorganisms) has developed in an empiric manner over many years. In many ways the control of processes was seen as more an art than a science. In more recent times most of these ancient biotechnological processes have been subjected to rigorous scientific examination and analysis; of particular significance has been growth of the science of genetics. Better understanding of genetics has led to more effective application of the genomic potential of the organisms used in various industries. However, in the past 10 years, major new developments in the ability to select and manipulate genetic material (recombinant DNA technology, cell fusion, etc.) has led to interest in the creative uses of living organisms. These new genetic techniques coupled with advances in fermentation technology and downstream processing will have major economic impact on biologically based industries, agriculture and forestry throughout the world.

Over the next two decades biotechnology will have a major impact on health, pharmaceuticals, agriculture, food and the environment. The development of new drugs, human and animal vaccines, diagnostics, transgenic crops and a range of new processes to clean-up and manage the environment must bring substantial improvements in the living standards of most people.

GLOSSARY

Activated sludge process Aerobic sewage treatment process using aerobic microorganisms present in sewage sludge to break down organic matter in sewage.
Aerated pile Microbial composition of organic waste matter, where the wastes are heaped in piles and forced aeration supplies oxygen.
Aerobic Living or acting only in the presence of oxygen.
Amino acids The building blocks of proteins.
Anaerobic Microorganisms that can grow and multiply in the absence of oxygen.
Anaerobic digestion A microbial fermentation of organic matter to methane and CO_2 that occurs in near absence of air; a sewage treatment process.
Antibiotic A specific type of chemical substance that is used to fight microbial infections usually in humans or animals. Most antibiotics are produced by microorganisms. Semi-synthetic antibiotics are natural antibiotics modified chemically.
Antibody A protein produced by the immune system as a result of exposure to a specific antigen, characterised by specific reactivity with its complementary antigen.
Antigen A molecule introduced into an organism and recognised as foreign material, resulting in the elicitation of antibody production (immune response) directed specifically against the foreign molecule.
Antisense genes Genes in which the mirror image of the normal nucleotide base sequences are inserted, preventing expression of the natural genes.
Ascites Liquid accumulation in the peritoneal cavity, widely used as a method for propargating hybridoma cells for monoclonal antibody formation.
Bacteriophage A virus that multiples in bacteria.
Biolistics A method to introduce DNA into a host plant cell. It involves precipitating DNA onto microscopic particles or projectiles. The particles are then accelerated and penetrate the plant cells depositing DNA.
Biological oxygen demand (BOD) The oxygen used in meeting the metabolic needs of aerobic organisms in water containing organic compounds.
Biomass All organic matter that derives from the photosynthetic conversion of solar energy.
Bioreactor (fermenter) Containment system for fermentation purposes.
Biosensor An electronic device that uses biological molecules to detect specific compounds.
Bovine somatotropin (BST) Growth hormone that can be produced by recombinant DNA technology and used to increase the milk yield on cows.
Callus Unorganised plant cell mass capable *in vitro* of repeated cell division and growth.
Cell line Cells that acquire the ability to multiply indefinitely *in vitro*.
Cellulose Complex polysaccharide forming the cell walls of plants.
Chymosin An enzyme used to clot milk and is used in the manufacture of cheese.
Conjugation The transfer of genetic material from one cell to another by cell-to-cell contact.
Continuous fermentation A fermentation process that can run for long periods, in which raw materials are supplied and products and microorganisms are removed continuously.
Chromosomes The threads of DNA in the nucleus that carry genetic inheritance.
Clone A collection of genetically identical cells or organisms derived from a common ancestor; all members of the clone have identical genetic composition.
Complementary DNA (cDNA) DNA strand formed from messenger RNA using the enzyme reverse transcriptase.
Downstream processing Separation and purification of product(s) from a fermentation process.
DNA probes Isolated single DNA strands used to detect the presence of the complementary (opposite) strands, and used as very sensitive biological detectors.
Electroporation Transitory opening of membrane pores by electrical pulses.

Embryo transfer Implantation of embryos from donor animals or generated by *in vitro* fertilisation into the uteri of recipient animals.
Enzyme A class of proteins that control biological reactions.
Enzyme bioreactor A reactor in which a chemical conversion reaction is catalysed by an enzyme.
Fermentation The process by which microorganisms turn raw materials such as glucose into products such as alcohol.
Fibroplast Flattened connective tissue cell.
Gene A unit of heredity; a segment of DNA coding for a specific protein.
Gene transfer The use of genetic or physical manipulations to introduce foreign genes into host cell to achieve desired characteristics in progeny.
Genetic engineering Technologies, including DNA technologies, used to isolate genes from an organism, manipulate them in the laboratory and insert them into another cell system.
Genome The genetic endowment of an organism.
Haploid cell Cell with one set of chromosomes as opposed to diploid, having two sets.
Hybridoma A unique fused cell that produces quantities of a specific antibody, and reproduces endlessly.
Immobilised enzyme An enzyme that is physically defined or localised in a defined region, enabling it to be reused in a continuous process.
Ligase Enzyme used by genetic engineers to join cut ends of DNA strands.
Lignin A complex mixture of substances found in woody tissue.
Lignocellulose The composition of woody biomass, including lignin and cellulose.
Metabolite Product of biochemical activity.
Micropropagation Use of small pieces of tissue such as meristem grown in culture to produce large numbers of plants.
Monoclonal antibody Antibodies derived from a single source or clone of cells that recognises only one kind of antigen.
Mutation Stable changes of a gene inherited on reproduction.
Organoleptic An aspect of sensory perception: taste, smell, mouth-feel.
Phenotype Physical characteristics of an organism, affected by both genotype and environment.
Plasmid Loop of DNA found in bacteria and some other organisms, e.g. yeasts, that carries non-essential genes and replicates independently of the chromosomes.
Polymerase chain reaction (PCR) The action of an enzyme (polymerase) to produce many copies of a polynucleotide sequence of DNA.
Probiotics Health-giving properties derived from microorganisms.
Promoter sequence A regulatory DNA sequence that initiates the expression of a gene.
Proteins Large molecules consisting of amino acids; the products of genes.
Protein engineering Generating proteins with subtly modified structures, conferring properties such as higher catalytic specificity or thermal stability.
Protoplast Microbial or plant cell whose wall has been removed so that the cell assumes a spherical shape.
Recombinant DNA The hybrid DNA produced by joining pieces of DNA from different organisms.
Restriction enzymes Enzymes used by molecular biologists to cut through DNA from different organisms.
Restriction fragment length polymorphism (RELP) Fragments of different lengths of DNA that are produced by cutting DNA with restriction enzymes.
Single cell protein (SCP) Cells or protein extracts of microorganisms grown in large quantities for use as human or animal protein supplements.

Somaclonal variation Genetic variation produced from the culture of plant cells from a pure breeding strain.
Splicing Gene splicing, manipulation, the object of which is to attach one DNA molecule to another.
Scale-up Expansion of laboratory experiments to full-sized industrial processes.
Tissue culture A process where individual cells, or clumps of plant or animal tissue, are grown artificially. ***Transduction*** The transfer of bacterial genes from one bacterium to another by a virus (bacteriophage).
Transformation The acquisition of new genetic markets by the incorporation of added DNA.
Transgenic organism Animals, plants or microorganisms where hereditary DNA has been augmented by the addition of DNA from a source other than parental plasm.
Vectors Vehicles for transferring DNA from one cell to another.

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Textbook

Larissa V. Timoschenko

Editor I.A. Cheremisina

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