Application

of

Microbial

Biotechnology

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Preface

Microbial biotechnology focuses on exploiting the beneficial microbial wealth for various human requirements. In recent years, several advances have been made in microbial biotechnology that exerted numerous impacts on the society. The developments are very fast and new dimensions are being added every day. Thus, it has become so imperative to collect research findings and prepare a publication that can indicate research directions and interventions.

I have been collecting literature on all aspects of Biotechnology, ever since I was assigned to establish research in biotechnology at Ambo PPRC since July 1994. My inclinations became more focused on microbial biotechnology after completing my PhD in the areas of food microbial biotechnology in 2005. I got additional chances to gather more information while I was serving as Coordinator of Plant Protection and Biotechnology Research Case Team between 2008 and 2011. My efforts were further intensified in July 2011, after joining Holetta Research Center to conduct research in microbial biotechnology. After searching for references, I realized that no publication exist that covers a wider spectrum of applications in one volume; hence, motivated to benefit others. This book was written with a primary objective of briefly showing the basic global trend and indicates possible areas of interventions in Ethiopia.

Chapters 1 to 4 illustrate the applications in agriculture. The applications in medicine are described in Chapter 5. Enzymes are and their applications in agriculture, medicine, industry, and environment are described in Chapter 6. Industrial applications are described in Chapters 7to 8 and environmental management is illustrated in Chapter 9. The recent advances in molecular biotechnology, being far reaching for us, are grouped together and described in Chapter 10.

This book could not have been written without the support of Dr. Belayneh Admassu, Coordinator of National Agricultural Biotechnology Program of EIAR. I am very grateful to him for all his overwhelming encouragements. I thank my colleague, Dawit Beyene, plant biotechnology researcher at National Agricultural Biotechnology Laboratory, for facilitating Internet access and enthusiastic support that enable me thrive until the end. Several researchers
working in plant and animal biotechnology, forestry, soil microbiology and animal nutrition have participated and discussed research topics that can be addressed in EIAR. Furthermore, the soft copy of my full presentation was dispatched to all my colleagues and former-supervisors for comments and suggestions. I am, therefore, very thankful to all those replied to my call and unanimously advised me to publish it. My sincere thanks and deepest gratitude go to Abebe Kirub, Director of Information, and Communication Directorate of EIAR for his appreciation and commitment in making this book readable and useful. He recognized the potential contribution of the compilation, thoroughly edit and made it possible to reach others in need.

Melaku Alemu, 2012
Biofertilizers

NITROGEN (N) and phosphorus (P) are the master key elements in crop production. N is the most abundant element in the atmosphere (78.08%), but plants are unable to use it as such. A greater part of soil P (95-99%) is present in insoluble form; dominant in alkaline soil and unable to be utilized by the plants. To increase the availability of N and P for plants, large amounts of fertilizers are used on regular basis, but after applications, a large proportion of fertilizer N & P is quickly transferred to the insoluble form and very little is useful. Thus, chemical fertilizers are often over-applied, and end up polluting the water because they are not used up. The chemicals are less expensive in the short term, but must be continuously reapplied, and are, therefore, more expensive over long-term usage. Both nutrients can be made available through biological means. A combination of chemical fertilizers and biofertilizers gives the plants a jump-start and maintains them until the microbes can be established.

Microbial inoculant or culture is the most appropriate name for biofertilizers. Biofertilizers can be defined as preparations containing high concentrations of specialized living microorganism which increase microbial activity in the soil and are able to fix N, solubilize and mobilize P and potash, including cellulose decomposing microorganisms for rapid decomposition. Biofertilizers are totally harmless, pollution-free, low-cost, and renewable agricultural inputs. Symbiotic N fixer and phosphate solubilizing microorganisms play an important role in supplementing N and P to the plant, allowing a sustainable use. Use of these microbes as fertilizers in the field has been reported to increase crop yield.

The other important functions of soil microbes include:

- Increasing soil porosity by gluing soil particles together and aids water infiltration;
Defending plants against pathogens by outcompeting pathogens for food; and
Breaking down of leaf litter into usable nutrients by saprophytic fungi in the soil.

Inputs containing efficient strains of specific microorganisms, which are capable of mobilizing nutritive elements, are required for the plants by fixing atmospheric N, solubilizing and enhancing up take of soil P and stimulating plant growth through synthesis of growth promoting substances.

Nitrogen-fixing Microbes

Biological nitrogen fixation is the reduction of N\(_2\) (atmospheric N) to NH\(_3\) (ammonia). It is a complicated enzymatic process mediated by the enzyme nitrogenase, which is found only in prokaryotes. The conversion of N gas to ammonia introduces N into the biological N cycle. Some soil microbes release N that plants need for growth and emit gases that maintain the critical composition of the earth's atmosphere.

The N-fixing microorganisms convert N from the atmosphere into ammonium (NH\(_4\)) or nitrate (NO\(_3\)) ions. The most important biofertilizer commercially available is the rhizobial inoculant used for legume seed inoculation. It has been a practice for almost a hundred years to add commercially produced rhizobia to soil as legume inoculants to reduce the need for nitrogenous fertilizer. N-fixing bacteria are classified into the symbiotic and the non-symbiotic (free living). Symbiotic N\(_2\) fixers include *Rhizobium* and *Bradyrhizobium*, which form nodules on the roots of legumes within which the rhizobia proliferate. Leguminous plants form symbiotic associations with species of *Rhizobium*, *Bradyrhizobium*, and *Frankia* that fix atmospheric molecular N. *Rhizobium* and *Bradyrhizobium* are Gram-negative aerobes related to the pseudomonads. The actinomycete, *Frankia*, forms nodules on the roots of several types of trees and shrubs and fix N, which they provide to their host in a useful form. This allows these plants to be "pioneer plants" even in N-deficient soils.

The non-symbiotic forms include genera such as *Azotobacter*, *Azospirillum*, *Acetobacter*, *Azoarcus*, and cyanobacteria. *Azoarcus* and Cyanobacteria are associated with several grasses. The cyanobacteria, especially *Anabaena*, occur in association with the small floating water fern *Azolla*, which forms masses on the paddies. Because of the nearly obligate association of *Azolla* with *Anabaena*, paddies covered with *Azolla* remain rich in fixed N. These microbes are extensively used to enrich the cultivable lands with N thereby minimizing the use of chemical fertilizers.
Phosphate Solubilizing Microbes

The bound form of phosphate is made available by soil microorganisms like bacteria and fungi, which solubilize the bound form and make it available to the plants. Phosphate solubilizing microorganisms (PSM) include bacteria and fungi, which grow in the presence of insoluble phosphates. These not only assimilate P but also solubilize them and the P thus released can be utilized by plants. This can reduce the use of Phosphate fertilizers and can increase the yield of crop plants. Phosphate solubilizing microorganisms are capable of solubilizing Ca, Al and Fe phosphates, as well as rock phosphates and mineralizing organic P making the P present in the soil available to the crops.

The most efficient and dominant phosphates solubilizers belonging to bacterial groups are *Bacillus* species and *Pseudomonas* species as PSB, which are grown in field, adopted for a particular environmental factor and soil texture. PSB are capable of solubilizing unavailable organic and inorganic forms of P (80%). The Organic P is slowly mineralized by the action of phosphatases and inorganic P solubilized by the action of organic and inorganic acids.

Decomposers

Composting has long been used by farmers and gardeners to make soils more fertile and improve crop yields. Composting is defined as the aerobic (oxygen requiring) microbiological process in which a succession of mixed microbial populations is decomposing heterogeneous organic matter. It refers to the decomposition and stabilization of manure or other organic substrates under conditions that allow developed met of thermophilic temperature (40–65 °C) because of biologically produced heat. Compost, a mixture of soil, partially decayed plants, and sometimes manure is very rich in microorganisms. The description of the microorganisms that participate in the composting process is complex, because the populations and communities change continuously as a function of the evolution of temperature, nutrient availability, oxygen concentration, water content, and pH in the course of composting. Nature provides an extensive, native population of microorganisms that are generally attached to all organic wastes. Selected microbial inoculants can be applied to maximize the benefits. When conditions are right, these microbes grow and multiply by decomposing the material to which they are attached. When managed properly, composting improves the handling characteristics of any
organic residue by reducing its moisture content, volume, and weight. Uses of decomposers include:

- they are reliable to break down organic waste and dead organisms;
- releases key ions such as nitrates, phosphates and sulfates for use by other organisms;
- Composting relies on bacterial action; and
- Many types of bacteria participate in the composting process, thriving at different temperatures and on different materials.

The composting process increases the value of raw manures by destroying pathogens and weed seeds and creating a media for the production and proliferation of beneficial organisms. Improving the quality of reclaimed soils requires an active population of microorganisms, which can promote plant growth. Increasing the activity of microorganisms can be done by adding nutrients, making agrotechnical soil improvements and by the inoculation of beneficial microorganisms.

The concept of effective microorganism (EM) was developed by Professor Teruo Higa of University of the Ryukys in Japan in 1982. EM is a consortium of microbes consisting of 3 major groups of microorganisms—bacteria, yeast and fungi. The predominant bacteria are lactic acid bacteria: *Lactobacillus plantarum; L. casei; Streptococcus lactis*; photosynthetic bacteria: *Rhodobacter sphaeroides*, and yeasts: *Saccharomyces cerevisiae* and fermentative fungi. EM technology is found to be useful in a wide range of activities including crop production, animal husbandry, environment, and industrial waste disposal. It has been used in more than 120 countries for various purposes. EM- compost has a good texture and a pleasant odour, leads to the improvement of soil nutritional status, physical, chemical and microbiological properties, helping crops to grow healthy and strong. In Ethiopia, EM is produced by Woljeeji Agro-Industry PLC as of 2010, in Debre Zeit. EM formulations were evaluated for composing of various biomasses and found to be encouraging.

**Vesicular Arbuscular Mycorrhizae**

Vesicular Arbuscular Mycorrhiza (VAM) or mycorrhizal fungi form a bridge between the roots and the soil, gathering nutrients from the soil and giving them to the roots. VAM is a mutualistic association between fungal mycelia, plant roots and the soil. VAM help in nutrient transfer mainly of P, Zn, and S. They
also mobilize different nutrients like Cu, K, Al, Mn, Fe, and Mg from the soil to the plant roots.

Advantages of VAM include:

- Maximizing plants absorption area and can penetrate smaller crevices than root hairs;
- Improving soil texture;
- Increasing water uptake in plants;
- Increasing mineral uptake; especially P, Cu and Zn;
- Sequester heavy metals (Os, Pb); and
- Limiting uptake of Al, As, Ti, Ba and Cd.

Mixed inoculation with diazotrophic bacteria and VAM creates synergistic interactions that may result in a significant increase in growth, in the P content in plants, enhanced mycorrhizal infection, and an enhancement in the uptake of mineral nutrients. Mycorrhizae also benefit plants indirectly by enhancing the structure of the soil. Endomycorrhizal fungi hyphae excrete gluey, sugar-based compounds called glomalin, which helps bind soil particles, and make stable soil aggregates. This gives the soil structure, and improves air and water infiltration, as well as enhancing carbon and nutrient storage and water infiltration, as well as enhancing carbon and nutrient storage.

**Plant Growth Promoting Microbes**

**Rhizobacteria**

Root colonizing bacteria (rhizobacteria) that exert beneficial effects on plant development via direct or indirect mechanisms have been defined as plant growth promoting rhizobacteria (PGPR). They enhance plant growth by direct and indirect means. PGPR have been reported to enhance plant growth by a variety of mechanisms such as:

- fixation of atmospheric N that is transferred to the plant;
- production of siderophores that chelate iron and make it available to the plant root;
- solubilization of minerals such as P; and
- direct synthesis of phytohormones (auxins, cytokinins, gibberellins) that stimulate plant growth. Some strains of *P. fluorescens* produce several PGP phytohormones [auxins, e.g. indole-3-acetic acid (IAA)].
PGPR that indirectly enhance plant growth via suppression of phytopathogens do so by a variety of mechanisms. These include the ability to

- produce siderophores that chelate iron, making it unavailable to pathogens;
- synthesize anti-fungal metabolites such as antibiotics, fungal cell wall-lysing enzymes, or hydrogen cyanide, which suppress the growth of fungal pathogens;
- successfully compete with pathogens for nutrients or specific niches on the root; and
- induce systemic resistance.

Biopriming plants with some PGPB can provide systemic resistance against a broad spectrum of plant pathogens. Diseases of fungal, bacterial, and viral origin and in some instances even damage caused by insects and nematodes can be reduced after application of PGPB. Certain bacteria trigger a phenomenon known as induced systemic resistance (ISR). ISR phenotypically similar to systemic acquired resistance (SAR). SAR develops when plants successfully activate their defense mechanism in response to primary infection by a pathogen, notably when the latter induces a hypersensitive reaction through which it becomes limited in a local necrotic lesion of brown, desiccated tissue. As SAR, ISR is effective against different types of pathogens but differs from SAR in that the inducing PGPB does not cause visible symptoms on the host plant.

Biochemical and molecular approaches are providing new insight into the genetic basis of these traits, the biosynthetic pathways involved, their regulation, and importance for biological control in laboratory and field studies. Some PGPR of the genera *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Azospirillum*, *Klebsiella*, and Enterobacter have been isolated from the rhizosphere of various crops and noted for their synergistic effects on plant growth.

**Fungal Growth Regulators**

Gibberellic acid (GA) and related gibberellins are important growth regulators of plants. Commercial production of these acids helps in boosting agriculture. This acid is produced by the fungus *Gibberella fujikuroi* (imperfect state, *Fusarium moniliforme*) and can be produced commercially using aerated submerged cultures. A glucose-mineral salt medium, incubation at 25 °C and slightly acidic pH are used for fermentation. *Gibberella fujikuroi* is predominantly forming GA3, GA4, and GA7 as active GAs. Fermentations of high-yielding strains of this organism provide GAs for use in agriculture and
Endophytes

Microorganisms that reside in side the tissue of living plants and do not visibly harm the plants are known as endophytes. They live in different tissues of organs (roots, stems, leaves, flowers, fruits and seeds) of the host plants, mainly in inter- or intracellular spaces. Almost all classes of vascular plants and grasses examined to date are found to host endophytic organisms. Different groups of organisms such as fungi, bacteria, actinomycetes, and mycoplasma are reported as endophytes of plants. They have attracted increasing attention among taxonomists, ecologists, agronomists, chemists and evolutionary biologists. They produce an impressive array of secondary metabolites exhibiting a wide variety of biological activity, including plant growth promotion and biological control agents.

Plant Growth Promoting Endophytes

Endophytes are able to promote plant growth through many ways. These include phosphate solubilization activity, indole acetic acid production and the production of a siderophore and enzymes. Endophytic bacteria are also able to supply essential vitamins to plants, along with osmotic adjustment, stomatal regulation, modification of root morphology enhanced uptake of minerals along with alteration of nitrogen accumulation.

Role of Endophytes in Abiotic Stress Tolerance

Many evidence shows that these organisms can also accelerate seedling emergence and promote plant establishment under adverse conditions. Some endophytic fungi are able to protect their host plant from drought conditions and increase salt and heat tolerance. So it can be said that endophytes acts as biological trigger to activate the stress response more rapidly and strongly than non symbiotic plants. Mutualism interaction between endophytes and host plants may result in fitness benefits for both partners. The endophytes may provide protection and survival conditions to their host plant by producing a plethora of substances, which, once isolated and characterized, may also have potential for use in industry, agriculture, and medicine.

Role of Endophytes in Plant Protection
Endophytic bacteria colonize the internal tissues of the plant showing no external sign of infection or negative effect on their plant host. Recently, endophytes are viewed as outstanding source of secondary metabolites having antimicrobial activities. These microorganisms received considerable attention in last 20 years when their capacity to protect against insect and pest pathogens was noticed. Several reports have shown that endophytic microorganisms can have the ability to control plant pathogens and insect.

Endophytes have been shown to check progress of disease through endophyte mediated de novo synthesis of original compounds and antifungal metabolites. They represent a promising source of biocontrol agents and their use may be protective to crops and plants from microbial infection and herbivores. Endophytic fungi and bacteria are known to be rich sources of novel antimicrobial substances. The endophyte-associated plants produce some metabolites that induce resistance. Symbiotic plant activates defense system more quickly than non-symbiotic plants after pathogen challenge. The endophytes, which provide indirect defense against herbivores, may have come from a number of origins, including mutualistic root endophyte associations and the evolution of entomopathogenic fungi into plant-associated endophytes. The entomopathogenic view has gained support from observations of increased fungal growth in response to induced plant defenses and colonization of plant tissues.

Biofertilizers have been effectively utilized to improve the production and productivity of legume crops and soybean. There are many effective rhizobium isolates preserved, promoted, and even demonstrated during annual field days. Although few exotic biofertilizers were evaluated, there were found to be comparable but not superior to the indigenous/local isolates, meaning that there are many opportunities to expand the collections of effective/efficient biofertilizers but need to be further corroborated with molecular characterization so that reliable routine methods can be developed to obtain alternative new isolates.

Manure is solely depends on natural/spontaneous form. The use of superior inoculants needs to be exercised to improve the advantages of the compost.

There are only few reports on the existence of plant growth promoting bacteria (*Pseudomonas flourescens*) and thus require further investigation to exploit the potential.
Biopesticides

BIOLOGICAL CONTROL refers the deliberate use of natural living organisms (animals, plants, microorganisms) to suppress, reduce, or eradicate a pest population (pathogens, insect pests, and weeds). Biological control is considered as a potential cost-effective, safe, and environmentally beneficial means for reducing pest populations in crops, forests, or rangelands where low profit margins prevent large pesticide expenditure. Biological control agents (biopesticides) are effective and often quickly biodegradable and thus present no residue, inherently less harmful than conventional pesticides and mostly self-perpetuating.

Microbial pesticides consist of a naturally occurring microorganism; for example, a bacterium, fungus, virus, or protozoan as the active ingredient. These biopesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest(s). Some of the mechanisms of microbial biological control include:

- Antibiosis: producing a toxin specific to the pest;
- Suppression and causing a disease agent transfer;
- Direct parasitism, induced resistance; and
- Preventing establishment of other microorganisms through competition.

Among these, antibiosis is the most common mechanism defined as the antagonism brought about by the antimicrobial secondary metabolites or antibiotic-like substances, lytic enzymes, volatile compounds, siderophores or other toxic substances.

Some of the advantages of using biopesticides in reducing pesticides risks are:
Biopesticides are best alternatives to conventional pesticides and usually inherently less toxic or harmful than conventional pesticides;

Biopesticides generally affect only the target pest and closely related organisms in contrast to a broad spectrum of conventional pesticides that may affect organisms like birds, insects, and mammals. Biopesticides often have a narrow spectrum of pest activity, which means they have a relatively low direct impact on non-targets, including humans. Their use is often compatible with other control agents;

Biopesticides are often effective in very small quantities and decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides;

They can greatly decrease the use of conventional pesticides while crop yields remain high when they are used as fundamental components of IPM programs;

amenable to small-scale, local production in developing countries and products available in small niche markets that are typically unaddressed by large agrochemical companies; and

unlike most other tactics, biopesticides do not always have to be reapplied each time a pest outbreak occurs. Once natural enemies are released into a new environment, there is a good chance they will become established and provide a self-perpetuating form of control. In many cases, microbial insecticides are better than conventional insecticides because they suppress pest populations without eliminating natural populations of predators and parasites.

Several microbial biopesticides are commercialized and widely used to enhance agricultural productivity in many parts of the world. These are categorized as antimicrobials (to control pathogens), entomopathogens (to control insect pests), and mycoherbicides (to control weeds).

**Antimicrobials**

These inclue the following major categories:

- **Antibacterial agents**: *Bacillus subtilis, Pseudomonas fluorescens, Agrobacterium radiobacter*
- **Antifungal agents**: *Trichoderma spp., Psuedomonas fluorescens, Ampelomyces quisqualis, Gliocladium oleophila, Streptomyces spp., EM;*
- **Microbial parasites of root knot nematodes**: *Paecilomyces lilacinus, Trichoderma asperellum;*
Microbial Insecticides

- Entomopathogenic fungi: Beauveria bassiana, Metarhizium anisopliae, Verticilium lecani, Paecilomyces lilacinus;
- Entomopathogenic bacteria: Bacillus thuringiensis (Bt)
- Entomopathogenic viruses: Baculoviruses [Nuclear Polyhedrosis Viruses (NPV), Granuloviruses (GV)];
- Entomopathogenic nematodes: 2 genera—Steinernematidae and Heterorhabditidae

Weed Controlling Microbes

Mycoherbicides: Fusarium oxysporum and Fusarium arthrosporioides for the control of root parasitic weeds such as striga (Striga hermonthica) and Broomrape species (Orobanche species).

Research on microbial biopesticides have been conducted during the past two decades and resulted in some isolates, particularly Trichoderma species and entomopathogenic fungi (Beauveria bassiana, Metarhizium anisopliae). The mass production techniques for these biopesticides are well established at Ambo Research Center. However, these biopesticides have not been widely used in the country for various reasons. Molecular techniques are not employed to characterize these isolates and to obtain other additional potential superior isolates.

Exotic biopesticides have gained wider applications in the floriculture industry. Since 2007, the flower farms in Ethiopia have started to show their interest to import biopesticides for the control of pathogens and insect-pests. Consequently, several exotic predators/parasitoids and antimicrobials were tested and most of them were found to be effective for use in IPM. Some of the imported and effective microbial pesticides in Ethiopia are:

- Agrobacterium radiobacter, trade name Dygal, used for the control of crown gall (Agrobacterium tumafaciens) in flower farms;
- Trichoderma viride, trade name Nisarga, for the control of gray mold (Botrytis cinerea) in flowers
- Trichoderma viride, trade name Sanjeevnii, used for the control of damping off and wilt (Fusarium, Rhizoctonia and Pythium spp.) of tomato;
- Bacillus subtilis BS 01, trade name Real Bacillus, for the control of powdery mildew in pepper;
• **Beauveria bassiana**, trade name BotaniGard, white fly and thrips in flower farms;
• **Beauveria bassiana**, trade name Daman, for the control of diamondback moth of cabbage;
• **Metarhizium anisopliae**, trade name Kalichakra, for the control of termites, and
• **Verticillium lecanii**, trade name Verfire-L, for the control of aphids of pea.

These microbial biopesticides boosted the flower production. Accordingly, the Ministry of Agriculture has granted large-scale import permits for these biopesticides.
Fermented Foods and Beverages

The use of microorganisms to produce fermented foods and beverages has a very long history. Fermentation has been used for several thousand years as an effective and low-cost means to preserve the quality and safety of foods. In many developing countries, fermented foods are produced primarily using household methods based on spontaneous fermentations, and are carried out by microbes associated with raw food materials or back-slopping process, which makes use of samples of a previous batch of a fermented product as inoculants. Major limitations of spontaneous fermentation processes include inefficiency, low yields of product and variable product quality. The art of traditional processes needs to be transformed into a technology to incorporate objective methods of process control and optimization, and to standardize quality of the products without losing their desirable attributes. Fermentations can only be optimized when conditions like time, temperature, pH, substrate retreatment, inoculum-substrate ratio are controlled.

Microbe inoculants containing high concentrations of live microorganisms, referred to as starter cultures, are used to initiate and accelerate the rate of fermentation processes in a controlled manner. Biotechnology as applied to food bioprocessing makes use of microbial inoculants or starter cultures to enhance properties such as the taste, aroma, shelf life, safety, texture, and nutritional value of foods. Starter culture development and improvement is the subject of much research both in developed and in developing countries. This requires the isolation, characterization, and preservation of various microorganisms involved in each fermentation process. The role of each microorganism is identified and the best performing ones will be chosen and developed as starter cultures.

Fermentation is the slow decomposition process of organic substances induced by microorganisms, or by complex nitrogenous substances (enzymes) of plant...
or animal origin. In addition to processing foodstuffs, microbial fermentations can also result in the production of various products. There are 5 major application groups of commercially important microbial fermentation:

- fermented foods and beverages, microbial cells or biomass as the product; for example, single cell protein, baker’s yeast;
- microbial metabolites
- primary metabolites: ethanol, citric acid, glutamic acid, lysine, vitamins, polysaccharides and
- secondary metabolites: all antibiotic fermentation;
- microbial enzymes: catalase, amylase, protease, pectinase, glucose isomerase, cellulase, hemicellulase, lipase, lactase, streptokinase;
- recombinant pharmaceuticals: insulin, HBV, interferon, GCSF, streptokinase; and
- biotransformations or modification of compound, which is added to the fermentation process such as phenyl acetyl carbinol, and steroid biotransformation

Fermented foods are produced worldwide using various manufacturing techniques, raw materials, and mixed cultures of microorganisms that grow simultaneously or in succession. Animal and plant tissues subjected to the action of microorganisms and/or enzymes to give desirable biochemical changes and significant modification of food quality are referred to as fermented food.

The benefits of fermentation may include improvement in palatability and acceptability through:

- enrichment of the diet through development of a diversity of flavors, aromas, taste and textures in food substrates;
- preservation of substantial amounts of food through formation of acidulants, alcohol, and antibacterial compounds like lactic acid, alcoholic, acetic acid, bacteriocins and alkaline fermentations to make the product safe from pathogenic microorganisms;
- enrichment and enhancement of nutritive content of food substrates by microbial synthesis of essential nutrients like protein, essential amino acids, essential fatty acids, and vitamins;
- improvement of digestibility of protein and carbohydrates;
- detoxification during food fermentation, i.e. destroying undesirable components, removal of antinutrients, natural toxicants and mycotoxins;
- decrease of cooking times and fuel requirements.

Among the biopreservative agents, bacteriocins (proteinaceous antimicrobial compounds), have been the subject of intensive research for their diversified
use in food biopreservation and other purposes. Nisin is one of the commercialized bacteriocins used for a variety of purposes.

Fermented foods, whether from plant or animal origin, are intricate parts of the diet of people in all parts of the world. Biotechnology applications in the food-processing sector, therefore, target the selection and manipulation of microorganisms to improve process control, product quality, safety, consistency, and yield, while increasing process efficiency.

**Plant-Based Fermented Foods and Beverages**

Fermented plant products are among the most important sources of dietary proteins, carbohydrates, vitamins, minerals and fiber for many people in the developing world. Fermented plant foods may be classified into groups as

- those made from cereal grains like maize, sorghum, millet, rice and wheat;
- those made from pulses and nuts;
- those made from tubers like cassava, aroids, potatoes;
- those from fruits and vegetables; and
- beverages derived from tree saps. For simplicity, these can be re-categorized into three groups as cereal-, pulse- and legume-based; horticultural crops based, and fermented beverages.

**Cereal, Pulse, and Legume-based Fermented Foods**

Fermented foods contribute to about one-third of the diet worldwide and cereals are particularly important substrates for fermented foods in all parts of the world. The content and quality of cereal proteins may be improved by fermentation. Bacterial fermentations involving proteolytic activity are expected to increase the biological availability of essential amino acids more than yeast fermentations, which mainly degrade carbohydrates. Starch and fiber tend to decrease during fermentation of cereals. Cereal crops are important sources of dietary protein, carbohydrates, the B complex of vitamins, vitamin E, iron, trace minerals, and fiber. Cereals have a variety of uses as food. A variety of unique, indigenous fermented foods, other than leavened breads and alcoholic beverages, are also produced in regions of the world that rely mainly on plant sources of protein and calories. Cereals consist of 12-14% water, 65-75% carbohydrates, 2-6% lipids, and 7-12% protein. Cereals are quite similar in gross composition, being low in protein and high in carbohydrates. Together with oilseeds and legumes, cereals supply a majority of the dietary protein, calories, vitamins, and minerals to the bulk of populations in developing nations. Many of the indigenous fermentation products of cereals are valued for
the taste and aroma active components produced, and used as seasonings and condiments. A number of fermented products utilize cereals in combination with legumes, thus improving the overall protein quality of the fermented product. Cereals are deficient in lysine, but are rich in cystine and methionine. Legumes, on the other hand, are rich in lysine but deficient in sulfur containing amino acids. Thus, by combining cereals with legumes, the overall protein quality can be improved.

**Horticulture-based Fermented Foods**

Fermenting fruits and vegetables can bring many benefits to people in developing countries. Fermented foods play an important role in providing food security, enhancing livelihoods, and improving the nutrition and social wellbeing of millions of people around the world, particularly in the marginalized and vulnerable.

**Improving food security**

- Food preservation: when fruit and vegetables are harvested, they undergo rapid deterioration, especially in the humid tropics where the prevailing environmental conditions accelerate the process of decomposition. There are several options for preserving fresh fruit and vegetables, which include drying, freezing, canning, and pickling. However many of these are inappropriate for use on the small-scale in developing countries;
- Salvaging waste foods: fermentation can salvage waste food which otherwise would not be usable as food by changing the consistency of the product and making it digestible. This increases the range of raw materials available as food;
- Removal of anti-nutritional factors: many fruits and vegetables contain naturally occurring toxins and anti-nutritional compounds. These can be removed or detoxified by the action of microorganisms during fermentation; and
- Removal of cyanide of cassava through fermentation: the naturally occurring chemical in cassava, cyanogenic glucoside releases cyanide into the body, which can be fatal. Fermentation process can remove this chemical. At the beginning of the fermentation, *Geotrichum candida* acts on the cassava that makes the product acidic, which finally kills off the microorganisms, as they cannot exist in such medium. A second strain of microorganisms (*Cornibacterium lactii*), which can tolerate the acidic environment, then takes over and by the third day, 90-95% of the dangerous chemical will have been hydrolyzed. The cassava also develops its characteristic flavor.

**Improving nutrition**

The optimum health and nutrition of individuals depends on a regular supply of food and balanced diet. When diets are sub-optimal, the individual's capacity for work and achievements are greatly reduced. Availability of food, dietary
restrictions and taboos, misconceptions, limited time available for feeding or eating compound to create a group of individuals who are nutritionally disadvantaged.

- Vitamins: fermentation processes can result in increased levels of vitamins in the final product. *Saccharomyces cerevisiae* is able to concentrate large quantities of thiamin, nicotinic acid and biotin and thus form enriched products
- Digestibility: microorganisms contain certain enzymes such as cellulases, which are incapable of being synthesized by humans. Microbial cellulases hydrolyze cellulose into sugars, which are then readily digestible by humans. Similarly, pectinases soften the texture of foods and liberates sugars for digestion. Fermented foods are often more easily digestible than unfermented foods.

**Medicinal benefits**
There are many traditional beliefs about the medicinal properties of fermented food products.

**Improving cultural and social wellbeing**
Fermentation improves flavor and appearance of foods. One important area is the creation of meat-like flavor. The strong flavors of fermented food products can enhance a dull diet. Fermented vegetables are used as condiments to enhance the overall flavor of the meal.

**Fermented Beverages**

**Alcoholic beverages**
Alcoholic fermentation is conducted by yeast of the genus *Saccharomyces* that converts the glucose, fructose, and sucrose found in grape must and juice into ethanol via fermentation. These products of alcoholic fermentations originated in spontaneous fermentation processes are of great antiquity. However, it is only in recent years that modern methods of industrial microbiology have been applied to their manufacture. In beverage production, refinements are introduced with respect to flavor, aroma, color, and sanitation that are not necessary in the making of industrial alcohol. The type of beverage produced is determined by the nature of the plant material employed for fermentation. In all these processes, the method of preparing the fermentation medium is a factor of prime importance.

**Beer manufacturing**
Beer is made by the yeast fermentation of grains to ethanal and carbon dioxide. There are five major steps in the manufacture of beer from grain. These are
malting, mashing, fermenting, maturing, and finishing. Malting and mashing are concerned with the conversion of starch into fermentable form such as maltose or glucose. The chief raw material is malt, which is germinated barley that has been dried and ground. It contains starch, proteins, and high concentration of amylases and proteinases. Amylases convert the starch into fermentable sugar. Mould amylase derived from Aspergillus oryzae is sometimes used for the same purpose. Ground malt is mashed in warm water to bring about the digestion of starch and proteins.

Wine manufacturing
Wine is a product made by the normal alcoholic fermentation of the juice of sound, ripe grapes and the usual cellar treatment. Beverages produced by the alcoholic fermentation of other fruits and certain vegetable products are also called wines—for example, peach wine, orange wine, cherry wine. Wine can be made by a direct fermentation of sugars, i.e. glucose and fructose, instead of starch, which requires hydrolysis to yield sugars. Many fruits have the wine yeast Saccharomyces cerevisiae var. elliposideus on them. All that necessity is to crush the fruits. An, alcoholic fermentation starts spontaneously.

Animal Product-based Fermented Foods

Fermented Milk Products

Raw milk
The role of milk in nature is to nourish and provide immunological protection for the mammalian young. Milk and dairy products are generally very rich in nutrients that provide an ideal growth environment for many microorganisms. Most of the bacteria in fresh milk from healthy animals are harmless. However, rapid changes in the health of a milk animal or of the dairy farmer, or contaminants can make raw milk potentially dangerous if these factors introduce harmful bacteria into the milk. Moreover, since milk is nutritionally complete, it provides an excellent medium for growth of bacteria if it is mishandled. Pasteurization is a moderate but exact heat treatment of milk that kills bacteria that cause disease. Pasteurization and careful packaging in clean, sanitized containers help retard spoilage of milk so it lasts longer after it is purchased. Pasteurization does not completely sterilize milk and milk that is not properly handled can become re-contaminated after the heat treatment.
Fermented milk

Fermented milk can be stored for about 20 days compared with less than one day for fresh milk. Fermentation is the oldest means of preserving milk. Originally, unpasteurized milk was left to ferment naturally, and fermentation involved microorganisms present in the raw milk and surrounding air. With the development of modern technologies, specific lactic acid producing microorganisms are now introduced to carry out fermentations under controlled conditions. In this way, fermented products of superior nutritional, physical, chemical, and sanitary qualities are produced. Fermented milks are characterized by the accumulation of microbial metabolic products such as lactic acid, ethyl alcohol, and dozens of other chemicals collectively called flavor substances.

Starter cultures

The natural microflora of the milk is inefficient, uncontrollable, and unpredictable, or is destroyed altogether by the heat treatments given to the milk. A starter culture can provide particular characteristics in a more controlled and predictable fermentation. Commercial manufacturers provide starter cultures in lyophilized (freeze-dried), frozen or spray-dried forms. Lactic acid bacteria are often called dairy starter cultures, which are used for the production of various fermented milk products. Dairy starter cultures are carefully selected microorganisms deliberately added to milk to initiate and carry out desired fermentation under controlled conditions in the production of fermented milk products. Most of them belong to lactic acid bacteria (LAB) (Lactococcus, Lactobacillus, Streptococcus, and Leuconostoc). In some cases, few non-lactic starters (bacteria yeast and mold) are also used along with LAB.

Starter cultures can be used as single strain, mixed strain and multiple strains depending on the type of products to be prepared. The ability of starter culture to perform its functions efficiently during manufacture of fermented dairy foods depends primarily on purity and activity of starter cultures. The major roles of starter culture during fermentation of milk are:

- production of primarily lactic acid and few other organic acids, such as formic acid and acetic acid;
- coagulation of milk and changes in body and texture in final products;
- production of flavoring/aroma compounds such as diacetyl, acetoin and acetaldehyde;
- help in ripening of cheeses through proteolytic and lipolytic enzyme activities;
• produce antibacterial substances in the finished product; inhibition of undesirable organisms; and
• additional functional properties.

The main functions of the specific defined starters consist of providing adequate acidification and producing compounds that contribute to the development of sensory properties. Certain starter organisms are added specifically for the production of flavor compounds such as diacetyl, though the production of lactic acid and other compounds also contributes to flavor. Starters play a vital part in the manufacture of these products; they produce the lactic acid that influences important quality characteristics such as texture, moisture content, freedom from pathogenic microorganisms and their toxins, and taste.

Milk is composed of the following constituents

• **sugars**: lactose is a disaccharide when hydrolyzed by β D-galactosidase (lactase); the result is increased sweetness, most important functions being its utilization as a fermentation substrate;
• **proteins**: caseins and whey proteins;
• **enzymes**: consist of heat-stable enzymes produced by psychrotrophic bacteria: lipases, and proteinases;
• **minerals**: all the 22 minerals essential to the human diet;

**Yoghurt**

It is obtained by lactic acid fermentation of milk by the action of starter bacteria and is the most popular product throughout the world. It can be kept for up to ten days under refrigerated storage. Fermented milk products represent a rich source of nutrients and may improve lactose digestion through the breaking down of lactose into glucose and galactose by lactase. During the milk transformation in yogurt, pantothenic acid and vitamin B12 decrease, while other vitamins such as folic acid and niacin increase. Lactic microflora carries out a weak proteolytic activity, which causes the lysis of 1–2% of the casein and liberation of amino acid and peptide. Some of these compounds are metabolized by the microorganisms, while others are accumulated in yogurt. In particular, in comparison with milk, smaller amounts of methionine, lysine, threonine, valine, and thyroxine and larger amounts of free amino acids are present. In contrast, triglyceride lipolysis is negligible because of the absence of lipase in microorganisms contaminating the milk. Not only do fermented milks contain such nutrients as carbohydrates, fats, proteins, minerals and vitamins.
that allow for growth, development, and tissue differentiation, but they also contain growth factors, hormones like gastrin, insulin, IGF-I and IGF-II; and molecules with immune-stimulating effects that may have a variety of outcomes.

Fermented milk products also help enhance the immune system with modulation of the cellular immune response through bioactive peptides, whose activity may extend beyond the immune functions by some still unclear mechanisms. There is now proof that fermentation products, fermented milks, and probiotics used for fermentative purposes may all contribute to health benefits, but clear study designs are needed to clarify roles and specific domains for these activities. In the meantime, the role of fermented milk products in feeding infants and children should be carefully considered, starting at weaning.

**Butter**

To make butter, milk or cream is agitated vigorously at a temperature at which the milk fat is partly solid and liquid. Churning efficiency is measured in terms of the time required to produce butter granules and by the loss of fat in the buttermilk. Efficiency is markedly influenced by churning temperature and by the acidity of the milk or cream. As agitation continues, the whipped cream becomes coarser and eventually the fat forms semi-solid butter granules that rapidly increase in size and separate sharply from the liquid buttermilk. The organoleptic quality of butter can be described as the customer’s reaction to its color, texture, and flavor.

**Cheese**

The production of cheese from milk is a very ancient process. Cheese is made by adding acid or starter culture to milk, causing the sugar in the milk to ferment. Curdling (coagulating) milk causes it to separate into semi-solid curds and liquid whey. Cheese is nutritious as it is an excellent source of high quality proteins, dietary calcium, essential fatty acids, and other minerals such as Ca, Fe, and P, vitamins and essential amino acids and therefore is an important food in the diet of both young and old people. Cheese is a concentrate of the milk constituents, mainly fat, casein, and insoluble salts, together with water in which small amounts of soluble salts, lactose, and albumin are found.

Milk is coagulated by direct acidification, by LAB, by adding coagulants or a combination of acidification and addition of coagulants. One of the best-known coagulants and one that is used widely by cheese makers for many varieties of cheese is rennet. More than 2,000 varieties of cheeses are produced around the
The individual characteristics of each cheese variety are due to the type of milk, the microbial starter culture, and the make procedure used. The starter cultures contributed to acidity and microbial quality of the cheese. Cheese ripening is a complex process mediated by biochemical and biophysical changes during which a bland curd is developed into a mature cheese with characteristic flavor, texture, and aroma. The desirable attributes are produced by the partial and gradual breakdown of carbohydrates, lipids, and proteins during ripening, mediated by several agents including the following:

- residual coagulants;
- starter bacteria and their enzymes;
- nonstarter bacteria and their enzymes;
- indigenous milk enzymes, especially proteinases; and
- secondary inocula with their enzymes.

Proteolysis occurs in all the cheese varieties and is a prerequisite for characteristic flavor development that can be regulated by proper use of the above agents. Cheese ripening is essentially an enzymatic process, which can be accelerated by augmenting activity of the key enzymes.

**Whey**

Whey contains valuable nutrients, i.e., whey proteins, carbohydrate, and minerals. Wheys from cheese making vary according to the type of cheese made and, therefore, the content of protein, salts and lactose also vary. As whey contains about half of the total solids in the original milk, it should not be thrown away as waste but should be used as animal feed or for human nutrition.

Exopolysaccharides (EPS) produced by some lactic acid bacteria give the possibility to obtain high quality fermented milk products with desired rheological properties and various health benefits. EPS can efficiently replace commercial stabilizers for preventing or reducing syneresis, providing fermented milk products with suitable structure viscosity. The effect of EPS on food quality characteristics depends on the EPS properties themselves, as well as their interaction with various components of the food system. The influence of EPS on fermented products properties depend not only on the biopolymers properties, but also on their interaction with various components (e.g. protein) of the food system. Current research is focused on improving the production of EPS with specific structure and size to achieve the desired functionality.

The fermentation process of dairy products and its bacteria has received great attention over the decades after the discovery of the importance of viable
bacteria in food for health. More recently, probiotic cultures are finding their way into cultured milk products. These cultures that some have claimed health benefits for those consume them, i.e. better digestion, anticancer compounds, and prevention of heart disease. Probiotic cultures may be added as adjuncts or they may be directly involved in the fermentation process. Fermented milks are one of the oldest medical sciences and widely used for therapeutic benefits. Therapeutic activity of probiotic bacteria can be due to competition with pathogens for nutrients and mucosal adherence, production of antimicrobial substances, and modulation of mucosal immune functions.

Varieties and qualities of dairy products are dependent on the type of starter cultures used and the efficiencies of various processing techniques, which are determined by comparing the microbial, physicochemical, and sensory properties/characteristics.

- microbiological analysis (cfu/g): total aerobic content, (cfu/g), enumeration of lactic acid bacteria, enterobacteriacea, coliforms, mould and yeast;
- physicochemical properties: ripening period (days), total solid (%), fat (%), fat in total solid (%), ash (%), salt (%), salt in total solid (%), acidity (%), pH, protein (%); and
- sensory properties: color, texture, taste and aroma, strange taste and aroma, salt content and general acceptance.

Various molecular methods have been effectively used for routine detection and characterization of starter cultures. This has enhanced the efforts of improving the quality and variety of dairy products as well as wider alternative strains for use as probiotics.

**Sausage**

The preservation of meat by fermentation has been used for thousands of years. The shelf life was based on natural meat LAB, traces of nitrate and high amount of salt. Fermentation traditionally offers an easy and low-energy preservation method for meats that result in distinctive products that have an important part in the diet of people making them. Such fermented meats contribute both nutritional value and pleasure to meals. Fermented sausages are produced generally as dry or semidry products, although some are intermediate.

In their preparation, curing and seasonings are added to ground meat, followed by its stuffing into casings and incubation for varying periods at selected temperature. Dry sausage material is made from a mixture of pork and beef and pork. The curing mixture contains sugars, salt, nitrite and/or nitrate, ascorbates
and spices. Sugars are added for fermenting substrates for starter LAB and starter staphylococci and nitrates and/or nitrites as color stabilizers. Salt acts as one of the first hurdles against the growth of unwanted microorganisms. It also induces the solubilization and diffusion of myofibrillar proteins from muscle forming a gel texture between meat and meat as well as meat and fat particles of the raw sausage material. Some of the flavor is generated by meat endogenous enzymes and microorganisms via glycolysis, proteolysis, lipolysis, and lipid oxidation. The chemical and enzymatic reactions during dry sausage fermentation and ripening process degrade proteins into peptides, dipeptides, and amino acids and lipids into fatty acids. The amino acids contribute to the overall flavor of dry sausages. Amino acids are further decarboxylated into biogenic amines or aroma compounds, and fatty acids are oxidated into aldehydes, alkanes, alcohols, and ketones. These volatile compounds contribute to the flavor of dry sausage.

**Functional Foods and Nutraceuticals**

Functional foods are foods that have health promoting benefits over and above their basic nutritional value. Functional foods can be defined as foods that contain one or more added ingredients to provide a positive health benefit, over and above the normal functions of food to provide nutrients, satisfy physiological and psychological hunger and provide pleasure from eating. This definition excludes vitamins and minerals added to foods to replace losses in manufacture. Functional foods are considered as any food or food component that may provide demonstrated physiological benefits or reduce the risk of chronic diseases, beyond basic nutritional functions. Most of the functional foods are derived from dairy- and meat-based products that are good sources of valuable bioactive ingredients such as bioactive peptides with antimicrobial or antioxidant activity.

The term *nutraceuticals* was coined from *nutrition* and *pharmaceutical*. Functional foods that provide health benefits are also commonly referred to as nutraceuticals, which represents foods with medical health benefit, including the prevention and treatment of disease. While all foods are functional in that they provide nutrients, nutraceuticals contain health-promoting ingredients or natural components that have a potential health benefit for the body. When a functional food aids in the prevention and/or treatment of disease(s) and/or disorder(s) (except anemia), it is called a nutraceutical. The proposed definition can help form distinction between functional foods, nutraceuticals, and dietary supplements. Nutraceuticals include antioxidant and antimicrobial peptides.
from milk and galactooligosaccharides (GOS) from whey produced through microbial or enzymatic hydrolysis. The composition of nutraceuticals includes fatty acids, phenolics, flavonoids, carotenoids, ascorbic acid, and tocopherols. The global functional food and nutraceutical market is growing at a rate that is outpacing the traditional processed food market. Thus, nutraceuticals differ from dietary supplements in the following aspects:

- nutraceuticals must not only supplement the diet but should also aid in the prevention and/or treatment of disease and/or disorder and
- nutraceuticals are used as conventional foods or as sole items of a meal or diet. Dietary components play beneficial roles beyond basic nutrition, leading to the development of the functional food concept and nutraceuticals.

A functional food for one consumer can act as a nutraceutical for another consumer. Examples of nutraceuticals include fortified dairy products and fruits such as orange juice. Several naturally derived food substances have been studied in cancer therapies. Vitamin E, selenium, vitamin D, green tea, soy, and lycopene are examples of nutraceuticals widely studied in human health.

**Probiotics**

Probiotics are live microbial food ingredients that have a beneficial effect in human health. Probiotics are defined as live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host. They are non-pathogenic microorganisms that, when ingested in certain numbers exert a positive influence on the host physiology and health beyond inherent general nutrition. Lactic acid bacteria, which are found commonly as resident microflora of the gastro-intestinal and genitor-urinary tract of vertebrates, are considered as the major probiotic organisms. Most often, the bacteria come from two groups, *Lactobacillus* or *Bifidobacterium*. These bacteria help maintain a healthy digestive system and may strengthen the immune system. Mechanisms of action of probiotics include:

- competitive exclusion of pathogen binding or adherence and colonization of the host gut;
- production of antimicrobial substances such as bacteriocins;
- suppression of growth of pathogenic bacteria through competition for nutrients; and
- improvement of intestinal barrier function, and stimulation of host immunity.

The health benefits obtained through consumption of foods containing probiotic bacteria are well documented, and more than 90 probiotic products are available worldwide.
Prebiotics
These are food substances that are mostly dietary fibers and non-digestible carbohydrates like inulin, fructooligosaccharides, polydextrose, arabinogalactan, lactulose, and lactitol, which selectively stimulate and promote the growth of beneficial microorganisms such as probiotic bacteria, living inside the gut. When probiotics and prebiotics are mixed together, they form a synbiotic.

Synbiotics
They are products that contain both probiotics and prebiotics. These products have the ‘good’ bacteria (probiotics) and the non-digestible carbohydrate source (prebiotics) to encourage the growth of other beneficial bacteria. Fermented dairy products such as yogurt are synbiotic because they contain live bacteria and the food source needed for them to thrive. Without its food source, a probiotic would have a difficult time surviving in the digestive system because it cannot tolerate oxygen, low pH, and temperature. The most common synbiotic combinations available include Bifidobacteria and fructooligosaccharides (FOS), Lactobacillus GG and inulins, and Bifidobacteria and Lactobacilli with FOS or inulins.

Some of the potential health benefits of probiotics are:

- **Alleviation of lactose intolerance symptoms**: Individuals with low levels of the intestinal enzyme lactase (i.e., lactose maldigesters) have a limited ability to digest lactose (milk sugar), which can result in GI symptoms or lactose intolerance. Studies demonstrate that intake of yogurt and some probiotics can improve lactose digestion and alleviate symptoms of intolerance;

- **Anti-diarrheal effects**: The role of probiotics in the prevention and treatment of a variety of diarrheal illnesses such as acute diarrhea caused by rotavirus infections, antibiotic-associated diarrhea, and travelers’ diarrhea have been reported;

- **Protection against infections**: Help prevent or treat infections such as postoperative infections, respiratory infections, and the growth of Helicobacter pylori, a bacterial pathogen responsible for type B gastritis, peptic ulcers, and perhaps stomach cancer;

- **Reduced risk of colon cancer**: Some evidences, primarily from in vitro and experimental animal studies, indicate that probiotics may have the potential to reduce colon cancer risk. In experimental animals, intake of yogurt and specific probiotic cultures has been shown to reduce the development of precancerous lesions (aberrant crypts) and chemically induced tumors, although the findings appear to be both species- and strain-dependent;

- **Immune enhancement**: Yogurt and probiotics such as lactobacilli and bifidobacteria stimulate certain cellular and antibody functions of the immune system, which in turn may increase resistance to immune-related diseases for example, infections, GI disorders, cancer, allergies). Secretory immunoglobulin A,
which helps protect against microbial antigens at the intestinal mucosal surface, has been shown to be increased in mice fed *L. acidophilus*, *L. casei* or yogurt, and in humans consuming fermented milk;

- **Anti-inflammatory effects:** Because probiotics can influence the intestinal flora, they may have beneficial effects for patients with inflammatory bowel disease (IBD), which includes ulcerative colitis, Crohn’s disease, and pouchitis. Specific probiotic bacteria may alleviate or reduce symptoms of IBD. Intake of fermented milk for one year helped patients with ulcerative colitis maintain remission and had possible preventive effects, according to a randomized clinical trial;

- **Reduced symptoms of irritable bowel syndrome:** Probiotics may reduce symptoms of irritable bowel syndrome (IBS). A randomized controlled clinical trial in 25 patients with diarrhea-predominant IBS showed that intake of a probiotic formula twice daily for 8 weeks reduced abdominal bloating;

- **Prevention of allergic reactions:** Probiotics may help prevent allergic reactions in individuals at high risk of allergies, such as food allergies. Results of a randomized double-blind, placebo-controlled study demonstrated that administration of *L. rhamnosus* GG to pregnant mothers 2 to 4 weeks prior to delivery and to their newborn babies through 6 months of age led to a 50% decrease in the infants’ incidence of recurring atopic eczema, i.e., an indicator of food allergy later in childhood; and

- **Other potential health benefits:** Some experimental animal and human investigations suggest that probiotics may reduce the risk of heart disease by their beneficial effects on blood lipid levels and blood pressure. Different strains of lactobacilli and fermented milk products containing probiotic bacteria may help prevent and treat urinary tract infections, bacterial vaginosis, and yeast vaginitis in women. Probiotics may also help relieve constipation, reduce colic in infants, alleviate kidney stones, decrease inflammation associated with arthritis, and protect against dental caries.

### Single Cell Protein

Single Cell Protein (SCP) is a term coined in the 1960s to embrace microbial biomass products, which were produced by fermentation. SCP or microbial protein is the name given to a variety of microbial products that are produced by fermentation. SCP is the purified and dried cells of microorganisms such as algae, bacteria, yeasts, molds and higher fungi grown in large-scale hydrocarbon wastes culture systems for use as protein sources in food or feeds. When properly produced, these materials make satisfactory proteinaceous ingredients for animal feed or human food.

SCP can be produced by culturing different microorganisms on different substrates such as whey, starch, cellulose, hydrocarbons, alcohols, and molasses. One common feature of SCP processes was that they often eliminated waste products, thus covering the function of expensive waste treatment.
installations. The main components of agricultural waste are cellulose, hemicellulose, and lignin. These wastes can be converted after hydrolysis with acids, alkali, cellulase, and steam into fermentable sugars. Fermentable sugars can be used as a substrate for the growth of microorganisms and production of SCP. Agricultural wastes are the most abundant raw materials that consist of cellulose as a major component, which is suitable for the growth of microorganisms and production of SCP biomass. The production of SCP by certain strains of microorganisms in agricultural waste medium under optimum cultural and nutritional conditions increases the protein content of SCP, making it an ideal supplement for animal and poultry feed.

The choice of substrates that are normally abundant and in proximity to the production plant has determined the design and strategy of SCP processes. The most widespread and commonly used substrates for SCP production have been those where the carbon and energy source is derived from carbohydrates. This is because their building blocks (mono and disaccharides) are natural microbial substrates, and that carbohydrates are renewable and widely distributed resources. Molasses is a byproduct of the sugar manufacturing process. Baker’s yeast was the first microorganism to be produced in aerobic stirred fermentation on molasses as it is still produced today. However, this yeast has seldom been destined as food, but rather for baking purposes.

Advantages of SCP include:

- rapid growth rate and high productivity;
- high protein content (30-80% of dw);
- ability to utilize a wide range of cheap carbon sources, methane, methanol, molasses, whey, lignocellulose waste, etc.;
- relatively easy selection of cells;
- little land area required;
- production independent of season and climate;
- protein content and quality largely dependent on the specific microbe utilized and on the fermentation process; and
- fast growing aerobic microorganisms.

In spite of these advantages, only one SCP product approved for human consumption has reached the market as of 1964. This product is ‘mycoprotein,’ the processed cell mass preparation from the filamentous fungus Fusarium venenatum. Mycoprotein has been commercially available and marketed as meat-free burgers and fillets and prepared meals, such as stir-fries, curries, and pasta dishes, in which mycoprotein is the central component.
Mushrooms

People have harvested mushrooms from the wild for thousands of years for food and medicines. Of the estimated 1.5 million species of fungi, about 10,000 produce the fruiting bodies we call mushrooms. Mushrooms are the fruiting bodies of macrofungi. They are very nutritious products that can be generated from lignocellulosic waste materials. The vegetative part of the fungus, called the mycelium, comprises a system of branching threads and cord-like strands that branch out through soil, compost, wood log or other lignocellulosic material on which the fungus may be growing. After a period of growth and under favorable conditions, matured mycelium could produce the fruit structure, which we call the mushroom. Accordingly, mushrooms can be roughly grouped into the following four categories:

- edible mushroom such as *Agaricus bisporus*;
- medicinal mushrooms like *Ganoderma lucidum*;
- poisonous mushrooms like *Amanita phalloides*; and
- miscellaneous or other mushrooms

Mushroom biotechnology is concerned with the enhancement of human health through mushroom derivatives and environmental bioremediation.

In addition to their good proteins, mushrooms are relatively good sources of some nutrients: fat, phosphorus, iron, and vitamins including thiamine, riboflavin, ascorbic acid, ergosterine, and niacin. They are low in calories, carbohydrates, and calcium. Mushrooms also contain a high proportion of unsaturated fat.

**Medicinal value of mushrooms**
For example, hypotensive and renal effects, immunomodulatory and antitumor activities of polysaccharide-protein complex (PSPC) from mycelial cultures; immunomodulatory and antitumor activities of lectins from edible mushrooms; type I ribosome-inactivation protein from *V. volvacea* and medicinal effects of *Ganoderma lucidum*.

**Nutraceuticals and dietary supplements**
A mushroom nutriceutical is a refined/partially defined mushroom extractive, which is consumed in the form of capsules, or tablets as a dietary supplement (not as food) and which has potential therapeutic applications. A regular intake may enhance the immune responses of the human body, thereby increasing resistance to disease and, in some cases, cause regression of a disease state.

**Mushroom Bioremediation**

There are some benefits for improvement of the environment through mushroom mycelia. Environmental contamination could be ameliorated by applying mushroom mycelial technologies, i.e, the use of bioconversion processes to transform pollutanta into valuable foodstuffs, and the use of mushroom mycelia as tool for healing soil, called “mycorestoration.” This includes the selective use of mushrooms for mycofiltration or filtering water; mycoforestry to enact ecoforestry policy; mycoremediation to denature toxic wastes; and mycopesticides to control insect pests. Mycorestoration recognizes the primary role fungi/mushrooms can play in determining the balance of biological populations.

In Ethiopia, mushrooms are being produced by some individuals and sold to the hotels and supermarkets. The activity is attracting more people as it appears to be profitable with no competition and has the potential to be commercialized.

Microbes involved in fermentation processes were identified using conventional methods. The fermentation dynamics have also been studied, which were undertaken by universities and thus focused on the basic studies as summarized in the following table (Table 1).
Table 1: Summary of fermentation dynamics studies undertaken by universities

<table>
<thead>
<tr>
<th>Type of MB application</th>
<th>Achievements</th>
<th>Research gap to be addressed</th>
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<tbody>
<tr>
<td>food biotechnology</td>
<td>microbes involved in the fermentation processes were identified using conventional methods</td>
<td>use of superior starter cultures and modernizing the processes and methods</td>
</tr>
<tr>
<td>fermented cereals and pulses/legumes</td>
<td>tef dough, Siljo fermentation studied at AAU</td>
<td>starter cultures not developed; molecular characterization is needed</td>
</tr>
</tbody>
</table>
| fermented horticultural crops | • Kocho fermentation studied at AAU  
• fermented condiments (Awazie, Data, Kochkocha, etc) studied at AAU and other HLIs | starter cultures not developed; molecular characterization is needed |
| fermented beverages | microbes in Tella, Yej, Borde and Shameta studied at AAU | starter cultures not developed; molecular characterization is needed |
| fermented dairy products | microbes have been identified using conventional methods; products quality differences determined | starter cultures not developed; molecular characterization is needed |
| functional foods and probiotics | only 1 in vitro study conducted on metabolism of bile acids by LAB isolated from dairy products | probiotic lab strains are not identified and popularized |
| single cell protein | not attempted | not a priority need for Ethiopia but can be demonstrated in universities |
| mushrooms | • individuals have been producing on compost  
• new substrates are being studied at FRC-EIAR | no need of research effort as it is already a well established technology |
FORAGE PRESERVATION has been a fundamental aspect of livestock production for thousands of years. With any forage preservation technique, the quantity and quality of material available at the end of storage are always less than the original. Thus, the primary goal of forage preservation is to minimize spoilage and losses of dry matter. This is accomplished by minimizing the effects of plant enzymes and microorganisms either through drying (hay production) or through controlled fermentation (silage production). The choice between silage and hay production depends on climate and properties of a given crop. In wetter regions of the world, silage production dominates. On the other hand, rumen biotechnology deals with the improvement of the nutritive value of ruminant feeds thereby enhancing the efficiency of digestion of fibrous feedstuffs in the rumen through manipulation of the microbes in the rumen and use of direct feed microbials and enzymes as feed additives, which provide energy, protein, vitamins and detoxifying functions.

Silage

Silage making or ensiling is a fermentation process carried out under an anaerobic environment using appropriate bacterial inoculants. Silage fermentation is a preservation method for moist forage crops (typically grass, alfalfa, and corn) which is widely used all over the world. It is based on fermentation of forage crops whereby LAB convert/ferment water-soluble-carbohydrates (WSC) into organic acids under anaerobic conditions. The resulting low pH and toxicity of undissociated acids restricts the growth of spoilage microorganisms and ensure good forage preservation.
Although silage fermentation occurs naturally under anaerobic conditions due to the native bacteria on plants, the speed and efficiency of the fermentation (pH drop) is variable, depending on the numbers and types of lactic acid bacteria on the crop. The speed of pH drop affects the amount of sugar used by bacteria; the preservation of true protein; the quantities of lactic acid, acetic acid, and ethanol; and finally the quality of the silage. Recently, there have been some significant changes in the types of microbial inoculants available. An inoculant may contain one or more strains of lactic acid bacteria. The most common homofermentative species is *Lactobacillus plantarum*. Other common homofermentative species include various *Lactobacillus* or *Pediococcus* species and *Enterococcus faecium*. *Lactobacillus buchneri* is the heterofermentative species used to improve aerobic stability.

Silage has been a popular option for preserving the nutritive value of forages. When produced in excess during the wet season, fodder or forage can be ensiled and used during the dry season. Silage is produced by harvesting a forage crop at high moisture content of greater than 50% and subsequently fermenting that crop in a silo of various designs such as pit, tower, bunker, trench, or plastic silos and fed to animals throughout the year. Studies on silage inoculants focus on two important aspects: their effects on preservation and on the nutritional value of the silage. The ensiled product retains a much larger proportion of its nutrients than if the crop had been dried and stored as hay or stover. Silage is most often fed to dairy cattle, because they respond well to highly nutritious diets. Ideally, this process should occur in the total absence of oxygen. If good forage quality is assumed, the most important factor necessary to achieve desirable silage fermentation and subsequently maintain high-quality silage within the silo for indefinite periods is the elimination of oxygen (air) from the silage mass.

This is important for the following reasons.

- to limit plant cell respiration or oxidation of carbohydrates (plant sugars) into carbon dioxide, water and heat;
- to improve availability of plant sugars for production of lactic acid;
- to reduce the concentrations of forage fiber components and formation on nitrogen containing compounds that are generally assumed to be indigestible in the ruminant digestive system;
- to reduce dry matter losses, maintain the nutritive value and avoid production of mycotoxins and other substances that negatively affect animal health; and
to reduce the time interval between the removal of silage from the silo (oxygen access) and the onset of spontaneous heating in the feed bunk and increase the stability of silages in the feed bunk.

Some of the advantages of silage are to increase intake rate, improve the digestibility of forage, and result in increased milk quantity and quality.

Although the primary function of LAB has been to improve the preservation of crops in the silo, homofermentative LAB inoculants in particular have been shown in various studies to improve milk yield, gain, and feed efficiency. It has been found that milk yield increased in 47% of the studies when inoculated silage was fed, compared with untreated silage. The average increase in milk production for those studies in which the inoculant enhanced milk yield was 1.4 kg/cow per day. Some LAB strains have shown an even more consistent effect on milk yield.

EM has been used for silage preparation in many countries. EM-Silage is a formulation produced by commercial companies specifically designed for silage. In Ethiopia, EM has been produced as of 2010 by Woljeejii Agro-Industries PLC at Debre Zeit. A project financed by RCBP has been conducted in Jimma with the aim of demonstrating EM for ensiling coffee pulp/husk, and was found to increase the digestibility.

**Rumen Biotechnology**

The rumen is the dominant feature of the digestive tract of cattle. It represents a complex ecosystem in which feeds consumed by the ruminant animal are digested by an active, diverse, and densely populated microbial community. About two-thirds of feed digestion and 90% of fiber digestion takes place in the rumen—all with the aid of microbes. The microbial mix in the rumen is complex and highly dependent on diet. Rumen biotechnology deals with all mechanisms to improve the performance of rumen thereby enhancing the digestibility of feeds to make it more nutrition. Some of the strategies are manipulation of rumen ecology, detoxification of toxins and use of direct-fed microbials.

**Manipulation of microbes in the rumen**
The main agents that breakdown fiber, sugars, starches and proteins in the rumen are anaerobic, and include bacteria, protozoa, and fungi. These organisms ferment feed materials to produce mainly short chain organic acids or volatile fatty acids (VFAs), methane and carbon dioxide, and the process provides substrate (the feed) and ATP (energy) for the host and growth of microorganisms. It is the diversity, adaptability and mutualistic interactions among the ruminal microbes and the host that have given ruminants a competitive edge in their ability to digest and thrive on diets high in fiber but often low in protein.

Many benefits are derived from the symbiotic relationship between microorganisms and ruminant animals. Among these benefits are the utilization of cellulose and non-protein N, and synthesis of water-soluble vitamins and essential fatty acids by ruminal microorganisms.

Efficiency of fiber digestion in ruminants is critical for animal productivity. The nutritive value of the feed consumed and the efficiency with which rumen microorganisms convert dietary carbohydrate to product of fermentation determines the nutrient supply to the ruminant animal. Therefore, maximizing efficiency of breakdown and digestion of plant cell walls in the rumen can have a marked effect on animal productivity.

Modern feeding practices are geared towards of high milk and meat production; and have some challenges to rumen microflora. A deficiency of a nutrient needed by rumen microorganisms reduces microbial growth efficiency, which reduces microbial biomass and eventually reduces digestibility and feed intake, particularly of fibrous feeds.

The first priority in feeding ruminants is to ensure no deficiencies in the diet of nutrients for microbial growth in the rumen. Of major importance is that the efficiency of microbial growth also determines the proportion of digested feed that is converted to methane, acetate or butyrate, and VFA. Several methods are currently employed to manipulate rumen fermentation to enhance post-ingestion nutritive value of fibrous forages through use of biotechnology including inoculants of native and recombinant rumen microorganisms; and natural adaptation and microbial feed enzymes. Rumen biotechnology has the potential to improve the nutritive value of ruminant feeds that are fibrous, low in nitrogen and of limited nutritional value for other animal species.
Rumen fermentation mechanisms can modify the anti-nutritional factor (ANFs); for example, tannins, oxalates, fluoroacetate, and pyrrolizidine that limit the use of the feed or crop residues and unimproved pastures. Some of the applications of rumen biotechnology for improving animal production are:

- Manipulation of the microbial mix within the rumen such as defaunation that results in increased microbial cell outflow to the intestines; consequently improves the P/E ratio in the nutrients absorbed; and
- Microbial treatment of straw and other crop residues to improve digestibility: limited growing of non-toxic fungi such as white-rot on the straw to delignify the plant material

Understanding the factors controlling rumen microbial activity may allow scientists to modify the rumen ecology in order to create conditions that will optimize the use of poor-quality feed by ruminant livestock. The fastest way to improve rumen function in an animal is to introduce digestion-enhancing bacterial species from other animals or to increase populations of species that inhabit the rumen only at low levels. Bacteria from one ruminant species have been experimentally shown to colonize others successfully. It is therefore possible to isolate and identify microbes capable of tolerating/transforming ANFs from indigenous domestic ruminants

**Detoxification of Feeds in the Rumen**

The other actions of ruminal microorganisms may involve detoxification of plant constituents or the production of non-toxic compounds from plant substrates containing toxic compounds. Detoxification of secondary plant compounds in the rumen can be achieved by developing microbes that are capable of degrading the toxic substances of the feed in the rumen by introducing appropriate microbes. Ruminal bacteria can perform biochemical transformations on plant constituents that may affect the health of ruminant animals. Ruminants are more resistant to mycotoxin poisoning than monogastrics. Some microbes from the rumen have been identified for their ability to degrade mycotoxins or plant toxins. Among the first mycotoxins detoxified by ruminants were ochratoxin A and aflatoxin B1. The changes in the feed composition from roughage to concentrates and a high percentage of protein-rich concentrates in the daily diet modify the cleavage capacity of rumen microorganisms.
Direct-Fed Microbials

Feed additives are non-nutritive and are included in feed to improve the digestibility of feed, thereby improving the growth efficiency and feed utilization and preventing diseases. Commonly used feed additives include antimicrobials, antioxidants, emulsifiers, binders, and enzymes.

The digestive efficiency of livestock is targeted by administering the direct-fed microbials (DFM), preferably of rumen origin. DFM, the animal probiotics, strategically increase the numbers of beneficial bacteria in the rumen, concomitantly stimulating the production of desired fermentation products. The anaerobic fungi, an emerging class of DFM, play an active role in improved fiber digestion, dry matter intake, body weight gains and milk production.

There have been several hypotheses put forth to explain the usefulness and general modes of action of DFM. One of the most common explanations for improved animal health or production suggests that the addition of beneficial bacteria prevents the colonization of pathogens in the lower gut by competing for space and nutrients.

Some of the proposed mechanisms of DFM for improvements in animal performance are:

- production of antibacterial compounds (acids, bacteriocins, antibiotics);
- competition with undesirable organisms for colonization space and/or nutrients;
- production and/or stimulation of enzymes;
- stimulation of immune response by host; and
- metabolism and detoxification of undesirable compounds.

Many bacterial-based DFM is sold for use in ruminant diets with more specific applications. These products often contain lactobacilli with *Lactobacillus acidophilus* being one of the most common microorganisms used. Other commonly used bacteria include various species of Bifidobacterium, Enterococcus, and Bacillus. Most bacterial-based DFM are probably beneficial because they have effects in the lower gut and not in the rumen.

EM is included in animal feed to increase productivity. The improvement in the production performance of animals receiving EM is attributed to the better-feed conversion efficiencies achieved because of inclusion of EM. The quality of milk and sub-products of the milk such as cheese, yogurt, and cream have
vastly improved. Milk production has increased between 0.5 and 1 liter per cow per day. Overall health is very much improved.

**Enzymes as Feed Additives**

Enzymes are also used in a wide range of agro-biotechnological processes, such as enzyme-assisted silage fermentation, bioprocessing of crops and crop residues, fiber processing and production of feed supplements to improve feed efficiency. The potential for industrial enzyme products as animal feed additives has attracted substantial interest from feed manufacturers as a novel means of improving animal performance. Enzyme manufacturers have also targeted feed as an alternate outlet for their products, which have primarily been in the food, beverage, and detergent industries.

In the context of feed additives for ruminants, enzymes are employed to catalyze the degradative reactions by which substrates, i.e. feedstuffs, are digested into their chemical components; for instance, simple sugars, amino acids, fatty acids. These are in turn used for cell growth, either by ruminal microorganisms or by the host animal. The use of enzymes that degrade polysaccharides of the endosperm cell wall has become most prominent. The major cell wall polysaccharides are the β-glucans in barley and oats and arabinoxylans (pentosans) in rye, wheat, and triticale. In barley and rye particularly, the cell wall carbohydrates are prone to solubilization. The fiber processing, which includes the use of phytases to improve the efficiency of nutrient utilization and to reduce waste, is a rapidly growing sector. Fibrolytic enzymes hold great potential to improve feed utilization and productivity in ruminants.

Research has demonstrated that supplementing dairy cow and feedlot cattle diets with fiber degrading enzymes has a significant potential to improve feed utilization and animal performance. Ruminant feed enzyme additives, primarily xylanases and cellulases, are concentrated extracts resulting from bacterial or fungal fermentations that have specific enzymatic activities. Improvements in animal performance due to the use of enzyme additives can be attributed mainly to improvements in ruminal fiber digestion resulting in increased digestible energy intake. Animal responses are greatest when fiber digestion is compromised and when energy is the first limiting nutrient in the diet.

When diets of dairy and beef cattle are supplemented with commercial xylanases and cellulases, animal performance is significantly improved.
Because of the complexity of the rumen environment, it has been difficult to identify the exact mode of action for this beneficial response. Since xylanases and cellulases are the main activities that occur in efficacious enzyme mixtures, it may be assumed that the enzymes are having a direct, additive effect on the hydrolysis of plant fiber in the rumen. However, evidence to date suggests that the benefits of exogenous enzymes are synergistic to ruminal endogenous enzymes. This synergy may explain why relatively small amounts of enzyme can have such large effects on animal productivity. Limitations to the exploitation of this technology are the development of an adequate screening system for new enzymes, and the identification of the specific enzyme activities that are critical for efficacy.

Exogenous enzymes have been extensively used to remove anti-nutritional factors from feeds, to increase the digestibility of existing nutrients, and to supplement the activity of the endogenous enzymes of poultry.

Although enzyme products marketed for livestock number in the hundreds, they are derived primarily from only four bacterial (*Bacillus subtilis*, *Lactobacillus acidophilus*, *L. plantarum*, and *Streptococcus faecium*, spp.) and three fungal (*Aspergillus oryzae*, *Trichoderma reesei*, and *Saccharomyces cerevisiae*) species.

Silage was used at Bako and Holetta RCs several years ago but discontinued as it was expensive compared with hay. The silo is still available at Ada-Berga Livestock Substation, but it is not used. On the other hand, EM formulation supplied by Woljeejii Agro-Industry PLC in 2010 was evaluated as a feed supplement to lactating cows and found to be encouraging at Holetta RC. EM was also evaluated for its potential to improve the digestibility of coffee pulp and husk and was found to be effective. Little was done to improve digestibility of feeds. Unimproved forages/fodders are not ensiled and evaluated as feeds after developing appropriate LAB inoculants. Detoxification and Direct-fed-microbials are not well studied.
Pharmaceuticals Production

**Pharmaceutical** are the backbone of modern medicinal therapy. Most traditional pharmaceuticals are organic chemicals with low molecular weight. In addition to chemical-based drugs, a range of pharmaceutical substances are produced by or extracted from biological sources, including microorganisms. These include blood products such as coagulation factors, antibiotics, vaccines, antibodies, adjuvants, therapeutic enzymes, hormones including insulin, glucagon, human growth hormone, interferons, interleukin-based, anti-diabetic drugs, hypocholesterolemic agents, enzyme inhibitors, antihelminthics, antitumor agents, immune-suppressants, and immune-modulators, etc., which are used in medical and veterinary applications.

Many microorganisms represent attractive potential production systems for therapeutic proteins. Microorganisms can usually be cultured in large quantities, inexpensively and in a short time, by standard fermentation procedures. The bulk of biopharmaceuticals currently on the market are produced by genetic engineering using various recombinant expression systems. Expression systems, which could potentially be used for the production of recombinant biopharmaceutical products, include bacteria (*E. coli*), yeast (*S. cerevisiae*) and Fungi (*Aspergillus* spp.). Over half of all biopharmaceuticals thus far approved are produced in recombinant *E. coli* or *S. cerevisiae*.

**Antibiotic Manufacturing**

Antibiotics are antimicrobial drugs used against microbes. An antibiotic is a substance, usually produced by a microorganism, which, in very small quantity, inhibits or kills other microorganisms. Most of the antibiotics are made industrially by aerobic fermentations using appropriate strains of bacteria,
actinomycetes, and fungi. Microorganisms used in fermentation are rarely identical to the wild type. This is because species are often genetically modified to yield the maximum amounts of antibiotics. Mutation is often used, and is encouraged by introducing mutagens such as ultraviolet radiation, x-rays, or certain chemicals. Selection and further reproduction of the higher yielding strains over many generations can raise yields by 20-fold or more. Studies are continually being made on strain improvement, inoculum conditions, fermentation conditions, and various combinations of these factors. For example, improved mutant strains usually require adjustments in fermentation conditions in order to achieve the high yields in fermenters that are obtainable in shaken flasks.

**Penicillin Production**

Penicillin was first isolated from a mould *Penicillium notatum*. A variety of moulds belonging to other species and genera were later found to yield greater amounts of the antibiotic and a series of closely related penicillins. The naturally occurring penicillins differ from each other in the side chain (R group). Penicillin was produced by a surface culture method early in World War II. Submerged culture methods were introduced by 1943 and are now almost exclusively employed. Penicillin production needs strict aseptic conditions. The mechanism of antibiotic action of penicillins is inhibition of cell wall synthesis of bacteria. The cell wall is composed of peptidoglycan and murein. When bacteria are exposed to penicillin, the antibiotic binds to the penicillin binding proteins (PBPs) in the cell membrane followed by the release of autolytic enzymes that degrade the preformed cell wall and arrest further cell wall synthesis leading to death of bacteria.

The principal mode of action of tetracyclines is the inhibition of protein synthesis. The antibiotics bind to the 30S ribosomal sub-unit and prevent the attachment of aminoacyl-tRNA to be acceptor site on the messenger RNA (mRNA)-ribosome complex.

**Cephalosporin Production**

Cephalosporin C is made as the fermentation product of *Cephalosporium acremonium*. However, this form is not potent for clinical use. Its molecule can be transformed by removal of an aminoacidic acid side chain to form 7-α aminoccephalosporanic acid (7-ACA), which can be further modified by adding side chains to form clinically useful broad spectrum antimicrobials.
Streptomycin Production

Streptomycin and various other antibiotics are produced using strains of *Streptomyces griseus*. Spores of this actinomycete are inoculated into a medium to establish a culture with a high mycelial biomass for introduction into an inoculum tank, with subsequent use of the mycelial inoculum to initiate the fermentation process in the production tank. *Streptomyces* spp. are known to produce several antibiotics like Chloramphenicol, tetracycline, and others.

Steroids Translation in Pharmaceutical Industry

Microbial biotransformation of steroids is very important in the pharmaceutical industry. Steroids are used in treatment of various disorders and involved in regulation of sexuality. Chemical synthesis of steroids is very complex because of the requirement to achieve the necessary precision of substituent location.

Vaccine Preparations

Vaccines are essential to protect humans and animals from microbial diseases. The production of vaccines against the microbial pathogens, and more particularly the pathogenic viruses, in order to immunize the susceptible populations, is a safe and more certain recourse. Production of vaccines involves growing the microbes possessing the antigenic properties needed to elicit a primary immune response. Prophylactic treatment of serious pathogenic viruses and bacteria could become possible only by vaccines.

A vaccine is an agent, sourced from the pathogen, and deliberately introduced into the mammalian system in order to impart a ‘memory’ of the pathogen or its pathogenic component. Vaccines contain antigens that elicit the production of antibodies, or immunogens that trigger the cellular component of immune response. In the event of an encounter with the corresponding antibodies, only the antigens can bind with the antibodies, and form an antigen-antibody complex that neutralizes the harmful effects of the antigens or the organisms that produce them.

There are many types of preparations of vaccines and few examples of the diseases cured are:
• Live, attenuated vaccines: produced from whole living microbe weakened. Used against diseases such as measles, mumps, rubella, polio (Sabin vaccine), yellow fever;
• Inactivated or ‘killed’ vaccines: produced by killing the disease-causing microbe with chemicals and used against diseases such as cholera, flu, hepatitis A, Japanese encephalitis, plague, polio (Salk vaccine), rabies;
• Toxoid vaccine: toxins of the microbe are ‘detoxified’ by treating them with formalin and are used against diseases such as diphtheria and tetanus.
• Subunit vaccines: produced from a portion of the viral or bacterium structures (only the antigen or epitopes, usually proteins or lipids) and used against diseases such as hepatitis B, pertussis, pneumonia caused by *Streptococcus pneumoniae*;
• Conjugate vaccines: based on polysaccharide coatings used to disguise a bacterium’s antigens so that the immature immune systems of infants and younger children cannot recognize or respond to them. It is used for treatment of diseases such as *Haemophilus influenzae* type b and pneumonia caused by *Streptococcus pneumoniae*;
• DNA vaccines: plasmid that has been genetically engineered to produce one or two specific proteins (antigens) from a pathogen. Because these proteins are recognized as foreign, when they are processed by the host cells and displayed on their surface, the immune system is alerted, then triggers a range of immune responses. Although still in the experimental stages, these vaccines show great promise, and several types are being tested in humans; and
• Recombinant vector vaccines: use an attenuated virus or bacterium as a vector or carrier to introduce microbial DNA to cells of the body. In this case, the inserted genetic material causes the bacteria to display the antigens of other microbes on its surface. In effect, the harmless bacterium mimics a harmful microbe, provoking an immune response. It is under clinical testing stage.

The process of the deliberate introduction of a vaccine into the organism is vaccination. Since vaccination immunises the organism, the process is also called immunisation. When an organism is vaccinated, the immune system is readied to show an immune response by way producing antibodies against the pathogen. Chemicals or additives commonly used in the production of vaccines include (i) suspending fluid (sterile water, saline, or fluids containing protein); (ii) preservatives and stabilizers (for example, albumin, phenols, and glycine); and (iii) adjuvants or enhancers that help improve the vaccine's effectiveness. Vaccines also may contain very small amounts of the culture material used to grow the virus or bacteria used in the vaccine, such as chicken egg protein.

**Human Growth Hormones and Other Therapeutic Proteins**

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Human growth hormone (HGH) supplements are needed when, for some reason, there is a decrease in endocrine gland function. Gene defects in humans can lead to deficiencies in proteins, such as insulin, human growth hormone, and Factor VIII that may result in problems such as diabetes, dwarfism, and impaired blood clotting, respectively. Proteins for these chemicals can now be replaced by proteins manufactured through microbial biotechnology. HGH stimulates the production of protein in cells of most types, accelerates the production of the two master chemicals of heredity, DNA and RNA, stimulates the manufacture of red blood cells, and augments the flow of blood to the kidneys and the rate at which the kidney does its vital filtration work. The hormone seems to decrease the body's stores of fat and increase muscle mass and the tissues of liver, kidney, and heart. The best-known action is in stimulating bone growth and skeletal development, the effect that makes it valuable in correcting serious growth problems in some children. A deficiency in growth hormone results in hypopituitary dwarfism. This condition can be treated successfully by growth hormone produced by bacteria containing genes for HGH spliced into their genomes.

Some proteins and peptides of therapeutic value are difficult to purify in sufficient amounts from their human and animal sources. Recombinant DNA methods have had a revolutionary impact in the production of these compounds. Once the DNA sequences coding for these proteins and peptides have been cloned and amplified in microorganisms, the latter can continue to function as living factories for the inexpensive production of such compounds. Bacteria, especially *E. coli*, are used extensively as the host microorganism. Opting to use *E. coli* in the production of the hormone had several advantages like lowering the cost of production and higher output. Without the ability to recombine and produce HGH in the lab, there would be no way the demands would be met to provide effective treatments for various disorders. By creating it in a laboratory, it could be controlled and produced pure. Using *E. coli*, the production can be done in greater quantities with fewer health side effects. Improvements in this method of production will provide enough HGH for treatment to a larger mass of people.

**Insulin**

It is the first bacterially produced human hormone from recombinant *E. coli* and used for treatment of diabetes. For insulin production, two protein chains are
encoded by separate genes in plasmids inserted into bacteria. The protein chains are then chemically joined to form the final insulin product.

**Interferon** ($\alpha$, $\beta$, $\gamma$)

It is a family of 20 to 25 low molecular weight proteins called cytokines; which cause cells to be resistant for the growth of viruses. Interferon is an antiviral glycoprotein produced in *E. coli* cells, and it is currently used to fight certain types of cancers and for certain skin diseases. It is used as antiviral, antitumor and anti-inflammatory.

**Interleukins** (1, 2, 3)

They are produced by cells of the immune systems to regulate the interaction of these cells with other body cells. They stimulate cells in the immune system and are used as antitumor; treatment of immune disorders.

**Therapeutic Enzymes**

The pharmaceutical industry is being recognized as an important consumer of commercial enzymes. Enzymes are in great demand for use as therapeutic agents against many dreaded diseases. Accelerated and in-depth studies to utilize the vast microbial resources--both terrestrial and marine--as sources of novel therapeutic enzymes are highly significant. Microbial enzymes offer the potential to treat many important diseases, which are resurging after acquiring resistance to antibiotics. Some of these are:

- Urokinase and streptokinase (plasminogen to plasmin): to dissolve blood clot in heart attack; anticoagulant;
- Asparaginase and glutaminase to hydrolyze L-asparagine and L-glutamine to aspartic and glutamic acids, respectively, in the treatment of leukemia;
- Superoxide dismutase: antioxidant; anti-inflammatory;
- Penicillinase: to hydrolyze penicillin during acute penicillin allergy;
- Hyaluronidase: to hydrolyze hyaluronate during heart attack;
- Collaginase: to hydrolyze collagen in skin cancer;
- Uricase: to oxidize uric acid;
- Penicillin acylase Collagenase: synthetic antibiotic production to treat skin ulcers;
- Lipase: to digest lipids; and
- Ribonuclease: antiviral.

**Recombinant DNA Pharmaceuticals**
Genetic engineering, recombinant DNA technology, genetic modification/manipulation, and gene splicing are terms that are applied to the direct manipulation of an organism’s gene. The development of these new technologies has resulted in the production of large amounts of biochemically defined proteins of medical and pharmaceutical industries. The biochemically derived therapeutics use large extracellular proteins either for use in chronic replacement therapies or for the treatment of life-threatening indications. Some of the recombinant human proteins that are manufactured include:

- DNase I for treatment of cystic fibrosis;
- Drythro poietin for treatment of anemia; and
- Insulin for diabetes, IFN-α/2a for treatment of leukemia and Interleukin-2 for treatment of renal carcinoma.

**Marine Microorganisms**

Marine microorganisms are also other sources of pharmaceutically important secondary metabolites. A number of biologically active secondary metabolites and polysaccharides with varying degrees of action, such as antitumor, anticancer, anti-microtubule, anti-proliferative, cytotoxic, photo-protective, as well as antibiotic including antibacterial, antiplasmodial, anti-inflammatory, and antiviral agents and antifouling properties, have been isolated from marine bacteria, fungi, actinomycetes, cyanobacteria mainly blue green algae, and marine symbionts. The marine environment also represents a largely unexplored source for isolation of new microbes including bacteria, fungi, actinomycetes, microalgae-cyanobacteria, and diatoms are potent producers of bioactive secondary metabolites as lead compounds to develop novel drugs that may not be obtained from plants.

**Endophytes**

Numerous studies have indicated that these prolific actinobacteria appear to have a capacity to produce an impressive array of secondary metabolites exhibiting a wide variety of biological activity, such as antibiotics, antitumor and anti-infection agents. These microorganisms may represent an underexplored reservoir of novel species of potential interest in the discovery of novel lead compounds and for exploitation in pharmaceutical, agriculture, and industry.
Most of the endophytic bacteria belong to members of genera *Pseudomonas*, *Burkholderia*, and *Bacillus*. These genera are well known for production of their secondary metabolites, *viz.*, antibiotics, anticancer like Taxol, antiviral such as cytonic acid A and cytonic acid B, insecticidal, oocydin, and some immune suppressant agents.

Many secondary metabolites from endophyte-plant interactions have also been isolated and used in raw or derived forms to produce a variety of drugs treating many conditions. The toxic properties of ergot alkaloids also make them useful in the treatment of headaches and throughout the process of giving birth by inducing contractions and stemming hemorrhages. Drugs used to treat Parkinson’s disease have been created from isolates of ergot toxins, although health risks may accompany their use. Ergotamine has also been used to synthesize lysergic acid diethylamide because of its chemical similarity to lysergic acid. The generally chemically-based defense properties of endophytic fungi make them a perfect group of organisms to search for new antibiotic compounds within, as other fungi have in the past yielded such useful drugs as penicillin and streptomycin and plants use their antibiotic qualities as a defense against pathogens.

Some fungal and actinomycete isolates which were found to produce antifungal activities have been studied at AAU. In some cases, solid substrate fermentations gave better yields. However, best methods for large-scale production are not well developed.
Enzyme Production

NZYMES are biological catalysts in the form of globular proteins that drive and assist chemical reactions by increasing the rate at which they occur. They are produced by all living organisms, function as highly specialized catalysts, and accelerate the rate of a chemical reaction without being destroyed or changed. They are responsible for many essential biochemical reactions in the cells of all living organisms. Without enzymes, most chemical reactions within cells would occur so slowly that cells would not be able to work properly. Thus, enzymes catalyze biochemical reactions that are necessary to support life. Sources of commercial enzymes cover a wide range, from microorganisms to plants and animal sources. Nevertheless, for various reasons, microorganisms have become the major source of enzymes.

In commercial enzyme production, fungi and yeast contribute about 50%, bacteria 25%, animal 8% and plant 4% of the total. Microbes are preferred to plants and animals because:

- they are cheap sources with lower cost of production;
- their enzyme contents are predictable/consistent and growth substrates are obtained as standard raw materials;
- allow for ease of process modification and optimization; and
- enzymes are relatively more stable.

Enzyme Classification

Enzyme classification is the systematic arrangement and the naming of enzymes that is based on the 1972 recommendations of the Enzyme Commission (EC) of the International Union of Biochemistry. Reactions and the enzymes that catalyze them form 6 classes, each having 4-13 subclasses. The enzyme name has 2 parts. The 1st names the substrate(s). The 2nd, ending in
-ase, indicates the type of reaction catalyzed. Each enzyme is denoted by a number composed of 4 figures. The 1st figure denotes one of the 6 main divisions. The 2nd figure denotes the subclass and the 3rd figure denotes the sub-subclass. The 4th figure denotes the serial number of the enzyme in its sub-subclass. The enzyme number is preceded by the abbreviation E.C. There are 6 classes of enzyme reaction profile:

- **Oxidoreductases**: catalyze oxidation-reduction reactions, involving the movement of electrons from one molecule to another. In biological systems, the removal of hydrogen from the substrate is catalyzed by enzymes called dehydrogenases.
- **Transferases**: catalyze the transfer of groups of atoms (radicals) from one molecule to another. Aminotransferases or transaminases promote the transfer of an amino group from one amino acid to an alpha-keto-acid.
- **Hydrolases**: catalyze reactions between a substrate and water, and bind water to certain molecules. In this way, larger molecules are broken up into smaller units. This class of enzymes catalyses the cleavage of peptide bonds in proteins, glucosidic bonds in carbohydrates, and ester bonds in lipids.
- **Lyases**: catalyze the addition of groups to double bonds or the formation of double bonds through the removal of groups. Thus, bonds are cleaved using a different principle to hydrolysis. Pectate lyases, for example, split the glycosidic linkages by beta-elimination.
- **Isomerases**: catalyze the transfer of groups from one position to another on the same molecule. In other words, these enzymes change the structure of a substrate by rearranging its atoms.
- **Ligases**: join molecules together with covalent bonds. These enzymes participate in biosynthetic reactions where new groups of bonds are formed. Such reactions require the input of energy in the form of cofactors such as ATP.

### Applications of Enzymes

Enzymes have a wide variety of uses in the manufacturing industries, particularly in food and material manufacturing; for research purposes as reagents; in medical and commercial applications etc., which are briefly outlined as follows:

**Food enzymes**

- enzymes for starch processing (amylases for production of glucose);
- sweetener production (glucose isomerase for fructose production);
- bakery products (xylanase, α-amylase, glucose oxidase for dough conditioning, dough quality, loaf volume);
- dairy product (rennin, lactase for milk coagulation and hydrolysis of lactose);
- fruit juice (pectinase, cellulase, xylanase for juice clarification, juice extraction);
• wine making (glucanase and papain for haze clearance); and
• preparations of functional foods and nutraceuticals

Livestock production

As feed additives (xylanase for fiber solubility, phytase for removal of phosphate)

Enzymes for technological applications

• detergent enzymes (proteinase);
• enzyme for textile (cellulase and laccase for microfibril removal and for improving brightness of cloth);
• enzymes for leather processing (protease, lipase);
• enzymes for paper and pulp processing (xylanase); and
• for plastics, biopolymers, bulk organic chemicals and biosteel (silk)

Genetic Engineering and Biotechnological applications

• Alkaline phosphatase,
• Exonuclease,
• DNA polymerase 1,
• Klenow fragment of *E. coli* DNA polymerase 1,
• DNA polymerase III,
• T4 DNA polymerase,
• Terminal deoxynucleotidyl transferase,
• T4 Polynucleotide kinase,
• Reverse transcriptase,
• Restriction endonucleases,
• T4 DNA Ligase,
• Exonuclease III,
• l- Exonuclease,
• SI Endonuclease,
• Ligase,
• Polynucleotide kinase,
• Replicase,
• Reverse transcriptase,
• Ribonuclease (RNase),
• RNA Polymerase,
• RNase H,
• S1 nuclease,
• Terminal transferase,
• Topoisomerase,
• Transposase

Biochemical applications
• aa-tRNA synthetase,
• Adenylate cyclase,
• Aspartokinase,
• β-galactosidase,
• β-lactamase,
• Chloramphenicol acetyltransferase (CAT),
• Glutamate dehydrogenase,
• Luciferase,
• Resolvase

**Medical applications**

• Digestive enzymes: Pancreatic enzymes, mammalian protease (pepsin) plant proteases (Bromelain, papain), fungal amylases
• Enzymes with potential therapeutic applications, Asparaginase and glutaminase, Urokinase and streptokinase, Penicillinase, Hyaluronidase, Collaginase, Uricase

**Markers for disease**

• lactase dehydrogenase (LDH);
• creatine kinase (CK); and
• acetyl cholinesterase (AChE),

**Clinical diagnoses of diseases**

• Alkaline Phosphatase;
• Creatine Kinase (CK; EC 2.7.3.2);
• Alanine Aminotransferase (ALT; EC 2.6.1.2);
• Aspartate Aminotransferase (AST; EC 2.6.1.1);
• Sorbitol Dehydrogenase (SDH; EC 1.1.1.14);
• Lactate Dehydrogenases (LDH; EC 1.1.1.27);
• Cholinesterase (ChE);
• Lipase (Lip);
• α-Amylase (AMY; E.C. 3.2.1.1);
• γ-Glutamyltransferase (GGT; 2.3.2.2);
• Trypsin (EC; 3.4.21.4); and
• Glutathione Peroxidases (GPx; EC 1.11.1.9)

**Reagents in clinical chemistry**

• Glucose oxidase is utilized in a test-strip for the screening of urine specimens; and
• Urease for blood lactate and pyruvate
Immunoassays

As enzyme-immunoassays for the determination of a variety of proteins and hormones used to replace the hazardous radioisotopes as markers

Analytical reagents

For the estimation of specific substances, possibly present at very low concentrations, in the presence of other, chemically similar, substances. E.g., uric acid by uricase, Ethyl alcohol by alcohol dehydrogenase, Ammonia by L-glutamate dehydrogenase, Cholesterol by cholesterol oxidase, Glucose by glucose oxidase, Urea by urease.

Antioxidants

- Superoxide dismutase; and
- Catalase

Sources of biofuels

- Alcohol from biomass using cellulolytic enzymes

Pharmaceuticals production

- For synthesis of chiral compounds, glycoprotein and as pharma targets

Research

- important for researches into diseases of humans and animals, biotechnology research and other research areas, that is, enzymes have been used in research for better and alternative treatment of diseases

Categories and Characteristics of Industrial Enzymes

Enzymes are categorized according to the compounds they act upon. Some of the most common seven categories of food enzymes and their activities include:

- **Amylases**: break down starches into simple sugars;
- **Cellulases**: break down fibers or cellulose;
- **Lactases**: break down dairy products;
- **Lipases**: break down/split fats (lipids) into glycerol and fatty acids;
• **Maltase**: breaks down grains;
• **Proteases**: breakdown proteins; and
• **Sucrase**: breaks down sugars

Enzymes have several characteristics that make them significant for use in industrial processes:

• they accomplish and accelerate a reaction efficiently;
• the rates of the reaction can be readily controlled by adjusting the temperature, pH and reaction time;
• enzymatic activity may be destroyed by heating to denaturing temperatures;
• enzymes are natural in origin and non-toxic and, therefore, may remain in the product without any harmful consequences; and
• enzymes exhibit great specificity and can be used generally at levels of less than 1% of the commercial product batch.

**Enzyme specificity**
Specificity is an important characteristic of enzymes. The three different types of enzyme specificity/specificities are

• stereochemical specificity;
• reaction specificity; and
• substrate specificity, which can be absolute or relative, substrate specificity.

**Industrially Important Microbial Enzymes**

Considerable progress has been made in recent times toward the improvement of microbial strains used in the production of enzymes. Microbial host strains developed for enzyme production have been engineered to increase enzyme yields by deleting native genes encoding extracellular proteases. Certain fungal producing strains have also been modified to reduce or eliminate their potential for producing toxic metabolites. Some of the common industrial enzymes that deserve research targeting large-scale production and characterization are described alphabetically as follows.

**Amylases**

Amylase converts polysaccharides into monosaccharides. Amylases are used for preparation of sizing agents in textile industry, preparation of starch sizing pastes for use in paper industry, bread production, chocolate and corn syrups and removal of spots in laundry. There are various types of amylases-α-β and
glucamylases. *Aspergillus oryzae, A. Niger, Bacillus subtilis,* and *B. diastaticus* are principally used. The conversion of starch to high fructose syrup utilizes amylases, i.e. in producing sweeteners. Various other enzymes produced by different microbes also have industrial applications. These are rennin used in cheese production and *Mucor pusillus* is used for its commercial production. Fungal pectinases are used in clarification of fruit juices. Glucose oxidase is used to remove oxygen from soft drinks, salad dressings, etc.

**Catalase**

The enzyme catalase has found limited use in one particular area of cheese production. Hydrogen peroxide is a potent oxidizer and toxic to cells. It is used instead of pasteurization, when making certain cheeses such as Swiss, in order to preserve natural milk enzymes that are beneficial to the product and flavor development of the cheese. These enzymes would be destroyed by the high heat of pasteurization. However, residues of hydrogen peroxide in the milk will inhibit the bacterial cultures that are required for the actual cheese production, so all traces of it must be removed. Catalase enzymes are typically obtained from bovine livers or microbial sources, and are added to convert the hydrogen peroxide to water and molecular oxygen.

**Cellulases**

Cellulases are enzyme complex capable of degrading crystalline cellulose to glucose. Cellulase is component of sequentially acting enzymes used for the bioconversion of cellulose, nature’s most abundant polysaccharide, produced by bacteria and fungi. Cellulases and hemicellulases have numerous applications and biotechnological potential for various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture. Mechanistically, cellulase is a family of at least three groups of enzymes: endo-(1, 4)-β-D-glucanase (EC 3.2.1.4), exo-(1, 4)-β-D-glucanase (EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21).

**Chitinases**

Chitinase is involved in the process of producing mono- and oligosaccharides from chitin. Furthermore, chitinase is a potential antifungal agent through its chitin degradation activity. The major applications of chitosan include wastewater clearing, preparation of cosmetics, paper production, textile
finishes, photographic products, cements, heavy metal chelating agents and for medical and veterinary purposes. Endo-acting chitin hydrolase chitinase A, the exoacting hydrolase chitinase B and N-acetyl-D-glucosaminidase are responsible for chitin degradation. Chitin is the second most abundant natural biopolymer after cellulose, which is found attached to other polysaccharides and proteins, covering layer of insects, cell walls of various fungi and crab and shrimp wastes. The thermophilic organisms Bacillus and Streptomyces species were reported to be the major sources of chitinases.

Dextranase

Dextranases are sometimes applied to hydrolyze dextran polysaccharide in sugar manufacture when bacterial (mainly Leuconostoc) deterioration of sugar eet has occurred. Unfortunately, dextranases only have a small market and low volume sales compared with many other industrial enzymes. Dextranase [(1-6)-α-D-glucan6-glucanohydrolases or glucanases (E.C. 3.2.1.11)] are enzymes that specifically hydrolyze α-(1-6) linkages in dextran. Since many dextrans contain a relatively high concentration of secondary linkages, other than α-(1-6), and enzyme that can break (1-2), (1-3) and (1-4) linkages in dextran, is also included together with true dextranase. The hydrolysis products of dextran by dextranase are glucose (with exodextranase), isomaltose, and isomaltodextran. Therefore, these are commonly called by common name as glucanases (E.C. 3.2.1.11). The prefixes show the nature of linkages attacked. In sugar production, dextrans are undesirable compounds synthesized by contaminant microorganisms from sucrose, increasing the viscosity of the flow and reducing industrial recovery, bringing about significant losses. The use of the dextranase enzyme is the most efficient method for hydrolyzing the dextrans at sugar mills. Some bacterial strains, filamentous fungi and a small number of yeasts have been shown to produce dextranase. The fungal dextranases showed the highest reaction rate.

Glucanase

Some yeast and many higher fungi contain (1→3)-α-linked glucan in their cell walls. In addition, there are β-glucans that contribute significantly to the strength and rigidity of the cell walls. β-Glucanases, produced by several fungi and bacteria are one of the most potent enzymes for degrading fungal cell walls. More recently, intensive efforts have been made to use the biocontrol agents for protecting fruit and vegetable crops from post-harvest diseases.
**Glucose Isomerase**

Glucose isomerase is produced from *Bacillus* and *Streptomyces* species and used for preparation of sweeteners. D-glucose ketoisomerase causes the isomerization of glucose to fructose to prepare high fructose corn syrup, which is 2x sweeter than sucrose. Since reaction is reversible, the ratio of glucose and fructose depends on the enzyme.

**Glucosidases**

Glucosidase is an enzyme of the hydrolase class that cleaves the glucosidic bond between two glucose molecules, occurring as α-, β- and α-1,3-glucosidase; α-glucosidase occurs in intestinal juice, and β-glucosidase (cellobiase) in the kidney, liver and intestinal mucosa. α-glucosidase (EC 3.2.1.20) is a glucosidase acting upon 1,4-α bonds (Syn. to Maltase). β-glucosidases (β-D-glucoside hydrolase, EC 3.2.1.21) are able to cleave the β-glucosidic linkages in di- and oligo glucosaccharides and several other glycoconjugates. β-glucosidase acts upon β1->4 bonds linking two glucose or glucose-substituted molecules (i.e., the disaccharide cellobiose). These enzymes are widely distributed and have important roles in many biological processes. The physiological role is diverse and includes ceramide catabolism in human tissues, cell wall, pigment and cyano-glycoside metabolism; defense against pathogens in plants; utilization of oligosaccharide substrates by different fungi and bacteria and liberates aroma-rich terpenes. In cellulytic microorganisms, β-glucosidase is involved in cellulose induction and hydrolysis.

The enzymatic hydrolysis of cellulosic material into glucose involves the synergistic action of at least three different enzymes: endo- and exo-cellulases and β-glucosidase or cellobiose.

- β-1,4-endoglucanase (1,4-β-Dglucan 4-glucanohydrolase; EC 3.2.1.4, cellulase), which cleaves internal β-1,4-glycosidic bonds;
- cellobiohydrolase (1,4-β-D-glucan cellobiohydrolase; EC 3.2.1.91, cellulase 1,4-β-cellobiosidase), an exo-acting enzyme which releases cellobiose from reducing and non reducing ends of cellulose; and
- β-glucosidase (β-Dglucoside glucohydrolase; EC 3.2.1.21, cellulase 1,4-β-glucosidase) that hydrolyzes cellobiose to glucose.

β-glucosidase is generally responsible for the regulation of the whole cellulolytic process and is a rate-limiting factor during enzymatic hydrolysis of cellulose, as both endoglucanase and exoglucanase activities are often inhibited by cellobiose. Thus, β-glucosidase not only produces glucose from cellobiose,
but also reduces cellobiose inhibition, allowing endoglucanase, and exoglucanase enzymes to function more efficiently. In recent years, interest in β-glucosidase has gained momentum owing to their ability to catalyze transglycosylation reactions. These types of reactions have great importance in wine industry because of its ability to improve the aroma of wines. In recent years, interest in β-glucosidase has gained momentum owing to their ability to catalyze transglycosylation reactions. These types of reactions have great importance in wine industry because of its ability to improve the aroma of wines.

**Keratinase**

Keratinases have different applications where keratins should be hydrolyzed, such as the leather and detergent industries, textiles, waste bioconversion, medicine, and cosmetics for drug delivery through nails and degradation of keratinized skin, feed, fertilizer, and pharmaceutical industries. In recent years, there have been many reports on the purification of keratinase from different microorganisms. Keratins are insoluble proteins that are major constituents of skin, hair, feathers, wool, horn, and nails. Feather contains over 90% of crude protein in the form of keratin. Because of a high degree of cross-linking by cystine disulfide bonds, hydrogen bonding, and hydrophobic interactions, keratin is insoluble and not degradable by proteolytic enzymes, such as trypsin, pepsin, and papain. However, it can be broken down by some keratinase-secreting microorganisms that turn native keratin into smaller molecular entities that, in turn, can subsequently be absorbed by cells.

**Laccase**

Laccase belongs to the bluemulti-copper oxidases and participates in cross-linking of monomers, degradation of polymers, and ring cleavage of aromatic compounds. It is widely distributed in higher plants and fungi. It is present in ascomycetes, deuteromycetes, and basidiomycetes and abundant in lignin-degrading white-rot fungi. It is also used in the synthesis of organic substance, where typical substrates are amines and phenols, the reaction products are dimers and oligomers derived from the coupling of reactive radical intermediates. In the recent years, these enzymes have gained application in the field of textile, pulp and paper, and food industry, synthetic chemistry, cosmetics, soil bioremediation, and biodegradation of environmental phenolic pollutant and removal of endocrine disruptors. Recently, it is also used in the design of biosensors, biofuel cells, as a medical diagnostics tool and
bioremediation agent to clean up herbicides, pesticides and certain explosives in soil. Laccases have received attention of researchers in the last few decades due to their ability to oxidize both phenolic and non-phenolic lignin-related compounds as well as highly recalcitrant environmental pollutants.

**Lactase**

Lactase or β-galactosidase is a glycoside hydrolase enzyme that cuts lactose into its constituent sugars, galactose and glucose. This process is used for milk products that are consumed by lactose intolerant consumers. Without sufficient production of lactase enzyme in the small intestine, humans become lactose intolerant, resulting in discomfort such as cramps, gas and diarrhea in the digestive tract upon ingestion of milk products. Lactase is used commercially to prepare lactose-free products, particularly milk, for such individuals. It is also used in preparation of ice cream, to make a creamier and sweeter-tasting product. Lactase is usually prepared from Kluyveromyces sp. of yeast and Aspergillus sp. of fungi.

**Lipases**

Lipases are produced by Micrococcus and used to degrade fats and oily substances when used in detergents. They also break down milk fats and give characteristic flavors to cheeses. Stronger flavored cheeses, for example, the Italian cheese, Romano, are prepared using lipases. The flavor comes from the free fatty acids produced when milk fats are hydrolyzed. Animal lipases are obtained from kid, calf, and lamb, while microbial lipase is derived by fermentation with the fungal species Mucor meihei. Although microbial lipases are available for cheese making, they are less specific in what fats they hydrolyze, while the animal enzymes are more partial to short and medium-length fats. Hydrolysis of the shorter fats is preferred because it results in the desirable taste of many cheeses. Hydrolysis of the longer chain fatty acids can result in either soapiness or no flavor at all.
Maltase

Maltase (EC 3.2.1.20) is an enzyme that catalyzes the hydrolysis of the disaccharide maltose to the simple sugar glucose. This enzyme is found in plants, bacteria, and yeast. In most cases it is equivalent to α-glucosidase, but the term ‘maltase’ emphasizes the disaccharide nature of the substrate from which glucose is cleaved, and ‘α-glucosidase’ emphasizes the bond, whether the substrate is a disaccharide or polysaccharide. Starch catabolism frequently depends on a secreted α-amylase that generates linear maltodextrins from starch as well as a cell-associated α-glucosidase (maltase) which converts maltose and maltodextrins to glucose. Extremely thermophilic α-amylases have been identified in members of the obligately anaerobic sulfur-reducing genus *Pyrococcus* (Archaea). A monomeric 125-kDa α-glucosidase has also been identified in this group. Several lines of evidence suggested that an α-glucosidase was similarly present in the crenarchaeote *S. solfatarius*. These included the ability of the organism to utilize maltose as the sole carbon and energy source and the presence of a *p*-nitrophenyl-α-D-glucopyranoside (PNPG) hydrolytic activity in crude cell extracts.

Pectinases

Pectinases have many applications in drink industry. Enzymes are also used in fruit juice manufacturing to clarify fruit juices. Pectins are substances in fruit lamella and cell walls. The cell wall also contains hemicelluloses and cellulose. Pectinase, xylanase and cellulase improve the liberation of the juice from the pulp. Pectinases and amylases are used in juice clarification. Brewing is an enzymatic process. Malting is a process, which increases the enzyme levels in the grain. In the mashing process, amylases are liberated and they hydrolyse (break down) the starch into soluble fermentable sugars like maltose, which is a glucose disaccharide. Similarly enzymes are widely used in wine production to obtain a better extraction of the necessary components and thus improving the yield.

Phytases

Phytases have important applications in human and animal nutrition because they hydrolyze the phytate present in legumes, cereal grains, and oil seeds. This results in an increased availability of minerals, trace elements and amino acids as well as phosphate. Phytates have been considered as a threat in human diet.
due to their antinutrients behavior, known as strong chelators of divalent minerals such as Ca2+, Mg2+, Zn2+, and Fe2+. Phytic acid is a major component of all plant seeds, constituting 1-3% by weight of many cereals and oilseeds and typically accounting for 60-90% of the total phosphorus. Phytates serve several physiological functions, especially in seed germination.

Phytic acid has a potential for binding positively charged proteins, amino acids, and/or multivalent cations or minerals in foods while preventing their assimilation through the digestive system. Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate) is the primary storage form of phosphate and inositol in plants and constitutes 3–5% of the dry weight of seeds in cereal grains and legumes. However, phytic phosphate cannot be digested by monogastric animals such as pig and poultry, and humans, because they lack the enzymes to hydrolyze phytate. The unabsorbed phosphorus passes into the environment through the feces and can be degraded by aquatic microorganisms, potentially causing eutrophication of water bodies downstream of agriculturally intensive areas. To alleviate the detrimental effects of high concentrations of phytate, swine, and poultry feed has been supplemented with phytase, which is the key biocatalyst for phytate degradation.

Phytases, which catalyze the hydrolysis of phytate into inorganic phosphate, inositol, and inositol mono- to pentaphosphates, appertain to the family of histidine acid phosphatases. Phytases are present in many plants and microorganisms (several bacteria and fungi). The biochemical properties of the phytases from thermophilic molds suggest that they are phosphate ester hydrolyzing enzymes. Phytases not only degrade phytic acid to increase the availability of phosphorus, but also improve the nutritional value of foods by making minerals such as calcium, iron, magnesium and zinc and proteins available for monogastrics. Thermophilic fungal phytases are known to hydrolyze insoluble phytates, to improve the bread-making process, to dephytinize food ingredients and to help in the promotion of plant growth.

**Proteases**

Proteases attack peptide bonds of proteins. The largest application of microbial proteases is in the laundry, in modern detergent formulations. They are used for removal of spots of milk, eggs, blood. Proteases are largely produced by *Bacillus licheniformis*. Other alkaline proteases are being developed using recombinant DNA technology, to function over a wide range of pH and temperature. On such recombinant .strain is Bacillus sp. Gx 6644, active for
milk casein. Another strain *Bacillus* sp. Gx 6638 produces several alkaline proteases. Through this technology a recombinant strain of a bacterium has also been developed. It produces the enzyme kerazyme, used for dissolving hair and opening hair clogged drains. In baking industry, also these enzymes have important application. Microbial proteases reduce mixing time and improve the quality of loaf. Fungal proteases are mainly obtained from *Aspergillus* spp. and bacterial from *Bacillus* spp. These enzymes are used as meat-tenderizer and in leather industry for bating of hides.

**Rennin-Chymosin**

Milk contains proteins, specifically caseins, which maintain its liquid form. Proteases are enzymes that are added to milk during cheese production, to hydrolyze caseins, specifically kappa casein, which stabilizes micelle formation preventing coagulation. Rennet and rennin are general terms for any enzyme used to coagulate milk. Technically, rennet is also the term for the lining of a calf’s fourth stomach. The most common enzyme isolated from rennet is chymosin. Chymosin can also be obtained from several other animal, microbial, or vegetable sources, but indigenous microbial chymosin from fungi or bacteria is ineffective for making cheeses. Site-specific proteolysis by chymosin detaches hydrophilic ‘tails’ of κ-casein resulting in coagulation (curdling) of milk proteins. It also traps bacteria that continue lactic acid. Calf chymosin (prochymosin) was cloned and expressed in *E. coli* to produce rennin (first genetically engineered or recombinant protein approved for human consumption). *Aspergillus* and *Candida* species are also known to produce rennin. Microbial rennets from various microorganisms under the trade names such as Rennilase, Fromase, Marzyme, and Hanilase being marketed since the 1970s have proved satisfactory for the production of different kinds of cheese.

**Sucrase**

Sucrase is the name given to a number of enzymes that catalyze the hydrolysis of sucrose to fructose and glucose. The enzyme invertase, which occurs more commonly in plants, also hydrolyzes sucrose but by a different mechanism. Sucrase is the enzyme that yeast use to digest sucrose into glucose plus fructose. Levansucrase (sucrose: 2,6-β-D-fructan 6-β-D-fructosyltransferase, EC 2.4.1.10), which synthesizes the polysaccharide levan from sucrose, is produced by a number of bacteria, including *Bacillus subtilis*1, *Aerobacter levanicum* (synonym: *Envinia herbicola*), *Gluconobacter oxydans*3, *Actinomyces viscosus*4, and others. Most of the original studies on this enzyme
were carried out on that from *Aerobacter levanicu*um, which has long been considered a constitutive, endocellular enzyme. Recent studies on levansucrase have focused on the extracellular enzyme from a constitutive mutant of *B. subtilis*.

**Tannase**

Tannin acyl hydrolase (TAH), also known as tannase, is an enzyme (EC 3.1.1.20) that catalyzes the hydrolysis of ester bonds present in gallotannins, complex tannins, and gallic acid. Tannase or tannin acyl hydrolase catalyzes the hydrolysis of the ester bonds present in the hydrolysable tannins and gallic acid esters. The major applications of tannase are in the elaboration of instantaneous tea and acorn liquor, as well as in the production of gallic acid from tannin-rich agrowastes. Tannase is also utilized as clarifying agent in wine, beer, fruit juices, and coffee-flavored soft drinks, feed, chemical and pharmaceutical industries. Moreover, it has been proposed the use of this enzyme for bioremediation of effluents from tanneries to improve the nutritional properties of tannin-rich forage. Tannase (tannin acyl hydrolase) hydrolyzes various gallic acid (GA) esters, such as gallotannin and tannic acid, which have strong protein-binding properties. One of the major commercial applications of tannase is the hydrolysis of tannic acid to gallic acid, a key intermediate required for the synthesis of an antibiotic drug, trimethoprim. Tannase is an extracellular, inducible enzyme produced in the presence of tannic acid by various fungi, bacteria, and yeast.

**Xylanase**

Xylanase is an industrial enzyme used in pulp and paper industry and needs to be stable at high temperature. Treatment of xylan (dominating component of hemicelluloses) with xylanase at elevated temperatures and basic pH disrupts the cell wall structure. Hydrolysis of hemicelluloses requires various types of enzymes. Briefly, xylan degradation requires endo-1-4-β-xylanase, β-xylanosidase, α-glucuronidase, α-L-arabinofuranosidase, as well as acetylxylan esterases. In glucomannan degradation, β-mannanase and β-mannosidase are required to cleave the polymer backbone. In the baking industry, xylanases are used for improving desirable texture, loaf volume, and shelf life of bread.

Thermostable xylanase were isolated from a number of bacterial and fungal sources. Members of the *Bacillus* sp., *Streptomyces* sp., *Thermoascus aurantiacus*, and *Fusarium proliferatum* produce xylanases that are active at
temperatures 50–80 °C. While the *Dictyoglomus* sp. were described to produce xylanases operating at an optimum temperature of 90 °C, a number of *Thermotogales* sp. were reported to secrete thermostable xylanases which can function at higher temperatures. Alkaliphilic and cellulase-free xylanases with an optimum temperature of 65 °C from *Thermoactinomyces thalophilus* and cellulase-free xylanases from *Clostridium abusonum* were also reported recently.

**Fibrolytic Enzymes**

Recent studies have shown that adding exogenous fibrolytic enzymes to ruminant diets increases milk production in some cases. These increases in animal performance are due to increases in feed digestion. Improvements in animal performance due to the use of enzyme additives can be attributed mainly to improvements in ruminal fiber digestion resulting in increased digestible energy intake. Cellulose and hemicellulose, the major structural polysaccharides in plants, are converted to soluble sugars by enzymes collectively referred to as cellulases and hemicellulases.

The major enzymes involved in cellulose hydrolysis are endocellulases, exocellulases, and β-glucosidase. In general, endoglucanases hydrolyze cellulose chains at random to produce cellulose oligomers of varying degrees of polymerization; exoglucanases hydrolyze the cellulose chain from the non-reducing end, producing cellobiose, and β-glucosidases hydrolyze short-chain cellulose oligomers and cellobiose to glucose. The main enzymes involved in degrading the xylan core polymer to soluble sugars are xylanases (EC 3.2.1.8) and β-1,4-xylosidase (3.2.1.37). The xylanases include endoxylanases, which yield xylooligomers and β-1,4-xylosidases, which in turn yield xylose. Other hemicellulase enzymes involved primarily in the digestion of side chains include β-mannosidase (3.2.1.25), α-L-arabinofuranosidase (3.2.1.55), α-D-glucuronidase (3.2.1.139), α-D-galactosidase (3.2.1.22), acetyl xylan esterases (3.1.1.72), and ferulic acid esterase (3.1.1.73).

**Lignolytic Enzymes**

Lignocellulosic biomass is a renewable and abundant resource with great potential for bioconversion to value-added bio-products. However, the bio-refining process remains economically unfeasible due to a lack of biocatalysts that can overcome costly hurdles such as cooling from high temperature, pumping of oxygen/stirring, and, neutralization from acidic or basic pH. Some enzymes offer a framework for enhancement of cellulases including specific
activity, thermal stability, or end-product inhibition. Lignocellulosic materials are composed primarily of cellulose, hemicellulose, and lignin due to photosynthetic activity of the plants. Lignocellulose is the major structural component of woody plants and non-woody plants such as grass and represents a major source of renewable organic matter. Lignocellulytic enzymes also have significant potential applications in various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper, and agriculture.

Lignin is a recalcitrant heteropolymer of phenylpropanoid units present in woody plant tissues, that confers them rigidity and resistance to biological attack. Lignin surrounds the cellulose and forming physical barrier restricting microbial enzyme attacks. In order to depolymerize and mineralize lignin, white-rot fungi consist of a group of basidiomycetes have developed an oxidative and unspecific system including extracellular enzymes including ligninolytic peroxidases, laccases and oxidases and other subsidiary enzyme such as AAO, Glox, etc., responsible for the production of extracellular hydrogen peroxide (H₂O₂). Among peroxidases, lignin peroxidase (LiP) is able to oxidize non-phenolic units whereas manganese peroxidase (MnP) and laccase preferentially oxidize phenolic units, but also act on non-phenolic units in the presence of mediators.

**Fibrinolytic Enzymes**
Fibrinolytic enzymes prevent or cure thrombotic diseases by degrading the fibrin in the blood clot. Fibrin is the main protein component of the blood clot, and is normally formed from fibrinogen by the action of thrombin (EC 3.4.21.5). Accumulation of fibrin in the blood vessels usually results in thrombosis, leading to myocardial infarction and other cardiovascular diseases.

Current thrombolytic therapies via injecting or orally administrating thrombolytic agents to lyses thrombi in blood vessels have been extensively investigated. Agents for therapeutic purposes include urokinase, a tissue-type plasminogen activator, and streptokinase. Despite their widespread use, all of these agents have undesired side effects and they are considered very expensive. Therefore, the search for fibrinolytic enzymes from various other sources is being continued. Microorganisms are important resources for thrombolytic substances. Various fibrinolytic enzymes were successively discovered from different microorganisms, including bacteria, actinomycyes, fungi, and algae. Among these, the most important one was bacilli fibrinolytic enzyme from different fermented foods.
Fibrinolytic enzymes were identified and studied among many organisms including snakes; earthworms; bacteria like *Streptococcus pyogenes, Aeromonas hydrophila, Serratia E15, B. natto, Bacillus amyloliquefaciens*; Actinomycetes; and fungi *Fusarium oxysporum, Mucor sp, Armillaria mellea*.

Some fungi and actinomycetes isolates have been investigated at AAU for their potential to produce enzymes. However, the studies were *at in vitro* level and did not attempt to develop mass production protocols for the enzyme-producing microbes, meaning the enzymes are not yet utilized at larger scale.
Industrial Biotechnology

MICROORGANISMS can function as efficient factories for the industrial scale production of the various primary metabolites. These include organic solvents/alcohols and acids, amino acids, vitamins, food additives (flavoring agents, sweeteners). The so-called ‘white’ biotechnology is a broad and expanding field that makes use of new enzymes for a variety of industrial uses, embraces the manufacture of non-oil-based and biodegradable bioplastics and biofuels, as well as artificial fibers, and encompasses many treatments of wastes and abatement of pollution, using microorganisms and plants, known as bioremediation.

Chemicals

Production of Organic Solvents

Most organic solvents are chemically synthesized. But a few solvents can also be produced commercially by microbial fermentation. Alcohol is an important solvent and raw material used in a variety of chemical industries. Although today industrial alcohol is also produced synthetically from ethylene, production of alcohol by fermentation of cheap sugary materials such as molasses by yeast is still an important industry.

Ethanol

Production of ethyl alcohol from sugary materials is one of the oldest known microbiological processes. Ethanol production by microbes has become very popular in those areas where plant residues (agricultural and other wastes) are available in abundance. For ethyl alcohol production, selected strains of
Saccharomyces cerevisiae are employed since not all the strains are equally efficient. Other efficient microbes are Zymomonas mobilis (fermenting carbohydrate and producing alcohol twice as rapidly as yeasts) and Thermoanaerobacter ethanolicus, a thermophilic bacterium. Corn sugar and plant starch are used as substrates. Two-step fermentation is used for conversion of cellulose to ethanol: (i) conversion of cellulose to sugars, generally by Clostridium spp., followed by (ii) conversion of these sugars to ethanol by yeasts, Zymomonas, or Thermoanaerobacter spp.

In recent years, because of the possibility of using ethyl alcohol as a fuel supplement and a chemical feedstock, there is an increased interest in increasing production but at a cheaper and economical rate. Gasohol, a 9:1 blend of gasoline and ethanol, has become popular fuel. Ethanol, although produced by fermentation for beverages and gasohol, industrial alcohol for use as solvent is mostly produced chemically.

A large amount of carbon dioxide is also produced during the fermentation, which is purified and compressed. In some distilleries, the yeast is recovered and used as animal feed while in most, it is discarded into the effluents, a procedure that is very undesirable. For this, a variety of improvements in the traditional batch fermentation has been described in literature. Among these, the one that has attracted attention is the cell recycle technique, which does not involve additional expenditure.

**Acetone-butanol fermentations**
The acetone butanol fermentation is one of the oldest fermentation known. The fermentation is based on culturing various strains of Clostridia in carbohydrate rich media under anaerobic conditions to yield butanol and acetone. Clostridium acetobutylicum is the organism of choice in the production of these organic solvents. Another species, C. saccharoacetobutylicum is able to convert the carbohydrates in molasses to acetone and butanol. These bacteria first synthesize acids (acetic and butyric) which are then converted to acetone and butanol. The solvents produced by fermentation are recovered by distillation.

**Glycerol**
Glycerol is an important solvent in flavoring and food coloring, and is used in production of explosives and propellants. Microbial production uses addition of sodium sulphite to a yeast-ethanol fermentation process. Sodium sulphite reacts with CO$_2$ to produce sodium bisulphite, which prevents the reduction of
acetaldehyde to ethanol. *Saccharomyces cerevisiae* and bacteria as *Bacillus subtilis* are used.

**Organic Acids**  
Many organic acids are used in foods such as acetic, lactic, citric, and malic, gluconic, itaconic, fumaric, ascorbic acids and monosodium glutamate, etc., which are all produced by microbial fermentations.

**Acetic acid fermentation**  
Acetic acid is produced by the oxidation of ethyl alcohol by bacteria such as *Acetobacter* species and the production of vinegar from fruit juices is perhaps one of the oldest organic acid fermentations known. Vinegar contains 4% to 8% acetic acid and is prepared from wine or other dilute solutions of alcohol. Acetic acid endows vinegar with its characteristic odor and sour taste. Acetic acid bacteria belonging to the genera *Acetobacter* and *Gluconobacter* are unique organisms that tolerate high acetic acid and ethanol concentrations. The commercial production of vinegar is still an empirical process, which involves a preliminary fermentation of the fruit juice to produce ethyl alcohol and its secondary fermentation into acetic acid under aerobic conditions. Various species of *Acetobacter* have the ability to oxidize alcohol to acetic acid.

**Lactic acid fermentation**  
Lactic acid is produced from various carbohydrates such as cornstarch, potato starch, molasses, and whey. When starchy materials are used, they are first hydrolyzed to simple sugars. The medium is then supplemented with a nitrogen source and calcium carbonate acid fermentation is carried out by the inoculation with homofermentative lactobacilli such as *L. bulgaricus* or *L. delbruckli*. After the completion of the fermentation (4–6 days), the fermented liquor is heated to 82 °C and filtered. The filtrate containing calcium lactate is spray dried after treating with sodium sulfide. To obtain lactic acid, the calcium lactate is treated with sulphuric acid and the lactic acid thus obtained is further purified.

**Gluconic acid fermentations**  
Gluconic acid used in pharmaceutical industries (carrier of Ca and Na), is produced by the fermentation of glucose either by strains of *Aspergillus niger*, *Penicillium* sp., or selected bacteria. In the commercial process, a nutrient solution containing 24–38% glucose, corn steep liquor, a nitrogen source and salts, with pH 4.5 is used to culture a selected strain of fungus in shallow pans or in submerged culture conditions to convert glucose into gluconic acid. The
pH of the medium is controlled by the addition of a strong solution of sodium hydroxide. Fermentation is carried out at 33 or 34 °C.

**Citric acid fermentations**
Citric acid, which is a key intermediate of the TCA cycle is produced by fungi, yeast and bacteria as an overflow product due to a faulty operation of the citric acid cycle. The ability of fungi to produce citric acid was first discovered by Wehmer in 1893 and today all the citric acid commercially produced comes from the mold fermentation. It is used in pharmaceuticals and food additives. Among the organisms used for citric acid production, *A. niger* has been the mold of choice for several decades. A variety of carbohydrate sources such as beet molasses, cane molasses, sucrose, commercial glucose, starch hydrolysates, etc., have been used for citric acid production. Among these, sucrose, cane, and beet molasses have been found to be the best. For citric acid production, the raw material is diluted to 20–25% sugar concentration and mixed with a nitrogen source and other salts. The pH of the medium is maintained around 5 when molasses is used and at a lower level (pH 3.0) when sucrose is used.

**Itaconic acid fermentation**
Itaconic acid is used as a resin in detergents. The transformation of citric acid by *Aspergillus terreus* can be used for commercial production of itaconic acid. Fermentation process involves a well-aerated molasses-mineral salts medium at a pH below 2.2. At higher pH, this microbe degrades itaconic acid. Like citric acid, low levels of trace metals must be used to achieve acceptable product yields.

**Gibberellic acid fermentation**
Gibberellic acid and related gibberellins are important growth regulators of plants. Commercial production of these acids helps in boosting agriculture. This acid is formed by the fungus *Gibberella fujikuroi* (imperfect state, *Fusarium moniliforme*) and can be produced commercially using aerated submerged cultures. A glucose-mineral salt medium, incubation at 25 °C and slightly acidic pH are used for fermentation. It takes normally 2–3 days.
Amino Acids, Vitamins, Food additives, and Flavoring agents

Biotechnology in the fermentation-processing sector makes use of microbial cultures for the production of a range of high value-added products such as flavor compounds, fragrances, vitamins, amino acids and food thickeners (microbial polysaccharides) and other food additives and ingredients. These high value products are increasingly produced in more technologically advanced developing countries for use in their food and non-food processing applications. Many of these high value products are also imported by developing countries for use in their food processing applications.

Amino Acid Fermentations
Amino acids produced through biotechnological processes are also of great interest as building blocks for active ingredients used in a variety of industrial processes. In recent years, there has been a rapid development of the production of particular amino acids by fermentation. Microorganisms can synthesize amino acids from inorganic nitrogen compounds. The rate and the amount of synthesis of some amino acids may exceed the cells need for protein synthesis, whereupon the amino acids are excreted into the medium. Some microorganisms are capable of producing sufficient amounts of certain amino acids, to justify their commercial production. The amino acids can be obtained from hydrolyzing protein or from chemical synthesis, but in several instances, the microbial process is more economical. Secondly, the microbiological method yields the naturally occurring L-amino acids. Amino acids have been used as flavor enhancers, sweeteners, antioxidants, and nutritive additives.

Vitamins Production by Fermentations
Vitamins are essential micronutrients for the metabolism of all living organisms, and they are synthesized by microorganisms and plants. Most vitamins and related compounds/coenzymes are now industrially produced and widely used as food or feed additives, medical or therapeutic agents, health aids, cosmetic and technical aids, and so on. Many biotechnological production processes (i.e., fermentation and microbial/ enzymatic transformation) as well as organic chemical synthetic ones have been reported.

Water-soluble vitamins
- riboflavin (vitamin B2);
- nicotinic acid, nicotinamide;
- pantothenic acid;
- pyridoxine (vitamin B6);
- biotin;
- vitamin B12;
- L-ascorbic acid (vitamin C);
- adenosine triphosphate and related nucleotides; and
- S-adenosylmethionine and related nucleosides

**Fat-soluble vitamins:**
- vitamin A (retinoids) and β-carotene (provitamin A);
- vitamin D;
- tocopherols (vitamin E);
- polyunsaturated fatty acids (vitamin F group);
- vitamin K compounds;
- ubiquinone Q (coenzyme Q).

**Food Additives**
A food additive is a substance not consumed as a food and not used as an ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in manufacturing, processing, preparing, treating, packaging, transporting or storing of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods. Many food additives are naturally occurring and some are even essential nutrients. It is the technical purpose that leads to these being classified as food additives and given an E number. Food additives play an important role in today's complex food supply as they carry out a variety of useful functions.

**Additives that maintain freshness and prevent deterioration**
Some food additives help keep foods fresh and safe as they help increase shelf-life. They can be divided into two categories based on their principal function:

- **Antioxidants:** prevent the oxidation of foods that results in rancidity or discoloration (used in baked foods, cereals, fats, oils and salad dressings)
- **Preservatives:** limit, retard, or arrest the growth of microorganisms that are present in or gain entry to the food, preventing spoilage or food poisoning (used in baked foods, wine, cheese, cured meats, fruit juices and margarine).

**Additives that amplify or promote sensory qualities**
Additives are also useful for imparting certain characteristics to foods, improving texture or helping in food processing.
Taste and texture modifiers: emulsifiers and stabilizers, thickeners, sweeteners, flavor enhancers; and
Colors: one of the first and most important sensory qualities; helps accept or reject a particular food item

Flavor/Aroma Chemicals and Sweeteners

Flavor is usually the result of the presence, within complex matrices, of many volatile and nonvolatile components possessing diverse chemical and physicochemical properties. Whereas the nonvolatile compounds contribute mainly to the taste, the volatile ones influence both taste and aroma. A vast array of compounds may be responsible for the aroma of the food products, such as alcohols, aldehydes, esters, dicarbonyls, short- to medium-chain free fatty acids, methyl ketones, lactones, phenolic compounds, and sulphur compounds. Volatile organic chemicals such as flavors and aromas are the sensory principles of many consumer products and govern their acceptance and market success.

Flavors produced using microorganisms currently compete with those from traditional agricultural sources. The metabolic end products produced by the microorganisms flavor fermented foods. For instance, mold-ripened cheeses owe their distinctive flavors to the mixture of aldehydes, ketones, and short-chain FA produced by the fungi. More than 100 commercial aroma chemicals are derived either through the screening for overproducers, the elucidation of metabolic pathways and precursors or through conventional bioengineering. LAB influences the flavor of fermented foods in a variety of ways.

Polysaccharides

Some bacterial species secrete mucoid substances of high molecular weight. When these viscous materials remain associated with the cell, they are called variously capsules, sheaths, or slime layer. Most of the extracellular polymers produced by bacteria are polysaccharides, although a few bacteria produce capsules made up of polypeptides of D-amino acid residues.

Polysaccharides are used to modify the flow characteristics (rheology) of fluids, to stabilize suspensions, to flocculate particles, to encapsulate materials, and to produce emulsions. Among many other examples is the use of polysaccharides as ion-exchange agents in chromatography and electrophoresis, as molecular
sieves, and, in aqueous solution, as hosts for hydrophobic molecules. Polysaccharides are used in enhanced oil recovery and as drag-reducing agents for ships. Only one microbial polysaccharide, xanthan, ranks among the 10 industrial polysaccharides utilized in the largest amount:

- xanthan is produced by Xanthomonas campestris and consist of a pentasaccharide repeating unit containing glucose, mannose, glucuronic acid, and acetyl and pyruvate substituent, which is used as emulsion stabilizer and thickener for foods and beverages; and emulsion stabilizers for pharmaceuticals and cosmetics; and
- dextran is produced by Acetobacter sp. Leuconostoc mesenteroides Streptococcus mutans and consists of polyglucose linked by α1,6-glycosidic bonds; some 1,2-, 1,3-, or 1,4-bonds are also present in some dextrans.

Among polysaccharides produced by bacteria and fungi, some have properties resembling those of agar whereas others have distinctive rheological (flow) properties valuable in certain pharmaceutical or industrial applications. Agar is a mixture of polysaccharides extracted from marine red algae, used to solidify a media while growing cells of microorganisms and plants.

Marine bacteria polysaccharides are another class of pharmaceutically important secondary metabolites. It has been found that exopolysaccharides (EPS) from marine bacteria contain novel formulations with a variety of properties like thickening, coagulating, adhesion, stabilizing, and gelling, which makes them suitable candidates for industrial applications.

**Biopolymers and Bioplastics**

Bacteria can synthesize a wide range of biopolymers that serve diverse biological functions and have material properties suitable for numerous industrial and medical applications. Over 250 different bacteria species have been reported to accumulate various polyhydroxyalkanoates (PHAs) during depletion of essential nutrients such as N, P or Mg and accumulation of excess C (glucose). The discovery that many bacteria synthesize large amounts of biodegradable polyester polymers of high MW, which can be used to manufacture plastics, has aroused considerable interest. Bacteria differ from one another in the type of reserve material they accumulate when they are grown with unbalanced supplies of nutrients. Many bacteria will accumulate glycogen and/or aliphatic polyesters, (PHAs), in amounts of 30–80% or more of their cellular dry weight. PHA are synthesized and intracellularly accumulated in most bacteria under unfavorable growth condition that can be extracted.
Polyester
The biopolymer poly-3-hydroxybutyrate (PHB), polyester that belong to PHAs, is produced by certain bacteria processing glucose, corn starch or wastewater. Its characteristics are similar to those of the petroplastic polypropylene. Some bacteria have been reported capable to produce PHA as much as 90% (w/w) of dry cells.

Polyethylene
The basic building block (monomer) of polyethylene is ethylene. This is just one small chemical step from ethanol, which can be produced by fermentation of agricultural feedstocks such as sugar cane or corn. Bio-derived polyethylene is chemically and physically identical to traditional polyethylene—it does not biodegrade but can be recycled. It can also considerably reduce greenhouse gas emissions.

Some of the applications of affordable biodegradable PHA polymers are

- as adhesives, absorbents, lubricants, soil conditioners, cosmetics, moisture barrier films;
- disposable utensils and dishes for use in the food industry;
- plastic wrap;
- coatings for paper products;
- components of fabrics for the textile industry;
- slow-release formulations for pesticides and fertilizers; and
- medical devices such as bone screws, pins, surgical sutures, stents, patches, controlled drug delivery devices.

Production of the primary metabolites and fine chemicals was not attempted but some dietary ingredients are produced during food fermentation process. The nutritional differences of diets are not related to the types of microbes involved in food processing. All additives are imported from abroad and used in the industries. Methods are not developed to enrich foods with key ingredients in the industries.
Biofuel Production

ENERGY can be extracted from biomass either by direct combustion or by first converting the biomass to another fuel and then combusting it. Biofuel production is part of ‘white’ biotechnology. Biofuels are drawing increasing attention worldwide as means of ‘modernizing’ the biomass, and providing greater access to clean liquid fuels and use substitutes for petroleum-derived transportation fuels to help address energy cost, energy security and global warming concerns associated with liquid fossil fuels (petroleum fuels).

Biological conversion of biomass to biofuel is well established, with the two main routes being fermentation and anaerobic digestion. Microbes produce fuels directly from biomass. Microbes convert biomass into chemicals that can be used to transport biofuels.

Useful fuels produced by microbes include ethanol, methanol, methane, hydrogen, and hydrocarbons. For microbial production of fuels, waste materials such as sewage and municipal garbage are used as fermentation substrates. Right strains are able to do the job. Biofuels are grouped in ‘generations’ according to the type of the technology they rely on and the biomass feedstock they convert into fuel. They are grouped as 1st, 2nd, 3rd, 4th or New Generation biofuels, in which microbes are involved in all of these technologies.

Generations of Biofuels

First-Generation Biofuels
They are obtained from food crops. The food crop and yeast could be mixed in a bioreactor for fermentation, which produces ethanol. Ethanol is used as fuel,
along with water and carbon dioxide byproducts including petroleum-gasoline substitutes—ethanol or butanol by fermentation of starches or sugar. Petroleum diesel also substitutes biodiesel by transesterification of plant oils, also called fatty acid methyl ester (FAME) and fatty acid ethyl ester (FAEE) from rapeseed (RME), soybeans (SME), sunflowers, coconut, palm, jatropha, recycled cooking oil and animal fats, and pure plant oils (straight vegetable oil).

**Second-Generation Biofuels**

Obtained from non-food crops such as lignocellulosic biomass as feedstock—found in crop residues, woody crops, or energy grasses. Cellulose from plant sources such as sawgrass is mixed in a bioreactor with yeast or bacteria for fermentation to produce ethanol, butanol, or other compounds for use as fuel, along with water and carbon dioxide byproducts like

- biochemically produced petroleum-gasoline substitutes—ethanol or butanol by enzymatic hydrolysis;
- thermochemically produced petroleum-gasoline substitutes (methanol, Fischer-Tropsch gasoline, mixed alcohols); and
- thermochemically produced petroleum-diesel substitutes—Fischer-Tropsch diesel, dimethyl ether (also a propane substitute).

**Third-Generation Biofuels**

Rely on biotechnological interventions in the feedstocks themselves. Rather than improving the fuel making process, they improve the feedstock. Plants are engineered in such a way that the structural building blocks of their cells (lignin, cellulose, hemicellulose), could be managed according to a specific task they are required to perform. Alternatively, when photosynthetic algae or cyanobacteria are exposed to sunlight and carbon dioxide, they produce and stockpile fats inside their cells. Exposing the cells to a chemical solvent frees these fat molecules, which can be refined into biodiesel.

**Fourth-Generation Biofuels**

They combine genetically optimized feedstocks, which are designed to capture large amounts of carbon with genomically produced microbes, which are made to make fuels. Genetically engineered photosynthetic cells under development, when exposed to sunlight and carbon dioxide, could produce and secrete energy-rich fats, which could then be refined directly into biodiesel fuel.
Microbial Production of Some Biofuels

Bio-ethanol

The most well known first-generation biofuel is ethanol made by fermenting sugar extracted from sugar cane or sugar beets, or sugar extracted from starch contained in maize kernels or other starch-laden crops. Sugar and starch crops provide the main feedstocks for the process of fermentation, in which a catalyst is used to convert the sugars into an alcohol, more commonly known as bio-ethanol. Glucose is the preferred substrate and conversion of other carbohydrates such as those in lignocellulose into ethanol requires genetic engineering of novel yeast and bacterial strains. Cellulose, hemicelluloses, and starches are a vast renewable source of sugars convertible to ethanol by microbial fermentation.

Lignocellulosic conversion would greatly increase the supply of raw materials available for bio-ethanol production. Lignocellulose biomass includes:

- agricultural waste such as straw, corn stover and bagasse;
- industrial waste such as sawdust and paper pulp;
- woody biomass from forestry;
- municipal solid waste including food and garden waste and paper product; and
- specific non-food energy crops such as switchgrass.

Saccharomyces strains produce high concentrations of alcohol with low levels of byproducts, and have the high cell viability and flocculation characteristics needed for repeated cell recycling. The waste stream from bio-ethanol production, known as vinasse, can be further converted through anaerobic digestion, creating a further step in a ‘cascade’ of energy extraction processes.

Biobutanol

Biobutanol is now recognized as a superior biofuel to ethanol. In addition to the beneficial fuel properties, the solventogenic clostridia used in the fermentation also have an inherent advantage. Clostridia can utilize a wide variety of carbohydrates, allowing all the carbohydrates, in addition to glucose, in biomass to be converted to fuel.

Methane

Methane produced by methanogenic bacteria can be used directly for cooking or heating or it can be used for electricity and/or heat production. It is used for generation of mechanical, heat, and electrical energy. Anaerobic decomposition
of waste materials produces large amounts of methane. Many sewage treatment plants produce this fuel. Efficient generation of methane can be achieved by using algal biomass grown in pond cultures, sewage sludge, municipal refuse, plant residue, and animal waste. Methanogens (archaebacteria) are obligate anaerobes and produce CH4 by reducing acetate and or CO2.

**Hydrogen**

Hydrogen gas is seen as a future energy carrier by virtue of the fact that it is renewable, does not evolve the ‘greenhouse gas’ CO2 in combustion, liberates large amounts of energy per unit weight in combustion, and is easily converted to electricity by fuel cells. Biological hydrogen production has several advantages over hydrogen production by photoelectrochemical or thermochemical processes. Biological hydrogen production has been achieved using biophotolysis of water by microalgae and cyanobacteria (hydrogenase or nitrogenase dependent), photosynthetic bacteria and genetically engineered bacteria. Biological hydrogen production is the most challenging area of biotechnology with respect to environmental problems. It depends on not only research advances, i.e. improvement in efficiency through genetically engineering microorganisms and/or the development of bioreactors, but also on economic considerations (the cost of fossil fuels), social acceptance, and the development of hydrogen energy systems.

**Biogas**

Biogas is obtained by anaerobic treatment of manure and other humid biomass materials; for example, in landfills, including food waste, and then upgraded to biomethane that can be feed-in into the natural gas grid and, for example, used in natural gas vehicles. Biogas, a mixture of different gases, is produced by anaerobic microbes using domestic and agricultural wastes. Bulk (50–70%) of biogas is CH4 and other gases are in low proportions. These include CO2 (25–35%), H2 (1–5%), N2 (2–7%) and O2 (0–0.1%). Leftovers of these plants are good fertilizers also. Cattle dung is the chief source of biogas. Animal waste is first hydrolyzed by hydrolytic bacteria. It is followed by acid formation by a group of acetogenic bacteria, which convert monomers into simple compounds like NH3, CO2 and H2. Finally methanogens reduce acetate and or CO2, to CH4.

Other fuels include hydrogen that could be developed as a major fuel produced by microbes in future. Photosynthetic microbes produce H2. They are able to convert solar energy into a fuel that can be stored.

**Next-Generation Biofuels Produced by Microbes**
Microbes can be engineered to produce biologically derived replacements for gasoline, diesel, and aviation fuel. Although much research has focused on ethanol as a bio-gasoline, many other biofuels offer advantages such as high energy density, low freezing point, and compatibility with the existing fuel storage and distribution infrastructure. Next-generation biofuels, such as long-chain alcohols, fatty-acid-derived, and isoprenoid-derived fuels offer promise as new biofuels and can be synthesized by microbes. These fuels are being developed as either supplements or drop-in replacements for existing petroleum fuels. Because there is active research on many next-generation fuels, this review highlights general tolerance strategies and discusses areas where mechanisms may only work classes of fuels.

Next-generation biofuels have many advantages, but the fuels are often toxic to microorganisms. Therefore, the inherent tolerance of the host may limit production potential. Microbes that can survive in hydrocarbon-rich environments have been isolated; however, these strains are rarely suitable for use as biofuel production hosts. Recent efforts have suggested that it may be possible to transfer tolerance mechanisms to a suitable production strain. The ideal host is a well-studied organism with good genetic tools available that can be engineered for both biofuel production and tolerance.

*E. coli* is a well-studied microorganism whose natural ability to synthesize fatty acids and exceptional amenability to genetic manipulation make it an ideal target for biofuels research. The biosynthesis of biodiesel-adequate fatty acid ethyl ester (FAEEs) referred to as microdiesel, in metabolically engineered *E. coli*. This was achieved by heterologous expression in *E. coli* of the *Zymomonas mobilis* pyruvate decarboxylase and alcohol dehydrogenase and the unspecific acyltransferase from *Acinetobacter baylyi* strain ADP1. By this approach, ethanol formation was combined with subsequent esterification of the ethanol with the acyl moieties of coenzyme A thioesters of fatty acids if the cells were cultivated under aerobic conditions in the presence of glucose and oleic acid. Ethyl oleate was the major constituent of these FAEEs, with minor amounts of ethyl palmitate and ethyl palmitoleate. Some rural communities are engaged in biogas production with the support of the Ministry Energy and some NGOs such as GIZ. Biogas production was carried out spontaneously without using any selected microbial inoculants that can maximize the yield. Ethanol is produced in most of the sugar estates from molasses.
Environmental Applications of Microbial Biotechnology

MICROORGANISMS are exposed to a variety of organic compounds in the natural environment, those derived from living organisms, and those generated by geochemical processes. Virtually every one of these myriad naturally occurring compounds is utilized as a source of energy and/or carbon by some microorganism. Microorganisms excel at using organic substances, natural or synthetic, as sources of nutrients and energy. Microorganisms mitigate a multitude of impacts that result from human use of the natural resources of the planet. The spectacular combined metabolic versatility of naturally occurring communities of microorganisms (bacteria and fungi) is exploited in some areas of environmental biotechnology: sewage and wastewater treatment, degradation of xenobiotics, mineral recovery, petroleum recovery, microbial desulfurization of coal, degradation of pulpwood and retting in textile industry.

Wastewater and Sewage Treatment

Wastewater originates from four primary sources: sewage, industrial effluents, agricultural runoff, and storm water and urban runoff. Treatment of wastewater is essential to prevent contamination of drinking water and the entry of pathogens and contaminants into the food chain. The microbial communities in a water treatment plant convert organic carbon to carbon dioxide, water, and sludge; convert some 80% of the ammonia and nitrate to molecular nitrogen; remove some soluble phosphate through incorporation into the sludge, either as polyphosphate granules within bacterial cells or as struvite (crystalline MgNH₄PO₄); and remove pathogenic bacteria. In sewage and wastewater treatment, near-complete mineralization of natural products through aerobic and anaerobic degradation by many different microorganisms leads to the reclamation of pure water. Many human-made chemicals (phenol,
chlorobenzenes) present in wastewater are also efficiently mineralized by this treatment. Others, however, are not completely degraded. For example, branched alkylbenzene sulfonate detergents, which are toxic to aquatic organisms, are only slowly degraded during sewage treatment. This example shows that a compound may be biodegradable, yet may not be degraded sufficiently and rapidly in a treatment facility or in the environment to prevent undesirable effects.

EM has been effectively used for treatment of wastes, effluents, and management of odor in many countries. EM is used in the integrated livestock for animal feed as well as sprayed for sanitation purpose within animal farms, poultry, swine, and fish farms to control foul and bad odors. In Ethiopia, EM produced by Wolejeeji Agro-Industry PLC has been demonstrated for treatment of coffee effluents and municipal wastes.

Treatment of Water Bodies

Marine biotechnology explores oceans, seas, ponds to develop novel pharmaceuticals drugs, chemical products enzymes, other industrial products, and processes. It also plays a vital role in the advancement of biomaterials, healthcare diagnostics, aquaculture and fishery, seafood safety, bioremediation and biofouling. Marine bacteria not only produce secondary metabolites against other organisms, but they also produce certain compounds, which help in cleaning their environment. Certain marine bacterial species are known as prolific producers of biosurfactants, bioemulsifiers, and exopolysaccharides. Degradation of a model polyaromatic hydrocarbon, anthracene, an organic pollutant, by a marine Bacillus circulans strain was studied. Marine fungi are capable of producing not only antimicrobial but also antifouling compounds, ensuring environmental hygiene (i.e. preventing the assemblage of marine organisms on human-made structures and devices submerged in the sea).

Bioremediation, Biodegradation, and Biotransformation

Natural biological and geochemical processes produce enormous quantities of organic compounds with a great diversity of structures and impacts. Cleaning up environmental pollutants like oil, gas, and heavy/toxic metals is a considerable challenge. However, biotechnology is coming to the aid of the environment through the development of bioremediation and biosorption, the
use of living organisms to return the environment to its natural state. Some microbes have an appetite for gas, oil or other toxic chemicals or xenobiotics that enter the environment through many different paths such as fertilizers, pesticides, combustion processes, and waste effluents. When the chemicals are degraded or absorbed and accumulated by the microbes, the chemicals’ structure can undergo changes. Bioremediation depends on the activities of living organisms to clean up pollutants dispersed in the environment. Physical or chemical treatments, such as vaporization, extraction, or adsorption, relocate rather than remove pollutants. In contrast, there are many instances in which the use of the right microbes, toxic, and sometimes explosive materials and organic pollutants can be converted into something harmless inorganic products like water, carbon dioxide and halide ions. Other advantages are that bioremediation is generally inexpensive and causes little disturbance to the environment. Naturally, occurring consortia, frequently dominated by bacteria, have the capacity to degrade a wide spectrum of environmental pollutants.

**Bioremediation**

Bioremediation is a key area of white biotechnology, because the elimination of a wide range of pollutants from water and soils is an absolute requirement for sustainable development. There are numerous processes of cleaning water, industrial effluents, and solid wastes, using microorganisms aerobically and anaerobically. Some of them are quite sophisticated, while others are simple and adapted to the conditions of developing countries. For instance, using microalgae, particularly blue-green algae or cyanobacteria, in ponds to eliminate nitrogen and phosphorous, after organic matter has been degraded by bacteria, leads to water that can be recycled for irrigating non-food crops (e.g. cotton) or for industrial purposes; in addition, microalgal biomass can be used as feed.

Bioremediation is a clean-up technology that uses naturally occurring microorganisms to degrade hazardous substances into less toxic or nontoxic compounds. Degradation means decay, and the prefix bio- means that the decay is carried out by a huge assortment of bacteria, fungi, maggots, worms, and other organisms that eat dead material and recycle it into new forms. Because the microorganisms already occur naturally in the environment, they pose no contamination risk. These microorganisms may:

- ingest and degrade organic substances as their food and energy source; and
• degrade organic substances, such as chlorinated solvents or petroleum products, that are hazardous to living organisms, including humans, and degrade the organic contaminants into inert products.

Metagenomics
Cataloguing the microflora of environments could help understand environmental problems such as carbon dioxide take up by bacteria to reduce its concentration, the breakdown of pollutants by microorganisms. It could also help detect environmental damage, because once we know what it is that is out there, it is a step to be able to determine any changes to the environment. After sequencing the DNA of single species—there are complete sequences for more than 300 organisms, researchers are trying to sequence the DNA of living species in defined environments—a science called environmental genomics, or metagenomics. Through better understanding of how myriads of bacteria combine and interact to influence the ocean, we shall be better able to monitor the conditions of these environments and possibly manipulate them to our advantage. It may even be possible to find microorganisms that can produce drugs or act as energy sources.

Biotransformation
In the context of environmental biotechnology, it refers to a process of biological changes of complex compound to simpler toxic to non-toxic or vice-versa. Several microorganisms are capable of transforming a variety of compound found in nature but generally, with respect to synthetic compound they are unable to show any appropriate action. Biotransfer appears to be one of the major detoxification methods known so far.

Composting
Composting shows promise in the treatment of high concentrations of resistant chemical wastes, as illustrated by a recent application to the degradation of explosives. The goal of composting is the production of a stabilized product that can be stored without further treatment, and can be applied to land without damage to crops. Degree of stabilization is synonymous with extent of decomposition, in that putrescible, phytotoxic material is decomposed through aerobic metabolism. Composting at industrial level also aims at maximizing the rate of decomposition to reduce the facility space necessary, and to shorten the phase where odor problems could arise. Furthermore, the required maturity depends on the potential utilization; compost that is applied to fields, where it continues the stabilization process, needs to be less mature than compost used in potting mixes.
Biomining/Bioleaching of Heavy Metals

Since high-grade ore deposits are easily accessible, they become rapidly depleted. Thus, it becomes necessary to recover mineral resources from low-grade ore deposits. However, no appropriate technology is still available for recovery of metals from low-grade deposits. It is encouraging to find some microorganisms that do it efficiently. This potential of microbes could only be realized recently and efforts are being made to use them for enhanced recovery of mineral resources from natural deposits. Microbes have been used for recovery of metals and petroleum. Biomining utilizes naturally occurring prokaryotic communities. Here, microorganisms are used to leach metals, principally Cu but also Ni and Zn, from low-grade sulfide- and/or Fe-containing ores. The process exploits the energy metabolism of various acidophilic chemolithoautotrophs that utilize inorganic compounds as energy sources and CO$_2$ as the source of carbon. These organisms use either ferrous iron or sulfide as an electron donor and oxygen as an electron acceptor with the formation of ferric iron or sulfuric acid. In the first case, the subsequent reaction of Fe$^{3+}$ with insoluble metal sulfides yields soluble metal sulfates; in the second, metal sulfides are oxidized directly to metal sulfates. The metals are readily recovered from the leachate by electrolytic procedures, and the residual solution is recycled. Research on biomining is directed to improving understanding of the microbiology of the leaching process and to exploring the use of microbes that grow at high temperatures. Microbiological mining can be achieved using S and Fe-oxidizing bacteria (*Thiobacillus, Acidithiobacillus, Beggiatoa spp*).

Petroleum Recovery

Besides metals, microbes can also be used to enhance recovery of petroleum hydrocarbons. The tertiary recovery of petroleum (the use of biological and chemical means to enhance oil recovery), and the enhanced recovery of hydrocarbons from oil shales are important due to depletion of recoverable oil resources. Tertiary recovery of oil uses solvents, surfactants, and polymers to dislodge oil from geological formations. Xanthan gums produced by some bacteria, such as *Xanthomonas campestris*, are useful compounds in oil recovery. These polymers have high viscosity and flow characteristics that allow them to pass through small pores in rock layers containing oil deposits. Xanthan gums are added during water flooding operations (water is pumped into oil reservoirs to force out oil). These help push the oil toward the
production wells. The polymers are produced by conventional fermentation in which *X. campestris* is grown and the gums are recovered.

Many oil shales contain large amounts of carbonates and pyrites and their removal increases the porosity of shale, enhancing recovery of oil. Acid dissolves the carbonates and these can be produced by *Thiobacillus* spp. growing on sulphur and iron in the pyrite. Thus, biolaleaching of oil shales by microbes has the potential for enhancing the recovery of hydrocarbons. Feedstock chemicals are the basic building blocks that serve as the raw materials used to synthesize other chemicals, ranging from small molecules to plastics and rubber, or that are used as solvents in a variety of industrial processes. The primary products of petroleum refining, such as ethylene, propylene, benzene, toluene, and xylene, are the dominant feedstocks for the chemical industry. Alternative renewable sources of feedstock chemicals are needed to conserve world oil reserves and, because of concerns about global warming, to minimize the increase in atmospheric carbon dioxide.

**Microbial Desulfurization of Coal**

Coal contains substantial amount of sulfur, both in pyrite (FeS$_2$) and in organic sulfur compounds, predominantly thiophene derivatives. The composition of coal varies considerably depending on the source. For example, Texas lignite coal contains 0.4% pyrite S and 0.8% organic S, whereas Illinois coal contains 1.2% pyrite S and 3.2% organic S, by weight. When coal is burned, most of this sulfur is converted to SO$_2$. The SO$_2$ combines with moisture in the atmosphere to form sulfuric acid (H$_2$SO$_4$), a major component of acid smog and acid rain. Microbial desulfurization of coal, by converting the pyrite to ferric sulfate and leaching it out of the coal, provides one way of ameliorating this problem. As much as one or two weeks are required to complete the desulfurization, and large areas of land are required for the leach heaps and the storage of coal.

**Fungal Removal of Pitch in Paper Pulp Manufacturing**

In the paper manufacturing industry, treatment of wood with certain white rot fungi to degrade certain wood extractives before pulping substantially decreases the toxicity of pulp mill effluent toward aquatic organisms. Compounds that are extractable from wood with organic solvents make up between 1.5% and 5.5% of the dry weight of softwoods (angiosperms) and hardwoods (gymnosperms).
These compounds, called *wood extractives*, consist mainly of triglycerides, fatty acids, diterpenoid resin acids, sterols, waxes, and sterol esters. Resin acids are present in most softwood but are generally absent or are minor components in hardwood species. During wood pulping and refining of paper pulp, the wood extractives are released, forming colloidal particles commonly referred to as *pitch* or *resin*. These colloidal particles form deposits in the pulp and in the machinery. These deposits can cause mill shutdowns and various quality defects in the finished paper products. Moreover, the resin constituents in pulp mill effluent show acute toxicity toward fish and aquatic organisms. Pretreatment of the wood with fungi to degrade some of the wood extractives before pulping has met with considerable success.

**Retting in Textile Industry**

One of the principal aspects of the microbiology of textiles is the use of microorganisms in preparing fibers such as flax and hemp. The fiber bundles are just held within the outer layers of cells and outside the central pithy and woody layers by an intercellular cement of pectin. A number of bacteria and moulds can digest pectin and used in retting. This permits the fiber bundles to be separated mechanically from the stems and from each other. Fibers can then be collected and woven into linen, or used in the form of ropes and packaging. The process, when properly operated, yields a nicer fiber, which can be made into linen of quite good quality.

**Environmental Monitoring**

The use of biological methods in environmental monitoring is essential in order to complement chemical analyses with information about actual toxicity or genotoxicity of environmental samples. Microorganisms are widely applied test-species in different bioassays because of the ease and low costs of their culturing as well as the lack of ethical issues often accompanying the use of higher organisms. Bioassay or ecotoxicity assay is an experiment in which living test-species are exposed directly to an environmental sample (soil, sediment, surface water, ground water, waste water), or extract of an environmental sample to measure a potential biological effect due to the presence of potential contaminants. Microbial bioassays can roughly be divided into toxicity assays and genotoxicity assays. The purpose of ecotoxicity bioassays is to assess the integral effect of an environmental sample on general
physiologic state of the test-species, while genotoxicity tests specifically show
the effects resulting in changes of genetic material.

Harmful effects of contaminants on the ecosystem and humans cannot be
assessed by standard chemical analyses of environmental samples, therefore
toxicity tests using live organisms or cells represent a vital part of
environmental monitoring. Many different biological methods based on native
or genetically modified microorganisms as test-species, have already
successfully been applied to environmental toxicity/genotoxicity assessment.
An important reason is the modern 3R concept (reduction, replacement,
refinement) in toxicology and ecotoxicology, which promotes the application of
microorganisms in biotests due to simple cultivation in axenic cultures and due
to the lack of ethical problems.

There are many reports that describe the important applications of bacteria,
protozoa, algae, and yeasts in biomonitoring, starting with classical physiology-
based toxicity bioassays, proceeding to genotoxicity assays, immunoassays and
docrine disruptor assays, and concluding with upcoming approaches of
toxicogenomics.

EM formulations are being evaluated for composting and management of
agricultural byproducts and municipal wastes, and for management of coffee
effluents, animal barn odor control.

Exotic/commercial microbial isolates are not investigated for treatment of
municipal and industrial wastes.
MICROORGANISMS are used as tools or devices to accomplish some processes due to their abilities to metabolize diverse substrates and express various genetic traits. In chemistry, they can be used to convert or transform organic compounds to more useful and complex analogues. On the other hand, the genetic traits of various microbes are used in genetic exchange, recombination and engineering, serving as vectors for creation of transgenic plants and animals. Microbial whole-cell based devices are also used as diagnostic tools in biosensing and bionanotechnology, as described below.

Biotransformation

Bioconversion of Compounds into More Useful Products

Biotransformation is the process whereby a substance is changed from one chemical to another by a chemical reaction within the body. Metabolism or metabolic transformations are terms frequently used for the biotransformation process. Microorganisms could modify certain compounds by simple, chemically well-defined reactions, which were further catalyzed by enzymes. These processes are called ‘biotransformations’. More recently, whole microbial cells have been used for specific chemical transformations. This should not be confused with anaerobic fermentation or conventional secondary metabolite production. Here, only part of the cell's metabolism is now being
utilized, usually a single pathway, and sometimes only a single enzyme. The advantage of using cells is that the expense of purifying the enzyme is avoided and, in some cases, the enzyme is more stable in its natural environment than after purification. Frequently, cells and enzymes are subjected to immobilization on an inert support.

When biotransformation results in metabolites of lower toxicity, the process is known as detoxification (described above). Enzymes carry out stereospecific reactions with high accuracy, whereas chemical technology results into many side-products from which the desired product is to be purified.

Bioconversion occurs when microorganisms modify a given compound to a structurally related compound. Microorganisms can use substrates that are not usually present in their environment. Synthesis of these unusual substrates can result in products that are useful compared to those resulting from synthesis of naturally occurring substrates. Bioconversions are useful when multistep chemical synthesis is more expensive or inefficient, or deemed impossible to achieve in the tube. Industrial conversions often employ a mixture of both chemical synthesis and cellular reactions to generate a given product. In general the advantages of biotransformation are to synthesize more useful or commercially important compounds and synthesize more complex compounds efficiently, with less expense and time, and accurately (enantiomerically pure).

Advantages of biocatalysts are well documented during the last decade. Among these, microbial biotransformation of steroids is very important in the pharmaceutical industry. Steroids are used in the treatment of various disorders and involved in regulation of sexuality. Chemical synthesis of steroids is very complex and expensive as the requirement to achieve the necessary precision of substituent location.

**Recombinant DNA Technology and Industrial Biotechnology**

It is genetically possible to ‘tailor’ the microorganisms for the production of any microbial metabolite by employing recombinant DNA technology and industrial biotechnology. Gene cloning extends the genome of the microorganism by allowing the introduction of novel genes from comparatively unrelated species. The cloning of genes from higher eukaryotes, particularly from human and domestic animals has been seen to offer even greater industrial potential, which microbes should then be used as universal recipients for such
genes and hence as production organisms. The major microbial hosts for production of recombinant proteins are *E. coli*, *B. subtilis*, *S. cerevisiae*, *Pichia pastoris*, *Hansenula polymorpha*, and *Aspergillus niger*.

It was not until genetic engineering came about that these biological methods became economically viable. Targeted genetic manipulation has not only enhanced the productivity of these methods, it has also resulted in the production of substances, which was imperviously impossible. Genetic engineering has dramatically expanded the potential of biotechnological methods. The production of a substance naturally found in a microorganism can be enhanced. The regulatory machinery controlling a gene can be changed for a stronger promoter. The enzyme coded by this gene can thus be produced in much greater quantities. The same effect can be achieved making sure that the desired substance is constantly produced. Microorganisms will usually stop producing a substance when enough is available. Genetic engineering can be used to deactivate this ‘stop signal’. Some important substances are only produced naturally by microorganisms that are difficult to culture. In this case, the gene used by the microorganism to produce the substance of interest can be given to a different microorganism that is easier to grow.

Genetically modified microorganisms are now not only used to produce pharmaceuticals, vaccines, specialty chemicals, and feed additives, they also produce vitamins, additives, and processing agents for the food industry. Here are a few examples:

- vitamin B2 (coloring, riboflavin E 101), vitamin C (preservative, ascorbic acid E 300);
- thickener, xanthan (E 415), acidity regulator, citric acid (E 330);
- preservative, natamycin (E 235), nisin (E 234), lysozyme (E 1105);
- various amino acids used to improve the quality of animal feed; also used in some foods, e.g. the flavor enhancer glutamate (E 621), the sweetener aspartame (E 951) or the flour treating agent cysteine (E 921); and
- numerous enzymes used in cheeses, bread and baked goods, alcoholic beverages, and juice, as well as in the production of glucose syrup (corn syrup), glucose, and other starch products.

Genetic engineering has expanded the industrial applications of microorganisms including production of human proteins. By using recombinant DNA technology, human DNA sequences that code for various proteins have been incorporated into the genomes of bacteria. By growing these recombinant bacteria in fermenters, human proteins could be produced commercially. Equally important is the development of new vaccines through gene cloning.
Genes for single antigens can be cloned and expressed by bacteria and a purified antigen, which has not been derived directly from the pathogenic organism, or virus may be used as a vaccine. In this way, vaccines for viral hepatitis and foot-and-mouth disease have been developed.

**Industrial Applications of Immobilized Systems**

Besides gene cloning, several commercial processes have used immobilized microbial cells and enzymes in the last few years. Immobilization means ‘imprisonment or confinement of a biocatalyst (enzyme/cell) in a distinct phase to a suitable inert support, where it can act upon its natural substrate repeatedly and continuously, and can be removed conveniently’. For a biocatalyst, the substrate is disposed in a bulk phase. The physically entrapped or covalently bonded biocatalyst is chemically bonded to an inert, insoluble matrix (support), which is a high molecular weight polymer. Immobilization methods include adsorption, covalent binding, cross binding, entrapment, and microencapsulation. Immobilized systems possess important practical applications in industry such as in antibiotic productions. A range of fungal cell protoplast immobilized systems has also been used in steroid transformations and various environmental applications.

**Microbes used as Vectors in Genetic Engineering**

Genetic engineering, recombinant DNA technology, genetic modification/manipulation, and gene splicing are terms that are applied to the direct manipulation of an organism’s gene. The development of these new technologies has resulted in the production of large amount of varieties of plants having very special traits that may not exist in nature. Since microbial cells have a much higher metabolic rate, genes of desired proteins/enzymes could be introduced into plasmid of bacteria and fungi. Plasmids are extra chromosomal genetic elements (small, circular DNA molecules) and considered as ‘replicons’ capable to replicate autonomously and to be maintained in a host. Plasmids can be found in all three major domains: Archea, Bacteria, and Eukarya. These replicons are commonly used in genetic engineering are called vectors (transfer genetic material). Thus, microbes are used as vehicles to express (show the characteristics of) genes.

The genetic traits of various microbes are used in genetic exchange, recombination and engineering. Viruses are often used to carry genetic material
into a cell. Sometimes the vectors are bacterial or yeast plasmids. These processes involve taking genetic material from one organism and transferring it to another. Plasmid host-to-host transfer requires direct, mechanical transfer by conjugation or changes in host gene expression allowing the intentional uptake of the genetic element by transformation. Plasmids serve as important tools in genetics and biotechnology labs, where they are commonly used to multiply (make many copies of) or express particular genes. Many plasmids are commercially available for such uses. Plasmids are frequently used in genetic engineering occur naturally bacteria, but are sometimes found in eukaryotic organisms too; for example, the 2-micrometre ring in Saccharomyces cerevisiae).

The gene to be replicated is inserted into copies of a plasmid containing genes that make cells resistant to particular antibiotics and a multiple cloning site (MCS, or polylinker), which is a short region containing several commonly used restriction sites allowing the easy insertion of DNA fragments at this location. Next, the plasmids are inserted into bacteria by a process called transformation. Then, the bacteria are exposed to the particular antibiotics. Only bacteria that take up copies of the plasmid survive, since the plasmid makes them resistant. In particular, the protecting genes are expressed (used to make a protein) and the expressed protein breaks down the antibiotics. In this way, the antibiotics act as a filter to select only the modified bacteria. Now these bacteria can be grown in large amounts, harvested, and lysed (often using the alkaline lysis method) to isolate the plasmid of interest.

Another major use of plasmids is to make large amounts of proteins. In this case, researchers grow bacteria containing a plasmid harboring the gene of interest. Just as the bacterium produces proteins to confer its antibiotic resistance, it can also be induced to produce large amounts of proteins from the inserted gene. This is a cheap and easy way of mass-producing a gene or the protein it then codes for, for example, insulin, or even antibiotics.

The major uses of plasmids contained in bacteria and yeasts are:

- vectors used for gene transfer and expression of foreign proteins and expression of recombinant antibodies;
  - bacteria: *Escherichia coli* and *Agrobacterium tumafaciens*;
  - yeast: *Saccharomyces cerevisiae*;
- sources of resistant genes to pathogens and pests;
  - *Bacillus thuringensis* produce toxic proteins against insect-pest;
- devices and vehicles for therapy of humans and animals;
- drug delivery devices
The use of recombinant microorganisms provided the techniques and experience necessary for the successful application of higher organisms, such as mammalian and insect cell culture, and transgenic animals and plants as hosts for the production of recombinant proteins. Bacterial expression of functional antibodies is a breakthrough in antibody technology.

**Biosensors**

**Principles of Biosensing**

In the past two decades, the biological and medical fields have seen great advances in the development of bioanalysis of materials using biosensors and biochips that are capable of characterizing and quantifying biomolecules.

A biosensor is an analytical device that combines a biological sensing element with a transducer and used for the analysis of biomaterial samples to gain an understanding of their bio-composition, concentration, structure, and function by converting a biological response into an electrical signal. The analytical devices composed of a biological recognition element directly interfaced to a signal transducer, which together relates the concentration of an analyte (or group of related analytes) to a measurable response. Biosensor represents biophysical devices, which will detect the presence and measure the quantities of specific substances in a variety of environments. These specific substances may include sugars, proteins and a variety of toxins in the industrial effluents. This signal can result from a change in protons concentration, release, or uptake of gases, light emission, absorption, and so forth, brought about by the metabolism of the target compound by the biological recognition element. The transducer converts this biological signal into a measurable response such as current, potential or absorption of light through electrochemical or optical means, which can be further amplified, processed, and stored for later analysis. Typically biosensors are comprised of three components:

- the detector, which identifies the stimulus;
- the transducer, which converts this stimulus to a useful output; and
- the output system, which involves amplification and display of the output.

The interaction of the analyte with the bioreceptor is designed to produce an effect measured by the transducer, which converts the information into a
measurable effect, such as an electrical signal. Biosensors and biochips can be classified by either their bioreceptor or their transducer type. The most common forms of bioreceptors used in biosensing are based on:

- **antibody/antigen interactions**: binding can be detected either through fluorescent labeling or by observing a refractive index or reflectivity change;
- **nucleic acid interactions**: the complementary relationships between A-T and G-C in DNA form the basis of specificity in nucleic acid-based biosensors;
- **enzymatic interactions**: composed of enzyme bioreceptors that use their catalytic activity and binding capabilities for specific detection;
- **cellular interactions** (i.e. microorganisms, proteins): cell behavior such as cell metabolism, cell viability, cell respiration, and bioluminescence can be used as indicators for the detection of heavy metal;
- **interactions using biomimetic materials** (i.e., synthetic bioreceptors): an artificial or synthetic sensor that mimics the function of a natural biosensor (e.g., aptamers which are synthetic strands of nucleic acid that can be designed to recognize amino acids, oligosaccharides, peptides, and proteins).

Among these, microorganisms offer advantages of ability to detect a wide range of chemical substances, amenability to genetic modification, relatively inexpensive to construct and broad operating pH and temperature range, making them ideal as biological sensing materials.

Microorganisms such as bacteria and fungi can be used as biosensors to detect specific molecules or the overall ‘state’ of the surrounding environment. For example, cell behavior such as cell metabolism, cell viability, cell respiration, and bioluminescence can be used as indicators for the detection of heavy metals. Proteins that are present in cells can also be used as bioreceptors for the detection of specific analytes. The use of live cells for biosensing applications is an exciting alternative to traditional biosensing approaches. These techniques may potentially enhance biosensor specificity and sensitivity. Cell-based biosensors are also particularly useful in detecting unknown compounds and toxins since the behavior of the candidate molecules can be directly observed in tissues.

**Types and Applications of Biosensors**

Microorganisms have been integrated with a variety of transducers such as amperometric, potentiometric, calorimetric, conductimetric, colorimetric, luminescence, and fluorescent to construct biosensor devices. Typically, biosensors belong to one of following classes of signal transduction:
- **Optical detection**: measures luminescence, absorption, surface plasmon resonance, etc.; use many different types of spectroscopy, such as absorption, fluorescence, phosphorescence, Raman, SERS, refraction, and dispersion spectrometry;
- **Electrochemical**: measures the current produced from oxidation and reduction reactions;
- **Mass-sensitive**: detects small mass changes caused by chemical binding to small piezoelectric crystals, i.e., surface acoustic wave, microbalance, etc.; and
- **Thermal detection**: measures the changes in temperature in the reaction between an enzyme molecule and a suitable analyte.

Microorganisms offer a form of bioreceptor that often allows a whole class of compounds to be monitored. Generally, these microbial biosensors rely on the uptake of certain chemicals into the microorganism for digestion. Often, a class of chemicals is ingested by a microorganism, therefore allowing a class-specific biosensor to be created. Microorganisms such as bacteria and fungi have been used as indicators of toxicity or for the measurement of specific substances. For example, cell metabolism (e.g. growth inhibition, cell viability, substrate uptake), cell respiration and bacterial bioluminescence have been used to evaluate the effects of toxic heavy metals. Some of the applications of biosensors are:

- Study of biomolecules and how they interact with one another (biospecific interaction analysis (BIA));
- In medicine, because biosensors work almost instantaneously, they allow clinical diagnosis to be performed at the bedside in critical care units and so there is no need to wait for lengthy procedures to be carried out in centralised labs, so treatment can often start immediately dramatically improving patient care;
- In industries, biosensors can be used to improve control of the manufacturing process, leading to a better yield and product quality. For example, monitoring the production of alcohol during the fermentation process; food quality analysis— to assay quantitatively substances such as vitamins, amino acids;
- Help meet environmental legislation through monitoring and measuring toxicity, e.g. monitoring the level of volatile organic compounds or pesticides in air, waste water and contaminated land;
- The Biological Oxygen Demand (BOD) is one of the most widely used and important tests in the measurement of organic pollution. The conventional BOD test takes a 5 day incubation period. A biosensor has been produced containing immobilised yeast (*Trichosporon contaneum*) which can now be used to measure the BOD;
- In mining, biosensors will detect the presence of poisonous or explosive gases in mines and so protecting the miners’ lives;
- In the pharmaceutical industry, there are many developments in medical diagnosis (monitor the health of a living body), e.g. the glucometer. An implantable glucose biosensor has been developed which monitors the person’s blood glucose level continuously without the need for all the finger pricking. The sugar level is displayed
as a digital readout in a wearable beeper-sized device. Ultimately the biosensor will be linked to an implanted insulin pump, which is a good news for diabetics;

- help maintain health and safety regulations by monitoring for microbial contamination—e.g. test kits can be used to detect the causative organism for Legionnaires’ disease in ventilation systems;
- military applications—scientific crime detection since September 11th. A biosensor called a ‘smart skin’ was developed in Dallas Texas and the US Airforce has been testing it since. It is a device worn by soldiers in the battlefield which recognises if the soldier’s body is responding to biological or chemical attack and alerts the wearer to such attack. A special biosensor for the detection of Anthrax has also been developed. Another security area is to detect drugs or explosives in the air, e.g. at airports.

The advantages of biosensors lie on the fact that they detect small amounts; are accurate, easy to use, fast, and cost effective; they are suitable for online and continuous monitoring.

Biosensors and Recombinant DNA Technology (Bioreporter technology)

The advent of genetic manipulation by recombinant DNA technology has created a broad range of specific microbial biosensors. The great majority of these are genetically engineered bacteria within which a promoter–operator (the sensing element) responds to the stress condition like toxic organic or inorganic compound, and DNA damage and changes the level of expression of a reporter gene that codes for a protein (the signal). The protein may be detected directly like green fluorescent protein or through its catalytic activity; for example, formation of a fluorescent or chemiluminescent product.

Bioreporters refer to intact, living microbial cells that have been genetically engineered to produce a measurable signal in response to a specific chemical or physical agent in their environment. Bacterial sensor reporters are genetically engineered to produce specific output in response to target chemicals, and offer an interesting alternative to monitoring approaches. Bacterial sensor-reporters detect bioavailable and/or bioaccessible compound fractions in samples. A variety of environmental pollutants can be targeted by specific biosensor-reporters. Bioreporters contain two essential genetic elements, a promoter gene, and a reporter gene. The promoter gene is turned on (transcribed) when the target agent is present in the cell’s environment. The promoter gene in a normal bacterial cell is linked to other genes that are then likewise transcribed and then translated into proteins that help the cell in either combating or adapting to the agent to which it has been exposed. In the case of a bioreporters, these genes, or
portions thereof, have been removed and replaced with a reporter gene. Consequently, turning on the promoter gene now causes the reporter gene to be turned on. Activation of the reporter gene leads to production of reporter proteins that ultimately generate some type of a detectable signal. Therefore, the presence of a signal indicates that the bioreporter has sensed a particular target agent in its environment.

Originally developed for fundamental analysis of factors affecting gene expression, bioreporters were early on applied for the detection of environmental contaminants and have since evolved into fields as diverse as medical diagnostics, precision agriculture, environmental monitoring, food safety assurance, process monitoring and control, and bio-microelectronic computing. Their versatility stems from the fact that there exist a large number of reporter gene systems that are capable of generating a variety of signals. Additionally, reporter genes can be genetically inserted into bacterial, yeast, plant, and mammalian cells, thereby providing considerable functionality over a wide range of host vectors.

Several types of reporter genes are available for use in the construction of bioreporter organisms, and the signals they generate can usually be categorized as colorimetric, fluorescent, luminescent, chemiluminescent, or electrochemical. Although each functions differently, their product always remains the same—a measurable signal that is proportional to the concentration of the unique chemical or physical agent to which they have been exposed. Bioreporter technology provides a robust, cost-effective, quantitative method for rapid and selective detection and monitoring of chemical and biological agents in very diverse applications. Their attractiveness lies in the fact that they can often be implemented in real-time, online bioassays within intact, living cell systems, thus providing a unique and revolutionarily new perspective on bacterial, plant, and mammalian physiology and intracellular interactions.

The study of infectious diseases, tumor progression and metastasis, gene therapy, mammalian development, and many other areas in which animal models are used as predictors for the human response to therapy can be greatly simplified and accelerated. The same ideals apply in cases of environmental monitoring and food safety, where rapid and remote monitoring devices can strategically pinpoint areas of biological hazard, whether in the form of biological warfare agents or pathogenic E. coli presence. Further advances in bioreporter genetics and miniaturized optics will clearly affect future monitoring and detection strategies in these fields as well as a host of others.
Bionanotechnology and Biosensors

Bionanotechnology, nanobiotechnology, and nanobiology are terms that refer to the intersection of nanotechnology and biology. The terms are often used interchangeably but with slight distinction. Bionanotechnology and nanobiotechnology have only emerged very recently, and serve as blanket terms for various related technologies. A very recent technological development enables to:

- understand and control matter at dimensions of approximately 1/1,000,000,000 (1 billionth) of a meter, or the space occupied by 3–4 atoms placed end-to-end;
- understand, create, and use structures, devices and systems that have fundamentally new properties and functions because of their nanoscale structure;
- image, measure, model, and manipulate matter on the nanoscale to exploit those properties and functions; and
- integrate those properties and functions into systems spanning from nano- to macroscopic scales.

This discipline helps indicate the merger of biological research with various fields of nanotechnology. Concepts that are enhanced through nanobiology include nanodevices, nanoparticles, and nanoscale phenomena that occur within the disciple of nanotechnology. Developing new tools for the medical and biological fields is another primary objective in nanotechnology. New nanotools are often made by refining the applications of the nanotools that are already being used. The imaging of native biomolecules, biological membranes, and tissues is also a major topic for the nanobiology researches. Other topics concerning nanobiology include the use of cantilever array sensors and the application of nanophotonics for manipulating molecular processes in living cells.

Bionanotechnology research offers the possibility of understanding the smallest structural features of a cell or tissue, and using that knowledge to develop medical devices or treatments that will work on a scale 100,000 times smaller than the diameter of the average human hair. This unprecedented opportunity to unlock the secrets of biology on an entirely new level opens a new era in healthcare. Integration of nano-scale technologies could lead to tiny, low-power, smart sensors that could be manufactured cheaply in large numbers. Bionanotechnology deals with biosensing the interaction of a small number of molecules, processing and transmitting the data with a small number of electrons, and storing the information in nanometer scale structures.
Future bionanotechnology will use computer chips inside living cells. Silicon chips smaller than cells can be produced, collected, and internalized inside living cells by different techniques (lipofection, phagocytosis or microinjection) and, most significantly, they can be used as intracellular sensors. The study of individual cells is of great importance in biomedicine. Many biological processes incur inside cells and these processes can differ from cell to cell. The development of micro- and nano-scale tools smaller than cells will help in understanding the cellular machinery at the single cell level. Nanodimension of new-generation biosensors will improve their spatial resolution down to the molecular levels, reduce their detection volume to few cubic micrometers, and speed up their signal response to few milliseconds for the diagnosis of clinical and environmental samples. In return, they will become simple, small, and low-cost diagnostic tools. Good cell viability after testing with nanoprobes has recently been demonstrated, which opens the possibility of non-harmful and continuous monitoring in real time.

Biotransformations of organic compounds are not attempted even in the universities. All kinds of genetic engineering experiments are hampered as the biosafety law is inhibitory. It is not possible to import bacterial cultures that harbor plasmids as vectors in gene cloning. Biosensing and bionanotechnology are not attempted and do not seem to be priority needs for Ethiopia. However, all need to be demonstrated and exercised in universities, as these subjects could not be comprehended on theoretical basis only.
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