National University of Life and Environmental Sciences of Ukraine

Faculty of biotechnologies

Department of ecobiotechnology and biodiversity

INDUSTRIAL BIOTECHNOLOGY

A course of lectures
for "Bachelor" students of
the 6.051401 — «Biotechnology» directions

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The educational edition

Industrial biotechnology
Course of lectures

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Introduction to the industrial biotechnology. Microbial Applications

Industrial biotechnology or microbial biotechnology is the application of scientific and engineering principles to the processing of materials by microorganisms (such as bacteria, fungi, algae, protozoa and viruses) or plant and animal cells to create useful products or processes.

The microorganisms utilized may be natural isolates, laboratory selected mutants or microbes that have been genetically engineered using recombinant DNA methods.

The terms “industrial microbiology” and “biotechnology” are often one and the same.

Areas of industrial microbiology include quality assurance for the food, pharmaceutical, and chemical industries. Industrial microbiologists may also be responsible for air and plant contamination, health of animals used in testing products, and discovery of new organisms and pathways.

For instance, most antibiotics come from microbial fermentations involving a group of organisms called actinomycetes. Other organisms such as yeasts are used in baking, in the production of alcohol for beverages, and in fuel production (gasohol).

Additional groups of microorganisms form products that range from organic acids to enzymes used to create various sugars, amino acids, and detergents. For example, the sweetener aspartame is derived from amino acids produced by microorganisms.

Industrial microbiologists may also deal with products associated with the food and dairy industries, with the prevention or deterioration of processed or manufactured goods, and with waste disposal systems.

Microbial Applications

1. Food and beverage biotechnology

• fermented foods, alcoholic beverages (beer, wine)
• flavors

2. Enzyme technology

• production and application of enzymes. Industrial applications of enzymes include the production of cheese, the clarification of apple juice, the development of more efficient laundry detergents, pulp and paper production, and the treatment of sewage. These processes have been dramatically enhanced by the use of recombinant DNA techniques to design enzymes of increased activity, stability, and specificity.

3. Metabolites from microorganisms

• amino acids
• antibiotics, vaccines, biopharmaceuticals
• bacterial polysaccharides and polyesters
• specialty chemicals for organic synthesis (chiral synthons)
• Some molecular sieves for purification/separation processes (e.g., dextran) and thickening agents (e.g., xanthan used in salad dressings), which are stable at high

Table 1 - Main application fields of immobilized cell cultures

Product	Examples
Enzymes	Amylases, cellulase and other cellulolytic enzymes, chitinolytic enzymes, cyclodextrin, glucosyltransferase, l-glutaminase, inulase, lipases, penicillin V acylase, peroxidases, polymethylgalacturonase, alkaline and acid proteases, pullulanases, ribonuclease and xylanase.
Antibiotics	Ampicillin, candicidin, cephalosporin C, clavulanic acid, cyclosporin A, daunorubicin, divercin, kasugamycin, nikkomycin, nisin Z, oxytetracyclin, patulin, penicillin G and rifamycin B
Steroids[a] , amino acids,	Androstenedione, hydrocortisone, prednisolone, progesterone, Alanine, arginine, aspartic acid, cysteine, glutamic acid, phenylalanine, serine, tryptophan
Organic acids, alcohols and polysaccharides	Acetic, citric, fumaric, gluconic, lactic, malic, propionic acids Butanol, ethanol, sorbitol, xylitol Alginate, dextran, levan, pullulan, sulfated exopolysaccharides Pigments, vitamins, flavors and aroma.
Environment water treatment	Carbon removal (COD), nitrogen removal (nitrification/denitrification, assimilation), heavy metal removal (Au, Cd, Cu, Ni, Pb, Sr, Th, U, etc), pollutant iodegradation (phenol and phenolic compounds, polycyclic aromatics, heterocycles, cyanide compounds, surfactants, hydrocarbons and oily products.
Biofertilisation	Soil inoculation with plant growth-promoting organisms (Azospirillum rasilense, Bradyrhizobium japonicum, Glomus deserticola, Pseudomonas fluorescens and Yarowia lipolytica).
Bioremediation	Degradation of pollutants in contaminated soils (e.g. chlorinated phenols), quifers and marine habitats (e.g. petroleum hydrocarbons) by microbial inocula.
Alternative fuels	Dihydrogen and methane productions, ethanol production, biofuel cells
Food processing, alcoholic beverages, milk products	Brewing, vinification, fermentation of cider and kefir, controlled in situ generation of bioflavors continuous inoculation of milk (lactic starters), lactose hydrolysis in milk whey.
Biosensors Electrochemical[b]	Acetic acid, acrylinitrile, amino acids, BOD, cyanide, cholesterol, chlorinated aliphatic compounds, ethanol, naphthalene, nitrate, phenolic compounds, phosphate, pyruvate, sugars, sulfuric acid (corrosion monitoring), uric acid, herbicides, pesticides, vitamins, toxicity assays.
Optical	Herbicides, metals, genotoxicant, polyaromatics and toxicity testing.

[a]Obtained by conversion of steroid parent compounds.
[b]Amperometric, potentiometric, conductometric.

temperatures, are examples of microbial carbohydrates. The latter are also used for secondary oil recovery in oil fields and as lubricants in drilling oil wells, gelling agents in foods, and thickeners in both paints and foods.

• Compounds such as acetone, methanol, butanol, and ethanol have multiple applications in industrial settings, often as raw materials for industrial processes. The microbiologist is involved in research on improvements in the production and detection of new metabolic pathways. Microbes will increasingly be used to supplant or replace those processes which rely on petroleum/natural gas for the production of these compounds.

4. Biological fuel generation

• ethanol or methane from biomass, single cell protein, production of biomass
• microbial recovery of petroleum

5. Environmental biotechnology

• water and wastewater treatment
• composting (and landfilling) of solid waste
• biodegradation/bioremediation of toxic chemicals and hazardous waste

6. Agricultural biotechnology

• soil fertility
• microbial insecticides, plant cloning technologies
• Conventional, recombinant DNA, and monoclonal antibody techniques are used to improve microbial inoculants which serve as fertilizer supplements by fixing atmospheric nitrogen to improve plant yields and to serve as plant pest controls. All of these require a microbiologist to insure product efficacy and quality.

7. Diagnostic tools

• testing & diagnosis for clinical, food, environmental, agricultural applications
• biosensors

1. The introduction in industrial biotechnology. The history of industrial biotechnology

There are many definitions of biotechnology. One of the broadest is the one given at the United Nations Conference on Biological Diversity (also called the Earth Summit) at the meeting held in Rio de Janeiro, Brazil in 1992. That conference defined biotechnology as "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use." Many examples readily come to mind of living things being used to make or modify processes for specific use. Some of these include the use of microorganisms to make the antibiotic, penicillin or the dairy product, yoghurt; the use of microorganisms to produce amino acids or enzymes are also examples of biotechnology.

Biotechnology is any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use (according to the definition of United Nations Conference on Biological Diversity in Rio de Janeiro, Brazil in 1992).

Industrial biotechnology or microbial biotechnology or industrial microbiology is the application of scientific and engineering principles to the processing of materials by microorganisms (such as bacteria, fungi, algae, protozoa and viruses) or plant and animal cells to create useful products or processes (according to the definition of European Federation of Biotechnology (EFB), 1978).

The terms “industrial microbiology” and “biotechnology” are often one and the same.

Periods of Biotechnology History:

I - Ancient Biotechnology (Pre-1800)

Throughout the history of agriculture, farmers have inadvertently altered the genetics of their crops through introducing them to new environments and breeding them with other plants — one of the first forms of biotechnology.

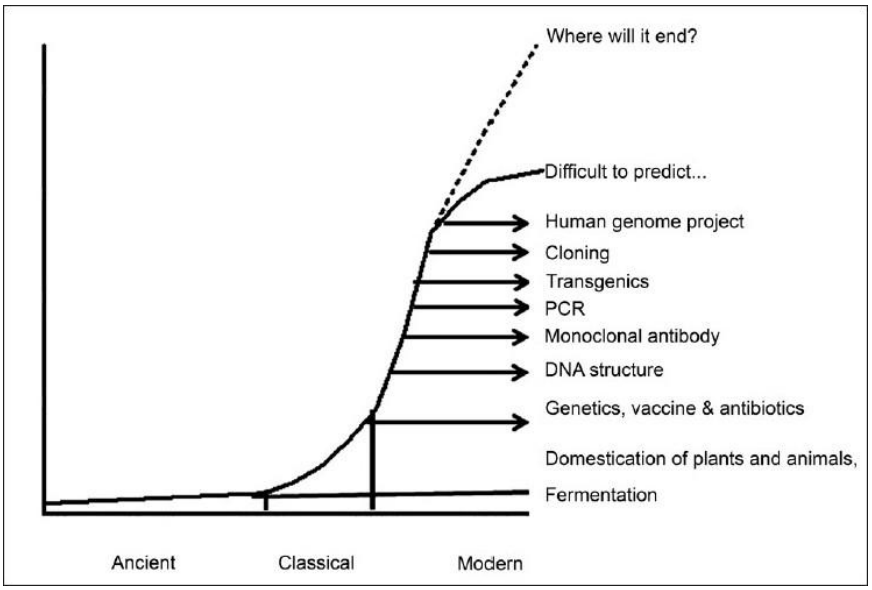


Fig.1 - History of the development of biotechnology

These processes also were included in early fermentation of beer. These processes were introduced in early Mesopotamia, Egypt, China and India, and still use the same basic biological methods. In brewing, malted grains (containing enzymes) convert starch from grains into sugar and then adding specific yeasts to produce beer. In this process, carbohydrates in the grains were broken down into alcohols such as ethanol. Later other cultures produced the process of lactic acid fermentation which allowed the fermentation and preservation of other forms of food, such as soy sauce.

Early fermentation process practiced by man included the leavening of bread, retting of flax, preparation of vinegar from wine, production of various alcoholic beverages like beer, wine, mead and the production of various fermented foods and milk.

Industrial microbiology came into existence, primarily, based on a naturally occurring microbiological process called **fermentation**. There are many evidences which clearly shows that ancient man knew fermentation process and practiced it more as an art rather than as a science.

After domestication of food crops and wild animals, man moved on to other new observations like cheese, curd, etc. Certainly, cheese can be considered as one of the first direct products (or by-product) of biotechnology, because it was prepared by adding rennet (an enzyme found in the stomach of calves) to sour milk, which is possible only by exposing milk to microbes (although this understanding was not there, at that time). Yeast is one of the oldest microbes that have been exploited by humans for their benefit. Yeast has been widely used to make bread, vinegar production, and other fermentation products, which include production of alcoholic beverages like whiskey, wine, beer, etc. Vinegar has a significant importance because of its low pH. Vinegar is

capable of preventing growth of certain microbes, and therefore, vinegar can be used successfully for food preservation. The discoveries and benefits of these observations led people to work on further improvement of the process. Fermentation was a powerful tool to improve their living conditions, even though they were ignorant about the principle behind it. One of the oldest examples of crossbreeding for the benefit of humans is mule. Mule is an offspring of a male donkey and a female horse.

II - Etiologic period (1856-1933) *Etiology* is the study of causation, or origination.

- **Louis Pasteur (1822 - 1895)** asserted that microbes are responsible for fermentation. **1859.** Louis Pasteur invented the process of pasteurization, heating wine sufficiently to inactivate microbes (that would otherwise turn the "vin" to "vin aigre" or "sour wine") while at the same time not ruining the flavor of the wine.
- **1873.** Robert Koch investigated anthrax and developed techniques to view, grow, and stain organisms. He then photographed them, aided by Gram, Cohn, and Weigart.
- **1910 BASIS OF MODERN GENETICS**
- **1928** Alexander Fleming noticed that all the bacteria in a radius surrounding a bit of mold in a petrie dish had died**.** The age of penicillin thus began, although it would be almost 15 years before it was made available to the community for medicinal use.
-

III – biotechnical period (1933 - 1972)

In the early twentieth century scientists gained a greater understanding of microbiology and explored ways of manufacturing specific products. In 1917, Chaim Weizmann first used a pure microbiological culture in an industrial process, that of manufacturing corn starch using *Clostridium acetobutylicum*, to produce acetone, which the United Kingdom desperately needed to manufacture explosives during World War I.

Biotechnology has also led to the development of antibiotics. In 1928, Alexander Fleming discovered the mold *Penicillium*. His work led to the purification of the antibiotic compound formed by the mold by Howard Florey, Ernst Boris Chain and Norman Heatley - to form what we today know as penicillin. In 1940, penicillin became available for medicinal use to treat bacterial infections in humans.

- 1940-1945: Large scale production of penicillin
- 1943-1953: Cortisone first manufactured in large amounts

IV – molecular or genotechnical period (1972 – 2000)

The field of modern biotechnology is generally thought of as having been born in 1971 when Paul Berg's (Stanford) experiments in gene splicing had early success. Herbert W. Boyer (Univ. Calif. at San Francisco) and Stanley N. Cohen (Stanford) significantly advanced the new technology in 1972 by transferring genetic material into a bacterium, such that the imported material would be reproduced.

- 1977: Genentech produced somatostatin (human growth hormone-releasing inhibitory factor), manufactured in bacteria. First time a recombinant gene was used to clone a protein.
- 1978: Harvard researchers produced rat insulin by recombinant DNA.

The commercial viability of a biotechnology industry was significantly expanded on June 16, 1980, when the United States Supreme Court ruled that a genetically modified microorganism could be patented in the case of *Diamond v. Chakrabarty*. Indian-born Ananda Chakrabarty, working for General Electric, had modified a bacterium (of the *Pseudomonas* genus) capable of breaking down crude oil, which he proposed to use in treating oil spills. (Chakrabarty's work did not involve gene manipulation but rather the transfer of entire organelles between strains of the *Pseudomonas* bacterium.

- 1982: FDA approves genetically engineered human insulin
- 1986: Orthoclone OKT3 (Muromonab-CD3) approved for reversal of kidney transplant rejection.
- 1986: first recombinant vaccine approved- hepatitis
- 1987: Genentech gets approval for rt-PA (tissue plasminogen activatior) for heart attacks

V – nanobiotechnological period (from 2000 to nowdays)

A series of derived terms have been coined to identify several branches of biotechnology; for example:

- **Bioinformatics** is an interdisciplinary field which addresses biological problems using computational techniques, and makes the rapid organization as well as analysis of biological data possible. The field may also be referred to as *computational biology*, and can be defined as, "conceptualizing biology in terms of molecules and then applying informatics techniques to understand and organize the information associated with these molecules, on a large scale."

  Bioinformatics plays a key role in various areas, such as ***functional genomics*** (functional genomics is a field of molecular biology that attempts to make use of the vast wealth of data produced by genomic projects (such as genome sequencing projects) to describe gene (and protein) functions and interactions. The goal of functional genomics is to understand the relationship between an organism's genome and its phenotype. The term functional genomics is often used broadly to refer to the many possible approaches to understanding the properties and function of the entirety of an organism's genes and gene products).

  Structural genomics seeks to describe the 3-dimensional structure of every protein encoded by a given genome. This genome-based approach allows for a high-throughput method of structure determination by a combination of experimental and modeling approaches. The principal difference between structural genomics and traditional structural prediction is that structural genomics attempts to determine the structure of every protein encoded by the genome, rather than focusing on one particular protein. With full-genome

sequences available, structure prediction can be done more quickly through a combination of experimental and modeling approaches, especially because the availability of large number of sequenced genomes and previously solved protein structures allows scientists to model protein structure on the structures of previously solved homologs.

and ***proteomics*** (**Proteomics** is the large-scale study of proteins, particularly their structures and functions. Proteins are vital parts of living organisms, as they are the main components of the physiological metabolic pathways of cells. The term *proteomics* was first coined in 1997 to make an analogy with genomics, the study of the genome).

- **Blue biotechnology** is a term that has been used to describe the marine and aquatic applications of biotechnology, but its use is relatively rare.
- **Green biotechnology** is biotechnology applied to agricultural processes. An example would be the selection and domestication of plants via micropropagation. Another example is the designing of transgenic plants to grow under specific environments in the presence (or absence) of chemicals. One hope is that green biotechnology might produce more environmentally friendly solutions than traditional industrial agriculture. An example of this is the engineering of a plant to express a pesticide, thereby ending the need of external application of pesticides. An example of this would be Bt corn. Whether or not green biotechnology products such as this are ultimately more environmentally friendly is a topic of considerable debate.
- **Red biotechnology** is applied to medical processes. Some examples are the designing of organisms to produce antibiotics, and the engineering of genetic cures through genetic manipulation.
- **White biotechnology**, also known as industrial biotechnology, is biotechnology applied to industrial processes. An example is the designing of an organism to produce a useful chemical. Another example is the using of enzymes as industrial catalysts to either produce valuable chemicals or destroy hazardous/polluting chemicals. White biotechnology tends to consume less in resources than traditional processes used to produce industrial goods.

The investment and economic output of all of these types of applied biotechnologies is termed as "bioeconomy"(**Biobased economy**, **bioeconomy** or **biotechonomy** refers to all economic activity derived from scientific and research activity focused on biotechnology - in other words, on understanding mechanisms and processes at the genetic and molecular levels and its application to industrial process).

2. Growth and microbial metabolism

Growth and Cell Division

Microbial Growth Defined:

- Mother or parent cell doubles in size
- Divides into two daughter cells

- Microbial growth is defined as the increase in the number of cells, which occurs by cell division

Cell Division

- Binary fission (equal cell division): A cell duplicates its components and divides into two cells
- Septum: A partition that grows between two daughter cells and they separate at this location
- Budding (unequal cell division): A small, new cell develops from surface of exisiting cell and subsequently separates from parent cell

Phases of Growth

Consider a population of organisms introduced into a fresh, nutrient medium. Such organisms display four major phases of growth.

- The lag phase
- The logarithmic phase
- The stationary phase
- The death phase

The Lag Phase

Organisms do not increase significantly in number
They are metabolically active
Grow in size, synthesize enzymes, and incorporate molecules from medium
Produce large quantities of energy in the form of ATP

The Log Phase

Organisms have adapted to a growth medium
Growth occurs at an exponential (log) rate
The organisms divide at their most rapid rate
a regular, genetically determined interval (generation time)

Stationary Phase:

Cell division decreases to a point that new cells are produced at same rate as old cell die.
The number of live cells stays constant.

Decline (Death) Phase:

Condition in the medium become less and less
supportive of cell division
Cell lose their ability to divide and thus die
Number of live cells decreases at a logarithmic rate

Serial Dilution and Standard Plate Counts

- Standard plate count: One method of measuring bacterial growth
- Agar plate: A petri dish containing a nutrient medium solidified with agar
- Serial dilutions are used to dilute the original bacterial culture before you transfer known volume of culture onto agar plate

Direct Microscopic Counts

- Another way to measure bacterial growth
- Petroff-Hausser counting chamber
- Bacterial suspension is introduced onto chamber with a calibrated pipette
- Microorganisms are counted in specific calibrated areas
- Number per unit volume is calculated using an appropriate formula

Most Probable Number (MPN)

- Method to estimate number of cells
- Used when samples contain too few organisms to give reliable measures of population size by standard plate count
- Series of progressively greater dilutions
- Typical MPN test consists of five tubes of each of three volumes (e.g. 10, 1, and 0.1ml)

Factors Affecting on the Bacterial Growth

The kinds of organisms found in a given environment and the rates at which they grow can be influenced by a variety of factors, both physical and biochemical.

Physical factors include:

- pH,
- temperature,
- oxygen concentration,
- moisture,
- hydrostatic pressure,
- osmotic pressure,
- and radiation.

Nutritional factors include: availability of carbon, nitrogen, sulfur, phosphorus, trace elements and, in some cases, vitamins.

pH

- Optimum pH: the pH at which the microorganism grows best (e.g. pH 7)
- According to their tolerance for acidity/alkalinity, bacteria are classified as:
  1. Acidophiles (acid-loving): grow best at pH 0.1-5.4
  2. Neutrophiles: grow best at pH 5.4 to 8.0
  3. Alkaliphiles (base-loving): grow best at pH 7.0-11.5

Temperature

- Obligate: organism must have specified environmental condition
- Facultative: organism is able to adjust to and tolerate environmental condition, but can also live in other conditions

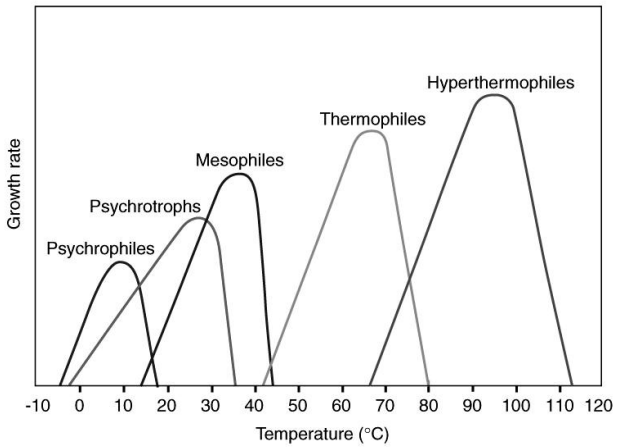


Fig.2 – Temperature Factors

- According to their growth temperature range, bacteria can be classified as:
  - Psychrophile
    - 0^o to 18^o C
  - Psychrotroph
    - 20°C to 30°C
      - Important in food spoilage
  - Mesophile
    - 25°C to 45°C
      - More common
      - Disease causing
  - Thermophiles
    - 45°C to 70°C
      - Common in hot springs and hot water heaters
  - Hyperthermophiles
    - 70°C to 110°C
      - Live at very high temperatures, high enough where water threatens to become a gas
      - Usually members of *Archaea*
      - Found in hydrothermal vents

Oxygen

- Aerobes: require oxygen to grow

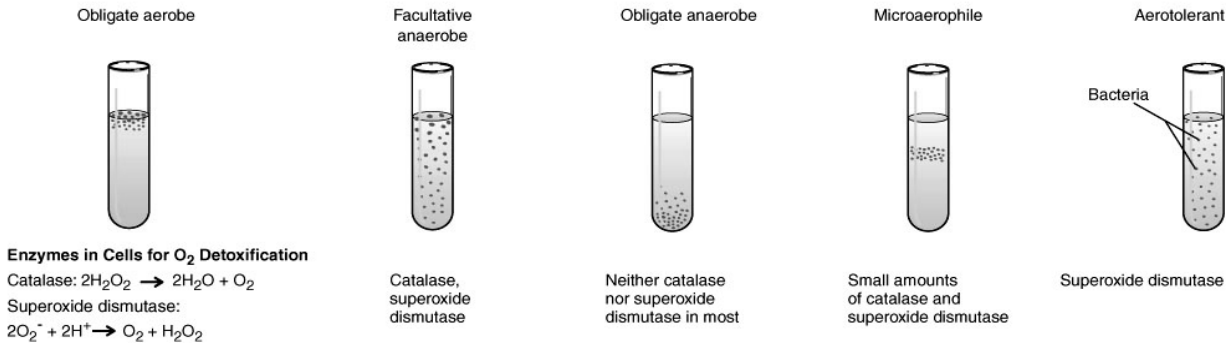


Fig.3 – Oxygen Factors

Obligate aerobes: must have free oxygen for aerobic respiration (e.g. Pseudomonas)

- Anaerobes: do not require oxygen to grow
  Obligate anaerobes: killed by free oxygen (e.g. Bacteroides)
- Microaerophiles: grow best in presence of small amount of free oxygen
- Capnophiles: carbon-dioxide loving organisms that thrive under conditions of low oxygen
- Facultative anaerobes: carry on aerobic metabolism when oxygen is present, but shift to anaerobic metabolism when oxygen is absent
- Aerotolerant anaerobes: can survive in the presence of oxygen but do not use it in their metabolism

Hydrostatic Pressure

- Water in oceans and lakes exerts pressure exerted by standing water, in proportion to its depth
- Pressure doubles with every 10 meter increase in depth
- Barophiles: bacteria that live at high pressures, but die if left in laboratory at standard atmospheric pressure

Osmotic Pressure

- Environments that contain dissolved substances exert osmotic pressure, and pressure can exceed that exerted by dissolved substances in cells
- Hyperosmotic environments: cells lose water and undergo plasmolysis (shrinking of cell)
- Hypoosmotic environment: cells gain water and swell and burst

Halophiles

- Salt-loving organisms which require moderate to large quantities of salt (sodium chloride)
- Membrane transport systems actively transport sodium ions out of cells and concentrate potassium ions inside

Why do halophiles require sodium?

- Cells need sodium to maintain a high intracellular potassium concentration for enzymatic function
- Cells need sodium to maintain the integrity of their cell walls

Nutritional Factors

- Carbon sources
- Nitrogen sources
- Sulfur and phosphorus
- Trace elements (e.g. copper, iron, zinc, and cobalt)
- Vitamins (e.g. folic acid, vitamin B-12, vitamin K)

Locations of Enzymes

- Exoenzymes: production of enzymes that are released through cell or plasma membrane

- Extracellular enzymes: usually produced by gram-positive rods, which act in the medium around the organism
- Periplasmic enzymes: usually produced by gram-negative organisms, which act in the periplasmic space

Sporulation
- The formation of endospores, occurs in Bacillus, Clostridium and a few other gram-positive genera
- Protective or survival mechanism, not a means of reproduction
- As endospore formation begins, DNA is replicated and forms a long, compact, axial nucleoid
- Core (living part of endospore): most of cell's RNA and some cytoplasmic protein molecules gather around DNA, Dipicolinic acid: contained in the core along with calcium ions

Germination
- A spore returns to its vegetative state, occurs in three stages:
  Activation, Germination proper, Outgrowth

Culturing Bacteria
- Culturing of bacteria in the laboratory presents two problems:
- A pure culture of a single species is needed to study an organism's characteristics
- A medium must be found that will support growth of the desired organism
- Pure culture: a culture that contains only a single species of organism

Types of Culture Media
- Natural Media: In nature, many species of microorganisms grow together in oceans, lakes, and soil and on living or dead organic matter
- Synthetic medium: A medium prepared in the laboratory from material of precise or reasonably well-defined composition
- Complex medium: contains reasonably familiar material but varies slightly in chemical composition from batch to batch (e.g. peptone, a product of enzyme digestion of proteins)

Commonly Used Media
- Yeast Extract, Casein Hydrolysate, Nutrient
- Serum, Blood agar, Chocolate agar

Selective, Differential, and Enrichment Media
- Selective medium: encourages growth of some organisms but suppresses growth of others (e.g. antibiotics)
- Differential medium: contains a constituent that causes an observable change (e.g. MacConkey agar)
- Enrichment medium: contains special nutrients that allow growth of a particular organism that might not otherwise be present in sufficient numbers to allow it to be isolated and identified

Preserved Cultures

- To avoid risk of contamination and to reduce mutation rate, stock culture organisms should be kept in a preserved culture, a culture in which organisms are maintained in a dormant state
  - Lyophilization
  - Frozen at -70^{o}C
  - Refrigeration
- Reference culture (type culture): a preserved culture that maintains the organisms with characteristics as originally defined

Control of Microorganisms

1. Preventing food spoilage

• sterilization methods, canning
• chemical & physical control of growth
• water activity, acidity, pickling, etc.
• fermented foods

2. Sanitation, prevention of waterborne disease

• disinfectants, antiseptics, etc.
• water treatment (filtration, chlorination…)
• wastewater treatment

3. Prevention of biodeterioration

1.Microbial cells or cell products
2. Enzymatic biotransformation products
3.Fermentation products (de novo synthesis)

Microbial metabolism

Primary vs. Secondary metabolism

Primary metabolites:

• produced during active growth
• generally a consequence of energy metabolism and necessary for the continued growth of the microorganism

Substrate A → Product
Substrate A →B → C → Product

• ethanol, lactic acid…

Secondary metabolites:

• synthesized after the growth phase nears completion
• a result of complex reactions that occur during the latter stages of primary growth

Substrate A → B → C → Primary metabolism (no product)

↓

D → E → Product of secondary metabolism

Substrate A → B → C → Primary metabolism (no product)

afterwards, the product is formed by metabolism of an intermediate

C → D → Product

• growth phase = trophophase
• idiophase = phase involved in production of metabolites
• citric acid, antibiotics,…

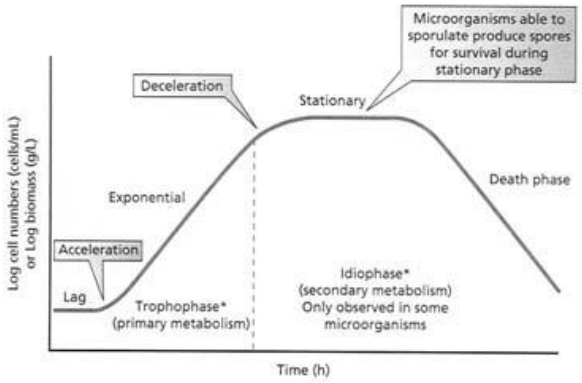


Fig.4 – Growth of a microorganism in a batch culture

Primary metabolite **Secondary Metabolite**

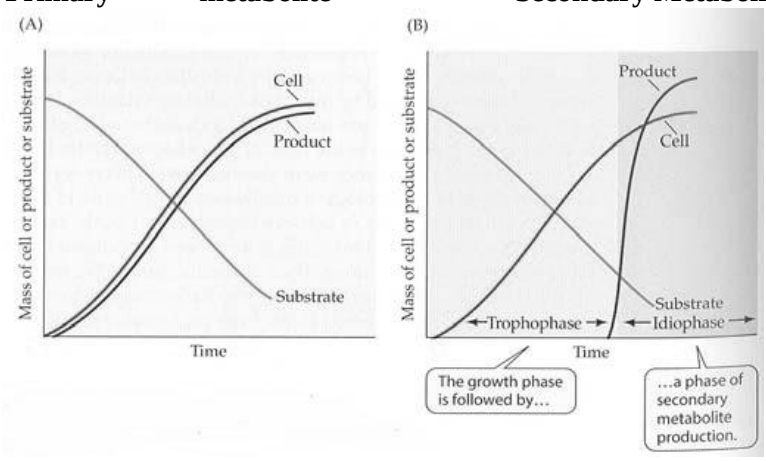


Fig.5 – Microbial metabolism

3. FERMENTATION BASICS

Types of Fermentation Process

The fermentation unit in industrial microbiology is analogous to a chemical plant in the chemical industry. A fermentation process is a biological process and, therefore, has requirements of sterility and use of cellular enzymic reactions instead of chemical reactions aided by inanimate catalysts, sometimes operating at elevated temperature and pressure.

Fermentation is the term used by microbiologists to describe any process for the production of a **product** by means of the mass culture of a **microorganism**.

The **product** can either be:
1. The cell itself: referred to as biomass production.
2. A microorganisms own metabolite: referred to as a product from a natural or genetically improved strain.
3. A microorganisms foreign product: referred to as a product from recombinant DNA technology or genetically engineered strain, i.e. recombinant strain.

3.1. Microbial kinetics

BATCH FERMENTATION

A **batch fermentation** can be considered to be a closed system. At time t=0 the sterilized nutrient solution in the fermentor is **inoculated** with microorganisms and incubation is allowed to proceed. In the course of the entire fermentation, nothing is added, except oxygen (in case of aerobic microorganisms), an antifoam agent, and acid or base to control the pH. The composition of the culture medium, the biomass concentration, and the metabolite concentration generally change constantly as a result of the metabolism of the cells.

After the inoculation of a sterile nutrient solution with microorganisms and cultivation under physiological conditions, four typical phases of growth are observed.

Lag phase - Physicochemical equilibration between microorganism and the environment following inoculation with very little growth.

Log phase - By the end of the lag phase cells have adapted to the new conditions of growth. Growth of the cell mass can now be described quantitatively as a doubling of cell number per unit time for bacteria and yeast's, or a doubling of biomass per unit time for filamentous organisms as fungi. By plotting the number of cells or biomass against time on a semilogarithmic graph, a straight line results, hence the term log phase. Although the cells alter the medium through uptake of substrates and excretion of metabolic products, the growth rate remains constant during the log phase. Growth rate is independent of substrate concentration as long as excess substrate is present.

Stationary phase - As soon as the substrate is metabolized or toxic substances have been formed, growth slows down or is completely stopped. The biomass increases only gradually or remains constant during this stationary phase, although the composition of the cells may change. Due to lysis, new substrates are released which then may serve as energy sources for the slow growth of survivors. The various metabolites formed in the stationary phase are often of great biotechnological interest.

Death phase - In this phase the energy reserves of the cells are exhausted. A straight line may be obtained when a semilogarithmic plot is made of survivors versus time, indicating that the cells are dying at an exponential rate. The length of time between the stationary phase and the death phase is dependent on the microorganism and the process used. The fermentation is usually interrupted at the end of the log phase or before the death phase begins.

FED BATCH FERMENTATION

In the conventional batch process just described, all of the substrate is added at the beginning of the fermentation. An enhancement of the closed batch process is the **fedbatch** fermentation. In the fed-batch process, substrate is added in increments as the fermentation progresses. In the fed-batch method the critical elements of the nutrient solution are added in small concentrations at the beginning of the fermentation and these substances continue to be added in small doses during the production phase.

CONTINUOUS FERMENTATION

In **continuous fermentation**, an open system is set up. Sterile nutrient solution is added to the bioreactor continuously and an equivalent amount of converted nutrient solution with microorganisms is simultaneously taken out of the system. In the case of a homogeneously mixed bioreactor we refer to a **chemostat** or a **turbidistat**. In the chemostat in the steady state, cell growth is controlled by adjusting the concentration of one substrate. In the turbidistat, cell growth is kept constant by using turbidity to monitor the biomass concentration and the rate of feed of nutrient solution is appropriately adjusted.

Nutrient requirements. All microorganisms need for their microbial activity the presence of several nutrients.

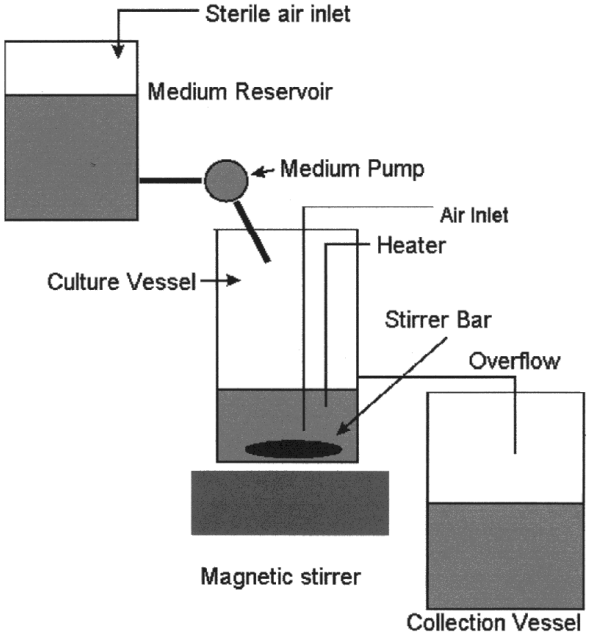


Fig.6 – Basic Chemostat System

Carbohydrates. Carbohydrates are capable of being used by all microorganisms, although in no case is there an absolute requirement for this group of organic compounds. Glucose is the most readily metabolized sugar. Most fungi can use disaccharides.

Lipides. Microbial requirements for steroids, and long-chain fatty acids can be summarized as follows. Long-chain fatty acids like linoleic acid and oleic acid are required for bacteria and fungi. Generally, steroids, other than cholesterol, are not required or utilized by microorganisms. In all fungi, and including yeast, ergosterol is a nutritional requirement.

Purines and pyrimidines. It is generally only in bacteria that cases of purine and pyrimidine metabolism have been reported. Algae do not utilize these compounds at all.

Vitamins and growth factors. There is considerable species variation in the requirements of vitamins and related factors by other microorganisms. Generally, vitamins A, C, D, and K are not necessary for growth.

Amino acids. Amino acids are not generally required by algae, although several algae species are capable of utilizing them. Species of other microorganisms are capable of utilizing all amino acids, except for yeast's, where there is no evidence of critrulline being used. It is usually the L-form of the acids that are biologically active but, unlike higher animals, some bacteria can also utilize the D-amino acids.

Nitrogen sources. It should be stressed that not all species require or utilize these compounds but rather that some species have been identified that are able to utilize these compounds. Fungi require ammonia, nitrate and nitrite.

Sulfur sources. Some species of yeast's can utilize elemental sulfur and sulfate. Generally yeast's do not require or utilize sulfur containing organic compounds. Bacteria require glutathione and thio-acetic acid while yeast's require sulphonic acid amides, thioacetate, thiocarbonate, thioglycolate and glutathione.

Chemical elements and inorganic ions. Mineral nutrients required by microorganisms are species dependent but consists generally of Fe, K, Mg, Mn. Sometimes S, N, Ca, Co, Cu, P, Zn is required.

Fermentor systems

A microbial fermentation can be viewed as a **three-phase system**, involving liquid-solid, gas-solid, and gas-liquid reactions.

The **liquid phase** contains dissolved nutrients, dissolved substrates and dissolved metabolites.

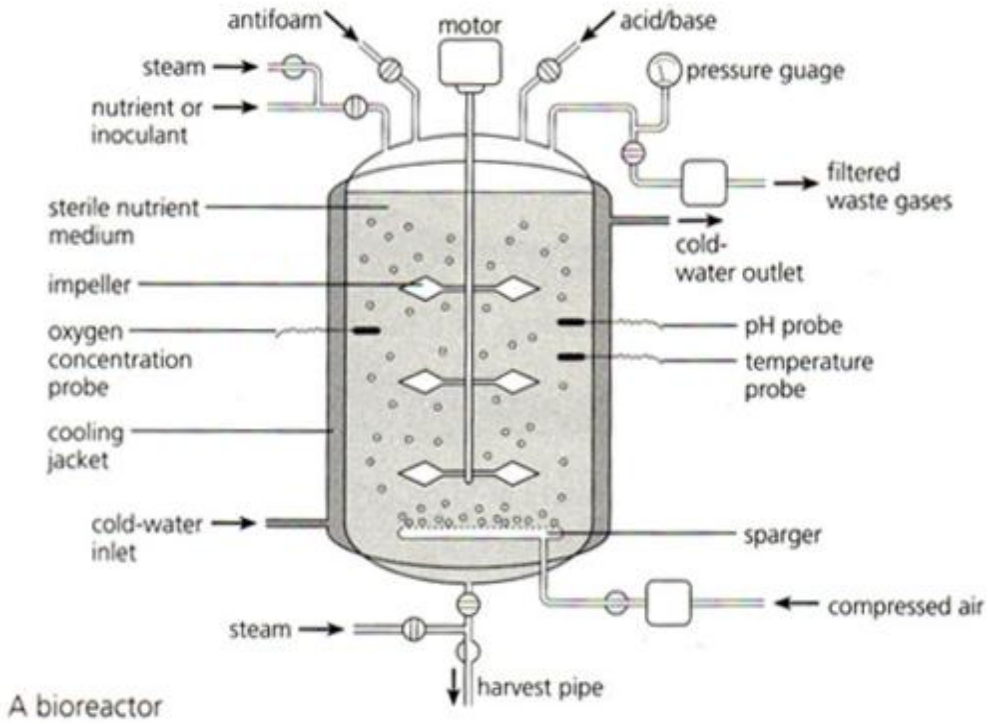


Fig.7 – Structure of the bioreactor

The **solid phase** consists of individual cells, pellets, insoluble substrates, or precipitated metabolic products.

The **gaseous phase** provides a reservoir for oxygen supply and for CO_2 removal.

3.2. Aeration & mixing

The transfer of energy, nutrients, substrate and metabolite within the bioreactor must be brought about by a suitable mixing device. The efficiency of any one nutrient may be crucial to the efficiency of the whole fermentation.

For the three phases, the stirring of a bioreactor brings about the following:
• Dispersion of air in the nutrient solution
• Homogenization to equalize the temperature and the concentration of nutrients throughout the fermenter
•Suspension of microorganisms and solid nutrients
• Dispersion of immiscible liquids 0.5 m/sec.

Nutrient solutions can be subdivided into two groups according to the way they behave when stirred: **viscous** solutions with Newtonian and non-Newtonian properties; and **viscoelastic** solutions, in which normal liquid-state properties are not observed in stirred vessels. The **viscosity**, the ability of a material to resist deformation, is the most significant property affecting the flow behavior of a fluid. Such behavior has a market effect on pumping, mixing, heat transfer, mass transfer and aeration.

There are only a few examples which fall into the second group, e.g. polysaccharides and certain antibiotic fermentation's. Most fermentation solutions fall into the first category. Uninoculated solutions and bacterial cultures often behave as simple Newtonian liquids.

With many mycelial organisms, changes occur during the fermentation not only in the amount of mycelium, but in the characteristics of the nutrient solution. Substrates are taken up during metabolism and the proportion of undisclosed substrates is reduced. At the same time, metabolites are excreted, thus affecting the viscosity of the solution.

Gas exchange and mass transfer

One of the most critical factors in the operation of a fermentor is the provision of adequate gas exchange. **Oxygen** is the most important gaseous substrate for microbial metabolism, and **carbon dioxide** is the most important gaseous metabolic product.

When oxygen is required as a microbial substrate, it is frequently a limiting factor in fermentation. Because of its low solubility, only 0.3 mM O_2, equivalent to 9 mg/l, dissolves in one liter of water at 20 degrees Celsius in an air/water mixture. This amount of oxygen will be depleted in a few seconds by an active and concentrated microbial population unless oxygen is supplied continuously. In contrast, during the same period the amount of other nutrients used is negligible compared to the bulk of concentrations. Therefore most aerobic microbial processes are oxygen limited. This is the reason why the concept of gas-liquid mass transfer in bioprocesses is centered on oxygen transfer even if other gasses such as carbon dioxide, hydrogen, methane and ammonia can also be involved.

Due to the influence of the culture nutrients, the maximal oxygen content is actually lower than it would be in pure water.

The solubility of gasses follows Henry's law in the gas pressure range over which fermentors are operated. This means that if the oxygen concentration in the gas phase

increases, the O_2 proportion of the nutrient solution increases. Consequently the highest O_2 partial pressure are attained during aeration with pure oxygen. Compared to the value in air (9 mg O_2/l), 43 mg O_2/l dissolves in water when pure oxygen is considered.

As temperature rises, the O_2 solubility decreases. For example the solubility at 33 degrees Celsius is 7,2 mg O_2/l.

For oxygen to be transferred from a gas bubble to an individual cell, several independent partial resistance's must be overcome.

*1 resistance within the gas film to the phase boundary
*2 penetration of the phase boundary between gas bubble and liquid
*3 transfer from the phase boundary to the liquid
*4 movement within the nutrient solution
*5 transfer to the surface of the cell

For fermentation's carried out with single celled organisms such as bacteria and yeast's, the resistance in the phase boundary between the gas bubble and the liquid is the most important factor controlling the rate of transfer.

Microbial cells near gass bubbles may absorb oxygen directly through the phase boundary and the rate of gas transfer to such cells is increased.

In cell agglomerates or pellets, the O_2 transfer within the agglomerate can become the limiting factor.

The mass transfer of **oxygen into liquid can be characterized by the oxygen transfer rate (OTR)** or by the **volumetric oxygen transfer coefficient (k_La)**. These values have been thoroughly examined as a critical parameter for bioreactor function. The oxygen transfer rate and the volumetric oxygen transfer coefficient are dependent on the following parameters:

* the vessel geometry: diameter, capacity
* mixing properties: power, impeller configuration and size, baffles
* aeration system: sparger rate, geometry, location
* the nutrient solution: composition, density, viscosity
* the microorganism: morphology, concentration
* the antifoam agent used
* the temperature

The baffles serve to disrupt the vortex pattern that develops around a single-shaft impeller rotating in an unconstrained fluid. The baffles produce a large planar liquid surface and a uniform flow pattern as well as increase the liquid hold-up for a given fermentor volume.

Surface-active substances such as **antifoam agents** reduce the oxygen transfer rate. In pure water, the bubble surface is constantly renewed through vibration and oscillation. As soon as surface-active substances are added, the renewal of the bubble surface by bubble movement ceases.

Microorganisms themselves have an effect on the oxygen transfer rate by acting as a barrier, thus inhibiting the O_2 transfer. With filamentous microorganisms, there are variations depending on whether the mycelium is in loose form or in pellets. While the oxygen transfer rate decreases gradually as the pellets increases in size, there is a much steeper decline with loose forms.

The gas bubbles are replenished in locations of the bioreactor where there is negative pressure, such as behind the agitator blades. As the aeration rate increases, various conditions can be characterized. At low aeration rates, large gas bubbles form behind individual turbine blades and smaller bubbles are spun off centrifugally into the nutrient solution. As the aeration rate is increased, gases bubbles collect behind all turbine blades and continue to accumulate. The energy input is one-third less than that used in unaerated systems. In this intermediate stirring range, gas dispersion is the best. At very high aeration rates, many large gases bubbles adhere to each other and the impeller is flooded with gas, resulting in sharply lowered gas dispersion.

The **critical oxygen concentration** is the term used to indicate the value of the **oxygen uptake rate** or o**xygen absorption rate** which permits respiration without hindrance. Generally the critical oxygen concentrations are 5-25% of the oxygen saturation value in cultures. At oxygen absorption rates which are lower than the critical concentrations, respiration rate is correlated with the O_2 concentration in the solution. Above this value, no dependence between respiration rate and dissolved oxygen has been observed. In Newtonian fluids, such as those occurring in yeast's and bacterial fermentation's, the critical oxygen concentration is constant and is not affected by fermentation conditions. In non-Newtonian solutions, such as those occurring with filamentous microorganisms, the critical oxygen concentration has been shown to be dependent on fermentation conditions.

Heat production

In order to obtain optimal yields, fermentation's must be carried out at **constant temperature**. We now discuss the parameters affecting the heat balance of a fermentation process.

The rate of heat production due to stirring, gassing/aeration and due to the metabolic activity of the microorganisms must be balanced by the heat loss resulting from evaporation and radiation plus heat removal by the cooling system.

During metabolism, heat evolution is a consequence of the thermodynamics of the overall microbial activity. Apart from anaerobic digestion and some other thermopiles microbial activity, the amount of heat produced is usually so high that if it is not removed it raises the temperature of the contents of the fermentor to a level beyond the optimum range for the system.

The evolution of heat during metabolic activity is related to the utilization of the carbon and the energy source. When the carbon source is being actively incorporated into biomass trough anabolism during growth, about 40-50 % of the available enthalpy in the substrate is conserved in the biomass, the rest being given off as heat. When the carbon source is being catabolized to provide energy for cell maintenance, all the enthalpy associated with the oxidation of the substrate is released as heat. If a biochemical product is formed, the heat evolved lies between the heat released during maintenance and that evolved during active growth. The amount of heat evolved is related to the stoichiometry for growth and product formation, whereas the rate of heat evolution is related to the rate of microbial activity.

3.3. Sterilization for fermentation processes

Sterilization cultures at all stages, from the preliminary culture to the fermentor. A fermentor can be sterilized either by destroying the microorganisms with some lethal agent such as heat, radiation, or a chemical, or by removing the viable microorganisms by a physical procedure such as filtration.

During fermentation the following points must be observed to ensure sterility:

- sterility of the culture media
- sterility of incoming and outgoing air
- appropriate construction of the bioreactor for sterilization and for prevention of contamination during fermentation

Sterilization of the culture media

Nutrient media as initially prepared contain a variety of different vegetative cells and spores, derived from the constituents of the culture medium, the water and the vessel. These must be eliminated by a suitable means before inoculation. A number of means are available for sterilization, but in practice heat is the most often used mechanism.

A number of factors influence the success of heat sterilization: the number and type of microorganisms present, the composition of the culture medium, the pH value, and the size of the suspended particles. Vegetative cells are rapidly eliminated at relatively low temperatures such as 60 degrees Celcius for 5-10 minutes, but for destruction of spores, temperatures of 121 degrees Celcius are needed during 15 minutes.

During heat sterilization there is always the possibility of destroying ingredients in the medium. Apart from the degradation of heat-labile components, also contributes to the loss of nutrient quality during sterilization. A common phenomenon is the occurance of the Maillard-type browing reactions which cause discoloration of the medium as well as loss of nutrient quality. These reactions are normally caused by carbonyl groups, usually from reducing sugars, interacting with amino groups from amino acids and proteins. Separate sterilization of the carbohydrate component of the medium may be necessary to prevent such reactions.

Filter sterilization is often used for all components of nutrient solutions which are heat sensitive. Sugars, vitamins, antibiotics or blood components are examples of heat-labile components which must be sterilized by filtration.

Most nutrient media are presently sterilized in batch volumes in the bioreactor at 121 degrees Celcius. Approximate sterilization times can be calculated from the nature of the medium and the size of the fermentor. Not only the nutrient media, but also the fittings, valves and electrodes of the fermentor itself must be sterilized. Therefor, actual sterilization times are significantly longer than calculated ones and must be empirically determined for the specific nutrient solutions in the fermentor. Smaller fermentors are sterilized in an autoclave while larger fermentors are sterilized by indirect or direct steam injection.

Sterilization of the fermentation air

Most fermentation's are operated under high aeration and the air supplied to the fermentor must be sterilized. The number of particels and microorganisms in air varies greatly depending on the location, air movement, and previous treatment of the air. On the average, outdoor air has 10-100,000 particles per m^3 and 5-2,000 microorganisms per m^3. Of these, 50% are fungus spores and 40% are Gram-negative bacteria.

Fermentors generally work with aeration rates of 0.5-2 vvm (air volume/liquid volume per minute). The methods available for sterilizing gases include filtration, gas injection (ozone), gas scrubbing, radiation (UV) and heat. Of these, only filtration and heat are practical.

Appropriate construction of the bioreactor

There should be a minimum number of openings in the fermentor to favor maintenance of sterility. Small openings must be made leak proof with O-rings, larger openings with flat gaskets. Whenever a movable shaft penetrates the fermentor wall, special problems of sterility maintenance should be solved.

Fermentation processes

An overall scheme of a fermentation process can be described as follows:

Stage 1: inoculums preservation
Stage 2: inoculums build-up
Stage 3: fermentor culture

Stage 1: inoculums preservation

The objective of preservation is to maintain strains as long as possible without cell division. The optimal method of preservation must be worked out for each strain. The following three techniques are most commonly used:

* Storage at low temperatures (2-6 degrees Celsius)
* Frozen storage (-18, -80 or -196 degrees Celsius)
* Lyophilization

Storage at 2-6 degrees Celsius is the least secure; there is a relatively high risk of contamination and reverse mutation through frequent transfer. The frozen storage is the most common and frozen cultures may be kept for several years. The proportion of survivors is critical because up to 95% of the microorganisms are generally killed during freezing and subsequent thawing. The best method of strain preservation is Lyophilization (freeze-drying).

Stage 2: inoculums build-up

The preserved culture is initially revived by growth in a erlenmeyer flask on a biological shaker or on a solid medium (if spore formation is needed). In order to obtain sufficient inoculum for small fermentors, a second series of shake cultures is usually made in more flasks. Out from lyophilized strains the growth of inoculum takes around 4-10 days, out from frozen cultures the growth of inoculum takes 4-48 hours for

bacteria and 1-7 days for fungi. Finally out of refrigerated cultures the growth of inoculum takes 4-24 hours for bacteria and 1-5 days for fungi.

Stage 3: fermentor culture

The nutrient media for production must be optimized not only in the ingredients used but also how the medium is prepared and sterilized, pH value before and after sterilization. The most important parameters during the fermentation are:

* Temperature
* Aeration
* Stirring

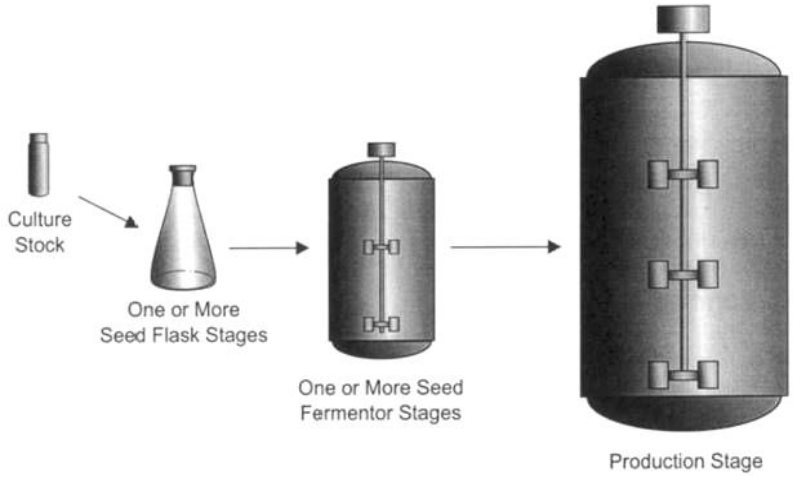


Fig.8 – Fermentor stages

Bacteria
- *Bacillus subtilis*
- *Lactobacillus bulgaricus*
- *Lactococcus lactis*
- *Leuconostoc oenos*

Yeasts
- *Candida utilis*
- *Kluyveromyces marxianus*
- *Kluyveromyces lactis*
- *Saccharomyces cerevisiae*

Filamentous fungi
- *Aspergillus niger*
- *Aspergillus oryzae*
- *Mucor javanicus (Mucor circinelloides* f. *circinelloides)*
- *Penicillium roqueforti*

Note: Normally, these microorganisms require no further testing if used under acceptable cultivation conditions.

Fig.9 – Examples of microorganisms classified as GRAS (generally regarded as safe)

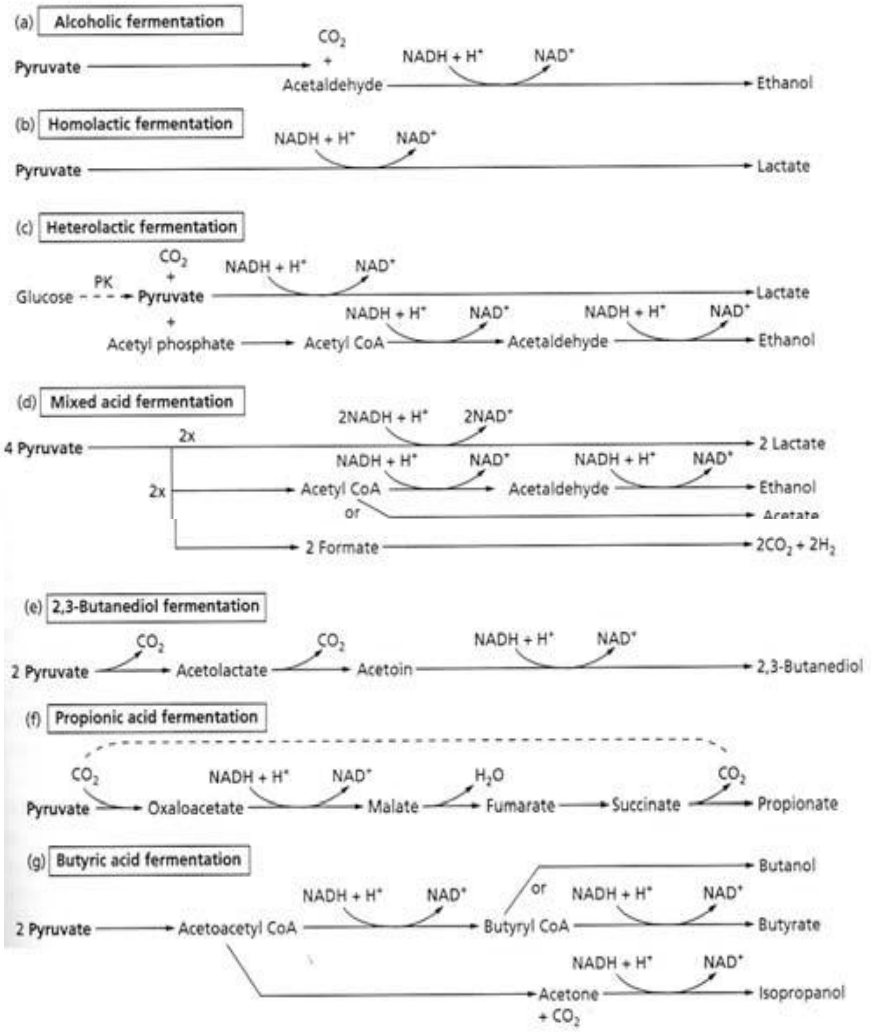


Fig.10 – Fermentation products from pyruvate

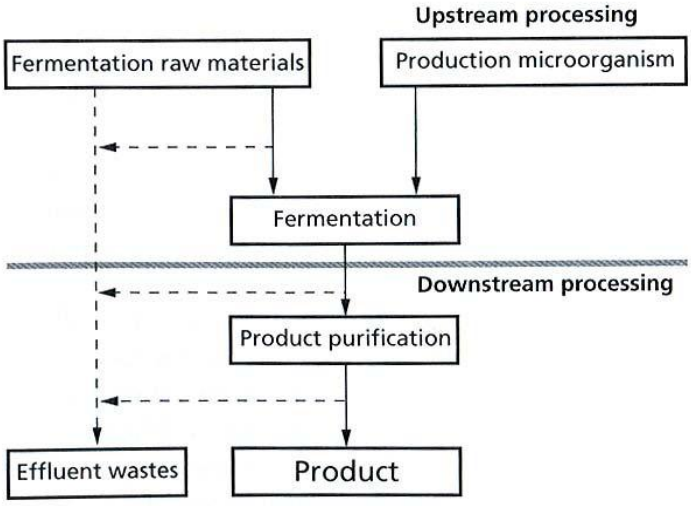


Fig.11 – Outline of a fermentation process

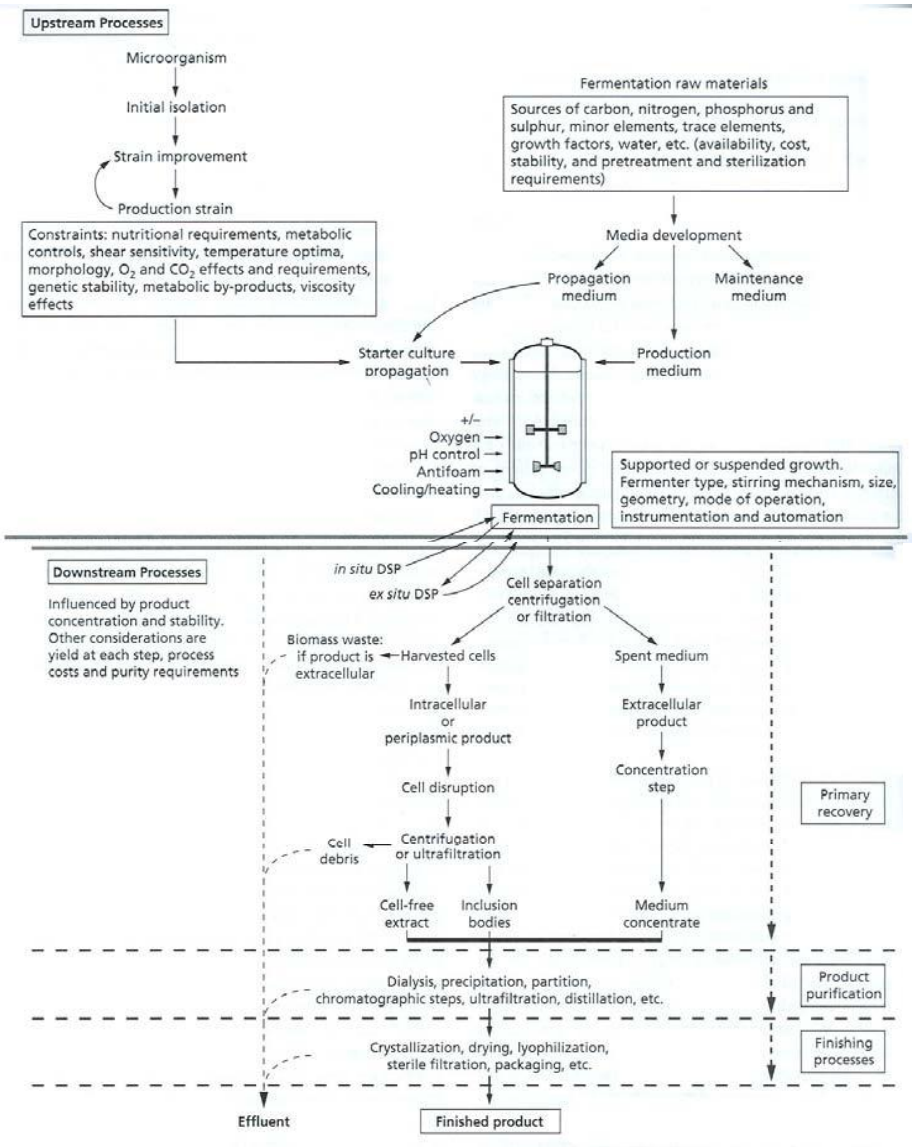


Fig.12 – An outline of upstream and downstream procewing operations

4. Microbial Products production
4.1. Alcoholic beverage industry

Ethanol

• *the* major microbial biotechnology: beer, wine, distilled beverages, industrial ethanol

Saccharomyces (sugar fungus)

• alcoholic (ethanolic) fermentation, principally by yeasts in the genus *Saccharomyces*

• Embden-Meyerhof-Parnas, glycolytic pathway

glucose + 2 ADP + 2 P_i → 2 EtOH + 2 CO_2 + 2 ATP

• not a facultative anaerobe, cannot grow anaerobically indefinitely (unsaturated fatty acids and sterols can be synthesized only under aerobic conditions)

• when oxygen present glucose oxidized via the Krebs cycle to CO_2 and water (much biomass and little alcohol produced)

Zymomonas mobilis
• Alphaproteobacterium
• osmotic tolerance, relatively high alcohol tolerance
• higher specific growth rate than yeast
• anaerobic carbohydrate metabolism through the Entner-Doudoroff pathway, yielding only 1 mol of ATP per mol of glucose → more glucose converted to EtOH
• limited substrate use, only 3 carbohydrates: glucose, fructose and sucrose
• genetic engineering to expand substrate range

Conversion of starch to fermentable sugars

1. Malting
• germination of barley to induce production of amylases
• beer

2. Amylolytic molds and yeasts
• filamentous fungus *Aspergillus oryzae*
• Japanese sake

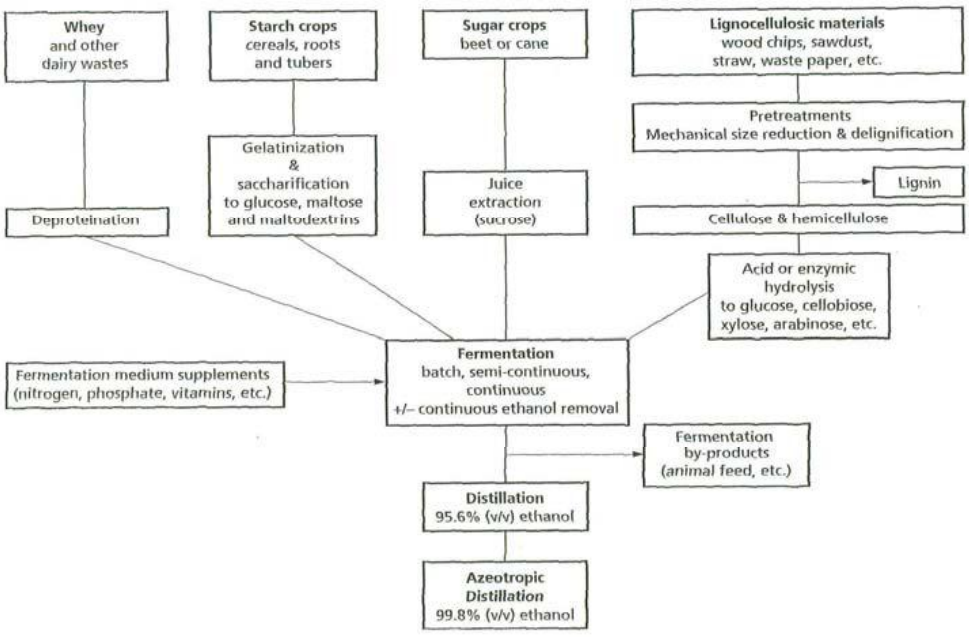


Fig.13 – Industrial ethanol production from various subsrates

4.2. Fermented food industry

Vinegar

• sour (spoiled) wine, vinegar (from French): vin and aigre (sour)
• production in the US about 160 million gallons per year

(2/3 used in commercial products such as sauces and dressings, production of pickles and tomato products)

• the acetic acid bacteria divided into two genera:
Acetobacter aceti and *Gluconobacter oxydans*
• obligate aerobes that oxidize sugar, sugar alcohols and ethanol with the production of acetic acid as the major end product
• ethanol oxidation occurs via two membrane-associated dehydrogenases: alcohol dehydrogenase and acetaldehyde dehydrogenase

Industrial Production of Acetic Acid

Trickling filter
• vinegar manufacturing industry near Orleans in 14th century
• trickling filter, wooden bioreactor (volume up to 60 m3) filled with beechwood shavings, acetic acid bacteria grow as biofilm
• the ethanolic solution is sprayed over the surface and trickles through the shavings into a collection basin, and recirculated
• temperature maintained at 29-35°C
• about 12% acetic acid produced in 3 days
• the life of a well-packed and maintained generator is about 20 years
Submerged, batch process (Frings acetator)
• stainless steel tank with a high-speed mixer microbes, air, ethanol and nutrients are mixed to provide a favorable environment for microbial growth
• 30°C maintained by circulation of cooling water
• 12% acetic acid in about 35 h
• production rate per m3 over 10 times higher than with surface fermentation" and over 5% higher than with trickling filter

The organisms employd in commercial vinegar production are *Acetobacter aceti* and *Gluconobacter oxydans*

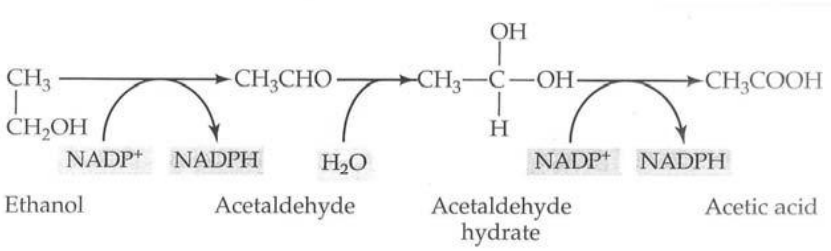


Fig.14 – Acetic Acid production

Lactic acid fermentation

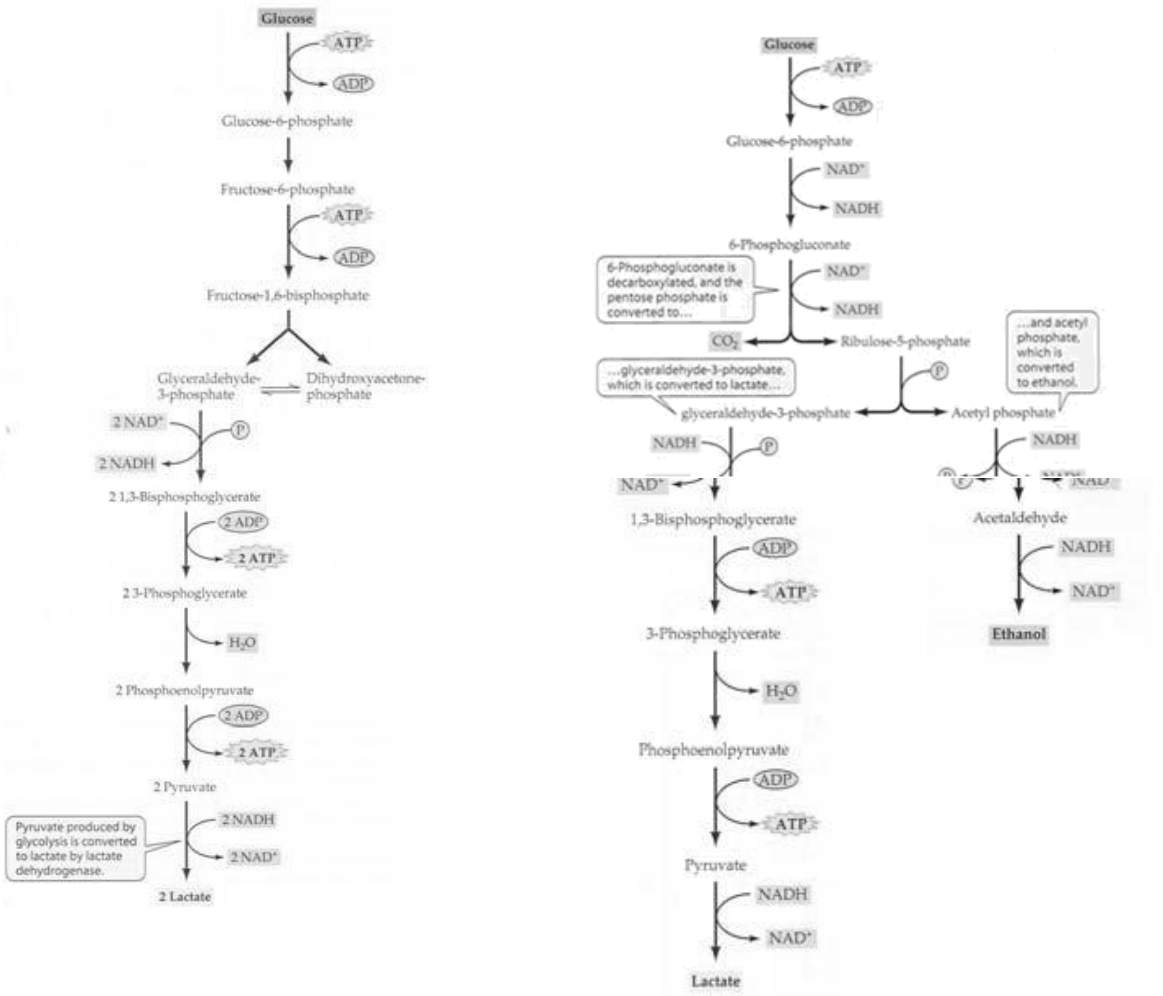
• pyruvate is reduced to lactic acid with the coupled reoxidation of NADH to NAD+
• lactic acid bacteria (e.g. *Lactobacillus, Streptococcus*) involved in many food fermentations
• fermented milk, cheese, fermented vegetables

Homolactic fermentation
• glucose degraded via EMP pathway, with lactic acid as the only end product

glucose + 2 ADP + 2 Pi → 2 lactic acid + 2 ATP
• carried out by *Streptococcus, Pediococcus, Lactococcus, Enterococcus* and various *Lactobacillus* species
• important in dairy industry (yogurt, cheese)

Heterolactic fermentation
• glucose degraded via pentose phosphate pathway
• in addition to lactic acid, also ethanol and CO_2 produced

glucose + ADP + Pi → lactic acid + ethanol + CO_2 + ATP



Homofermentation pathway

Net Gain 2 ATP 2 lactate per glucose

Heterofermentation pathway

Net Gain 1 ATP 1 lactate + 1 ethanol + 1 CO_2 per glucose

Fig.15 – Lactic acid fermentation

Lactic Acid Fermentation Lactic acid is produced from various carbohydrates such as corn strach, potato strach, molasses, and whey. When starchy materials are used, they are first hydrolysed to simple sugars. The medium is then supplemented with a nitrogen source and calcium carbonate arid fermentation is carried out by the inoculation with homofermentative lactobacilli such as Lactobacillus bulgaricus or Lactobacillus delbruckli. During the fermentation the temperature is controlled at 43-

50°C depending on the organism and the medium is kept in constant agitation to keep the calcium carbonate in suspension. 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.

Cheese making

Pre-ripening

- inoculation with starter culture of lactic acid bacteria
- acidification by fermentation of lactose to lactic acid

Table 2 - Microorganisms involved in the manufacture of cheeses and fermented milks

Products	Principal acid producers	Intentionally introduced secondary microflora
Cheeses		
Colby, Cheddar, cottage, cream	*Lactococcus lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*	None
Gouda, Edam, Havarti	*Lactococcus lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*	*Leuconostoc* sp., Cit$^+$ *Lactococcus lactis* subsp. *lactis*
Brick, Limburger	*Lactococcus lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*	*Geotrichum candidum*, *Brevibacterium linens*, *Micrococcus* sp.
Camembert	*Lactococcus lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*	*Penicillium camemberti*, sometimes *Brevibacterium linens*
Blue	*Lactococcus lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*	Cit$^+$ *Lactococcus lactis* subsp. *lactis*, *Penicillium roqueforti*
Mozzarella, provolone, Romano, Parmesan	*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lb. helveticus*	None; animal lipases added to Romano for picante or rancid flavor
Swiss	*Streptococcus thermophilus*, *Lactobacillus helveticus*, *Lb. delbrueckii* subsp. *bulgaricus*	*Propionibacterium freudenreichii* subsp. *shermanii*
Fermented milks		
Yogurt	*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*	None
Buttermilk	*Lactococcus lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*	*Leuconostoc* sp., Cit$^+$ *Lactococcus lactis* subsp. *lactis*
Sour cream	*Lactococcus lactis* subsp. *cremoris*, *Lc. lactis* subsp.	None

Coagulation of milk proteins (casein)

- addition of chymosin (rennin), an acid protease from calves' stomach - *renneting*
  since 1980s also recombinant enzyme produced in yeast
- catalyzes specific hydrolysis of κ-casein leads to coagulation in the presence of Ca^{2+} to form a gel (curd)
- also traps bacteria that continue lactic acid fermentation
- removal of liquid whey from the curd
- addition of secondary microbiota
- treatments vary for different cheese varieties

Separation of curd and whey

- accelerated by decrease in pH
- salting
- curd pressed and placed into mold

Ripening

• storage at controlled humidity
at ~9°C for up to a year
•modification of proteins and
fats by proteases and lipases
• complex development of flavor

4.3.Amino acids production

• annual worldwide production of over 400,000 tons
• uses as food additives, medicines, starting material in chemical synthesis
glutamic acid (80% of total), lysine (10%)
• production of glutamic acid (MSG) for use in foods; other amino acids (L-lysine)
• *Corynebacterium glutamicum*

Amino Acid Fermentations -In recent years there has been a rapid development of the production of particular amino adds 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, where upon 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 hydrolysing 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. The demand for amino acids for use in foods, feeds, and in the pharmaceutical industries is expanding; moreover. When production costs decrease, a new usage is anticipated, as raw material for amino acid polymers.

Table 3 - Amino acids used in the food industry

Amino acid[b]	Annual production worldwide (metric tons)	Uses	Purpose
L-Glutamate (monosodium glutamate, MSG)	370,000	Various foods	Flavor enhancer; meat tenderizer
L-Aspartate and alanine	5,000	Fruit juices	"Round off" taste
Glycine	6,000	Sweetened foods	Improves flavor; starting point for organic syntheses
L-Cysteine	700	Bread Fruit juices	Improves quality Antioxidant
L-Tryptophan + L-Histidine	400	Various foods, dried milk	Antioxidant, prevent rancidity; nutritive additives
Aspartame (made from L- phenylalanine + L-aspartic acid)	7,000	Soft drinks, chewing gum, many other "sugar-free" products	Low-calorie sweetener
L-Lysine	70,000	Bread (Japan), feed additives	Nutritive additive
DL-Methionine	70,000	Soy products, feed additives	Nutritive additive

Microbial production of amino acids shows two outstanding features which are not usually encountered in the development of other microbiological processes. One is the importance of auxotrophic micro organisms. These microorganisms were known only as useful tools in the analysis of metabolic pathways and in genetics but, today; they are

proving of great value. The second feature is that knowledge of metabolic control mechanisms can now be used to good purpose in industrial microbiological processes.

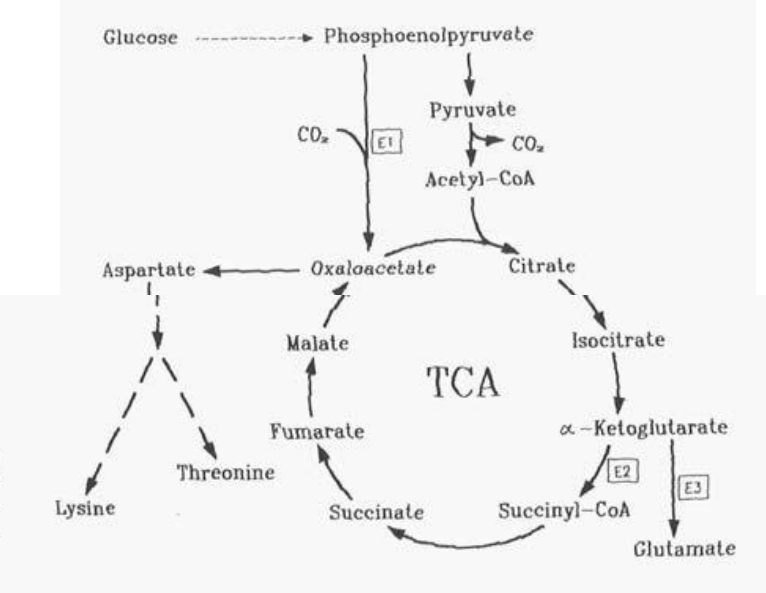


Fig.16 – Metabolic pathways in glutamic acid-producing corynebacteria

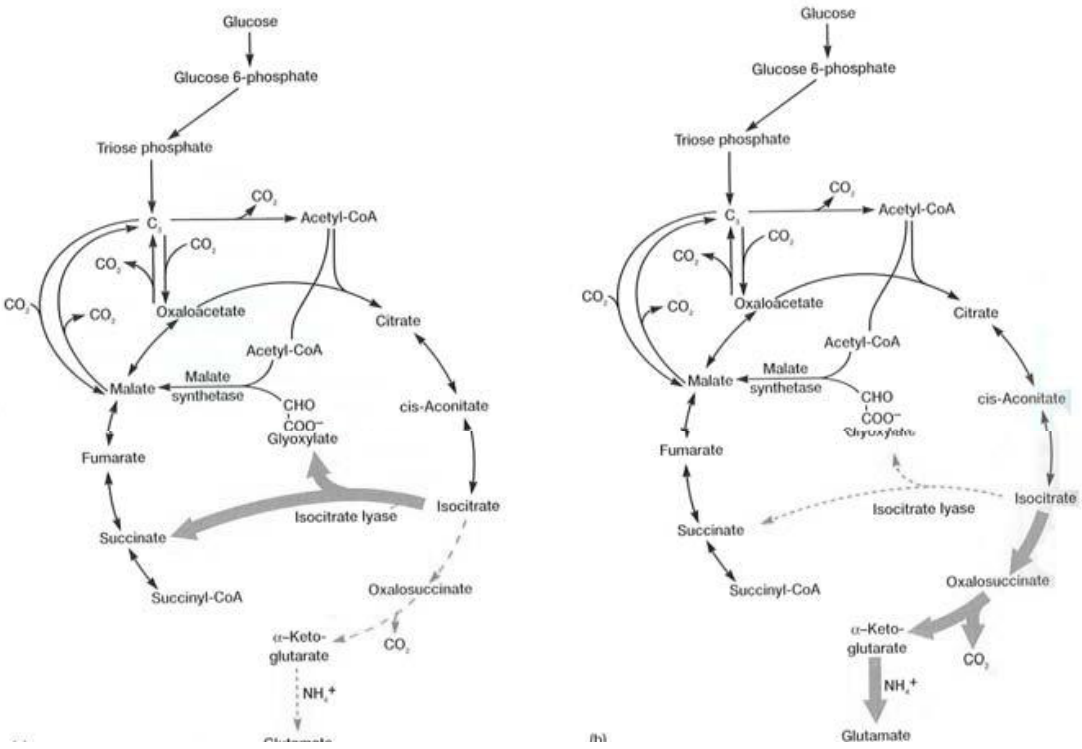


Fig.17 – Glutamic Asid Production

Gluconic Acid Fermentations - Gluconic acid used in pharmaceutical industries 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 per cent 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

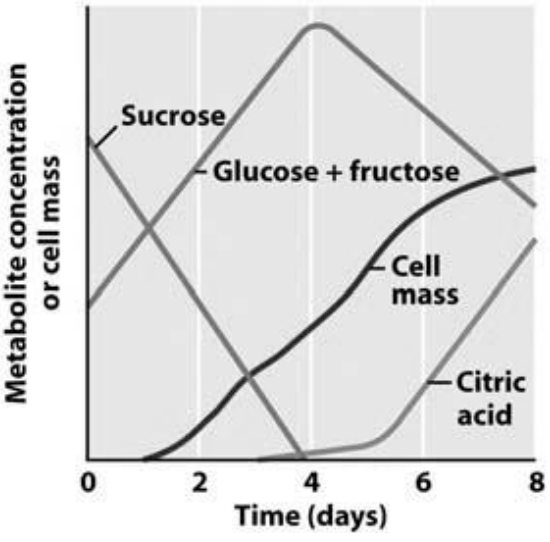
culture conditions to convert glucose into gluconic acid. The pH of the medium is controlled by the addition of a strong solution of sodium hydroxide. Fermentation is carried out at 33 or 34°C.The medium composition and fermentation conditions determine the production of acids other than gluconic acid (such as citric acid and oxalic acid) and hence it is important to select a mold strain and the fermentation conditions that will avoid the formation of unwanted organic acid. After the fermentation the cell free broth is centrifuged and processed to recover gluconic acid.

4.4. Organic acid production

• over 130,000 tons produced worldwide each year
• used in foods and beverages
• iron citrate as a source of iron preservative for stored blood, tablets, ointments,…
in detergents as a replacement for polyphosphates
• a microbial fermentation for production of citric acid developed in 1923
• today >99% of the world's output produced microbially
Aspergillus niger
• submerged fermentation in large fermenters
• sucrose as substrate, and citric acid produced during idiophase
• during trophophase mycelium produced and CO2 released
• during idiophase glucose and fructose are metabolized directly to citric acid

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. 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 per cent sugar concentration and mixed with a nitrogen source and other salts. The pH of the medium is maintained around five when molasses is used and at a lower level (pH 3.0) when sucrose is used.

The fermentations are carried out either under surface, submerged, or solid state conditions. In the surface culture method, shallow aluminium or stainless steel pans are filled with the growth medium, inoculated with the fungal spores and allowed to ferment. In the submerged culture method the mold is cultured in fermentors under

vigorous stirring and mixing, while in solid state fermentation, the mold is grown over carrier material such as bagasse etc., which is impregnated with the fermentation medium. The production of citric acid by A.niger is largely influenced by the concentration of trace metals such as iron, manganese, copper and zinc in the medium. An appropriate concentration of these elements is essential for good acid production. However, an excess is detrimental. To optimize the level of these trace metals, the raw materials are treated with either ferrocyanide, charcoal, chelating agents or catien exchange resins. Addition of methanol at 3-4 % concentration has been found to enhance the yield of citric acid. This fermentation is an aerobic fermentation and, therefore, adequate aeration is essential for successful citric, acid production

In recent years, the production of citric add by yeast is gaining importance because a variety of yeasts such as Candida, Hansenula etc. have been found to produce citric acid from carbohydrates and hydrocarbons. Strains of Candida lipolytica appear promising and good yields of citric acid from various raw materials has been reported. The mechanism by which these yeasts produce citric acid appears to be slightly different from the mechanism by which the fungi produce citric acid.

After the fermentation is over, calcium citrate is, precipitated from the fermented broth by the addition of calcium hydroxide. It is then filtered washed and treated with sulphuric acid to precipitate calcium sulphate. The solution containing citric acid is then purified by treatment with ion exchange resins, charcoal etc., and finally crystalized. Citric acid is used in food, beverage, textile, pharmaceutical and detergent industries. It is also increasingly used in the removal of toxic and corrosive gases in air. The variety of uses that it has, have increased the demand for this organic acid. Besides fungi and yeast, the possibilities of using bacteria to produce citric acid is also being explored.

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.

4.5. Antibiotic and vaccine production

• Antibiotics are small molecular weight compounds that inhibit or kill microorganisms at low concentrations

• often products of secondary metabolism

• the significance of antibiotic production is unclear, may be of ecological significance for the organism in nature

• antibiotics produced by various bacteria, actinomycetes & fungi *Bacillus*, *Streptomyces*, *Penicillium*

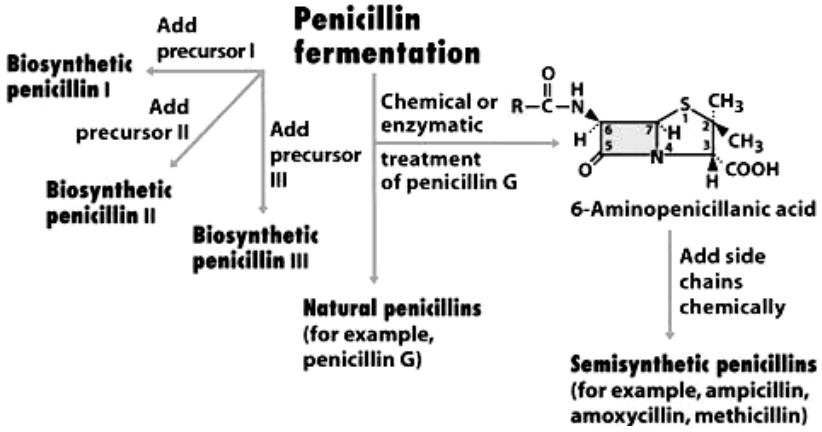


Fig.18 – *Penicillium* production

- **Pharmaceuticals**
  - **products of genetically engineered microbes** include:
    - **insulin** - treatment of diabetes
    - **growth hormones** - human (treat dwarfism), epidermal (promotes wound healing), bone (treat osteoporosis), animal (promotes livestock growth)
    - **tissue plasminogen activator** (TPA) - dissolves blood clots
    - **blood clotting factors** (VII, VIII, IX) - restore clotting mechanisms in hemophiliacs without chance of transmitting AIDS or hepatitis
    - **erythropoietin** - treatment of certain forms of anemia
    - **cytokines** - interferons (IFN), interleukins (IL), and other cytokines that act as anticancer agents or immune modulators
    - **IFN-gamma** stimulates cancer cells to produce tumor-associated antigens so they can be detected and eliminated by the immune system
    - **IL-2** stimulates T cells to promote immune responses
    - **tumor necrosis factor alpha** (TNFa) and **granulocyte-macrophage colony stimulating factor** (GM-CSF) work together with IL-2 in cancer therapy
    - **vaccine antigens** - prevention of bacterial, fungal, metazoan, viral diseases (e.g., recombinant Hepatitis B vaccine now in use)
    - **monoclonal antibodies** (mAb)
    - **diagnostic applications** - determine ovulation, pregnancy; identification of infectious agents
    - **therapeutic applications** - specific drug delivery in cancer therapy; destruction of platelet-catalyzed blood clots in heart disease therapy
  - **chemotherapeutic agents** -
    - **antibiotics are secondary metabolites** produced by bacteria (*Bacillus*, *Nocardia*, *Streptomyces*) or fungi (*Aspergillus*, *Cephalosporium*, *Penicillium*)
    - **more than 8000 known**, several hundred discovered per year (but most are unusable)

Table 4 - Important antibiotics produced by *Streptomyces* species

Antibiotic Group	Species	Common Name	Effective Against
Chloramphenicol	*S. venezuelae*	Chloramphenicol	Broad spectrum
Tetracycline	*S. aureofaciens*	Tetracycline	Broad spectrum
Chlortetracycline	*S. aureofaciens*	Chlortetracycline	Broad spectrum
Polyenes	*S. noursei*	Nystatin	Fungi
Macrolides	*S. erythreus*	Erythromcin	Most gram-positives
Legionella	*S. lincolnensis*	Clindamycin	Obligate anaerobes
Aminoglycosides	*S. griseus*	Streptomycin	Most gram-negative
	S. fradiae	Neomycin	Broad spectrum

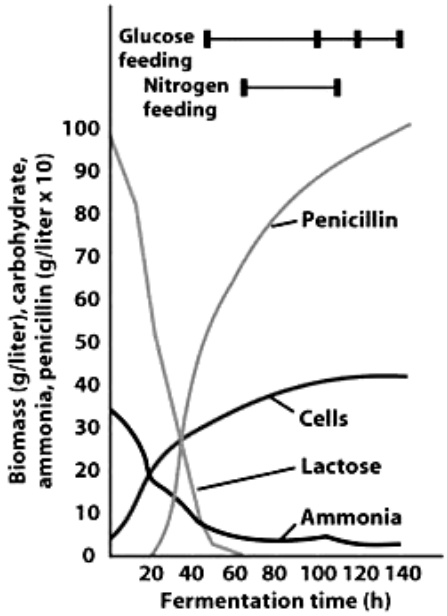


Fig.19 – Antibiotic and vaccine production

- **more than 100 tons produced annually**, worth more than $5 billion
- cheaper to produce by fermentation than by chemical synthesis, but their structures (and thus their activities) may be modified by subsequent chemical steps (semisynthetic antibiotics)
- **steps toward commercial production include:**
- **isolation** - usually by screening (cross-streak method)
- **testing** for toxicity and efficacy
- **optimization and purity of yield** - gene amplification, other genetic engineering of microbes
- **developing extraction and purification steps** - organic chemistry applications

examples:

- **ß-lactams**
- because they contain the ß-lactam group, they inhibit cell wall synthesis by blocking the transpeptidase that catalyzes peptide cross-linking
- examples - penicillin, ampicillin, cephalosporins
- **aminoglycosides**

- these molecules containing amino sugars bonded by glycosidic linkage inhibit protein synthesis in Gram (-) bacteria by binding to 30S ribosomal subunits
- examples - streptomycin, kanamycin, gentamicin
- **macrolides**
- these lactone rings connected to sugar moieties inhibit protein synthesis in Gram (+) bacteria by binding to 50S ribosomal subunits
- (examples - erythromycin, oleandomycin, spiramycin, tylosin
- **tetracyclines**
- these naphthacene ring systems inhibit protein synthesis by binding to 30S ribosomal subunits
- examples - chlortetracycline, oxytetracycline

Recombinant DNA Technology and Industrial Microbiology- It is genetically possible to "tailor" the microorganisms for the production of any microbial metabolite - vitamin, amino acid or enzyme. 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 man and his 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 two most ideal are the prokaryote, *Escherichia coli* and the eukaryote, *Saccharomyces cerevisiae.*

Some of the important products which gene cloning may make available in near future. The above proteins could be obtained on large scale through fermentation by methods, relatively cheaper than the conventional ones. For example, human growth hormone was previously extracted from the pituitary gland of cadavers and was mostly in short supply. Now, increase in supply should help more patients.

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.

4.6. Microbial enzyme industry and immobilization technology

Immobilization of Enzymes and Cells Related to Industrial Microbiology

Besides gene-cloning, in microbial biotechnology several commercial processes have used immobilised microbial cells and enzymes in the last few, years. Enzymes have been used in industry for over 70 years, initially in detergents that is still perhaps one of the largest bulk users of proteases and lipases.

In conventional biological industries also microbial enzymes have been used, e.g. proteases and amylases in malting and rennet in cheese manufacture. However, during last 15 years or so, immobilised cells and enzymes have been used as production systems.

1. Enzymes carry out stereospeciftc reactions with high accuracy, whereas chemical technology results into many side-products from which the desired product is to be purified.
2. Enzymes are cheap and carry out reactions "It low temperature and at atmospheric pressure, where as chemical catalysts require special and expensive conditions.
3. Since there is much diversity in microbial world, an enzyme for a desired reaction can be easily found out by merely screening a range of microbes.
4. Mutants with altered enzyme function can be isolated with appropriate genetic methods. Thus enzymes having different substrate specificities or with different physical properties (as temperature resistance) can be isolated. Also conventional genetics and gene cloning can be used for making specific changes in genes to increase the expression of the desired enzyme.

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 utilised, 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 immobilisation on an inert support.

The stability of an enzyme is improved after its immobilisation. Immobilisation affords a simple way of separating the enzyme or cell from the products when the reaction is complete and is likely to prove invaluable in the development of biological sensors (biosensors) - special electrodes based upto the selectivity and high affinity of enzymes for their substrates.

Immobilization Definition -Immobilisation means "imprisonment or confinement of a biocatalyst 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 (enzyme/cell) 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, such as glass beads, starch, cellulose, and polyacrylamide. Since1916, when the phenomenon was reported for the first time by J .M. Nelson and E.G. Griffin, this has been largely used in several manufacturing processes.

They had initially reported the immobilisation (adsorption) of an invertase on charcol/alumina without loss of activity. It was during 1950s and 1960s that the technique became very popular:

Immobilization Methods - These methods belong to the following five general categories.

1. Adsorption. The enzymes are adsorbed to several kinds of adsorbents with charged or neutral surfaces. Such materials are used for separation of proteins by adsorption chromatography. Calcium phosphate gels, carbon, carboxymethylcellulose (CMC), carboxymethyl sephadex, collagen, silica, gel, titania, alumina etc. are used.

2. **Covalent binding.** Enzyme is bound covalently to a support material using any of the various methods. The enzyme forms a covalent link with active groups of support material.

3. **Cross-binding.** Enzymes may be cross-linked to a multifunctional reagent without any solid support. Diazobenzidine, glutaraldehyde, toluene 2, 4-di-iso thiocyanate, hexamethylene di-isocyanate are some of the reagents used.

4. **Entrapment.** Enzyme is entrapped inside a cross-linked gel matrix. The gel is allowed to develop in an aqueous solution containing one or more enzymes.

5. **Microencapsulation**. This is modified entrapment where enzyme is immobilised within microcapsules prepared from organic polymers.

Industrial Applications of Immobilised Systems - Immobilised systems (enzymes and cells) possess important practical applications in industry.

A range of immobilised enzymes are produced by various molds. Immobilised systems have been used in antibiotic productions.

At least in case, of patulin and penicillin G production, immobilisation system can be operated on a continuous basis. Immobilised mycelia of Penicillium chrysogenum are used.

A range of fungal cell protoplast immobilised systems have also been used in steroid transformations. Quite recently immobilised fungal systems have also been applied to environmental problems. Microbial biotechnology has important applications in biodegradation, disposal of wastes and renewable sources of energy.

Commercially produced enzymes:

• enzymes used in ***industry***, such as amylases, proteases, catalases, isomerases

• enzymes used for ***analytical*** purposes, such as glucose oxidase, alcohol dehydrogenase, hexokinase, cholesterol oxidase

• enzymes used in ***medicine***, such as asparaginase, proteases, lipases

• different levels of quantity and quality

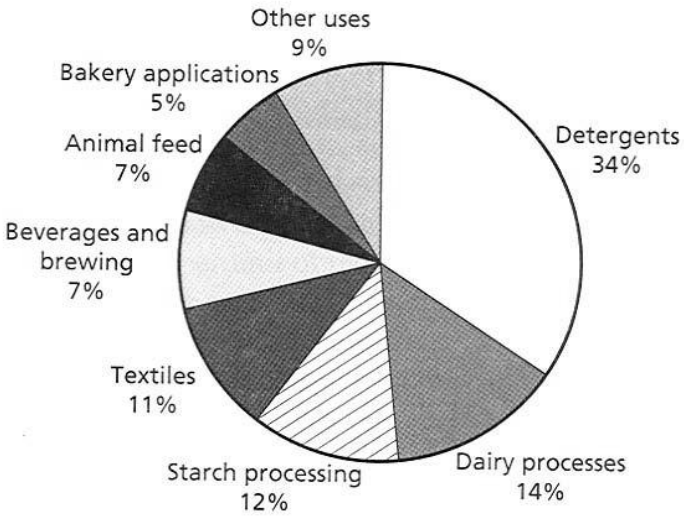


Fig.20 – Applications of bulk microbial enzymes

Amylases

• hydrolysis of starch (glucose polymer), one of the most readily available plant polysaccharides

Table 5 - Microbial enzymes and their commercial application

Enzyme	Genus of Producer	Use
Bacterial proteases	*Bacillus, Streptomyces*	Detergents
Asparaginase	*Escherichia, Serratia*	Antitumor agent
Glucoamylase	*Aspergillus*	Fructose syrup production
Bacterial amylases	*Bacillus*	Starch liquefaction, brewing, baking, feed, detergents
Glucose isomerase	*Bacillus, Streptomyces*	Sweeteners
Rennin	*Alcaligenes, Aspergillus, Candida*	Cheese manufacture
Pectinase	*Aspergillus*	Clarify fruit juice
Lipases	*Micrococcus*	Cheese production
Penicillin acylase	*Escherichia*	Semisynthetic penicillins
Taq polymerase	*Thermus aquaticus*	Polymerase chain reaction

Table 6 - Industrial enzymes produced by *Bacillus* species

Enzyme	Producer strains
α-Amylase	*B. amyloliquefaciens, B. circulans, B. licheniformis, B. stearothermophilus, B. subtilis*
β-Amylase	*B. polymyxa, B. cereus, B. megaterium*
Alkaline phosphatase	*B. licheniformis*
Cyclodextran glucanotransferase	*B. macerans, B. megaterium, Bacillus* sp.
β-Galactosidase	*B. stearothermophilus*
β-Glucanase	*B. subtilis, B. circulans*
β-Glucosidase	*Bacillus* sp.
Glucose isomerase	*B. coagulans*
Glucosyl transferase	*B. megaterium*
Glutaminase	*B. subtilis*
Galactomannase	*B. subtilis*
β-Lactamase	*B. licheniformis*
Lipase	*Bacillus* sp.
Metalloprotease	*B. lentus, B. polymyxa, B. subtilis, B. thermoproteolyticus*
Metalloprotease	*B. amyloliquefaciens*
Penicillin acylase	*Bacillus* sp.
Pullulanase	*Bacillus* sp., *B. acidopullulans*
Serine protease	*B. amyloliquefaciens, B. amylosaccharicus, B. Licheniformis, B. subtilis*
Urease	*Bacillus* sp.
Uricase	*Bacillus* sp.

• amylases are enzymes that hydrolyse starch production of sweeteners from starch: maltose or glucose syrups (further transformation to high fructose syrup with glucose isomerase)
• starch hydrolyates used as additives in the manufacture of candies, baked goods, canned goods, and frozen foods

Table 7 – Microbial enzymes with industrial-scale applications and some of their sourses

Microbial Enzymes with Industrial-Scale Applications and Some of Their Sources

Enzyme	*Source*	*Action*	*Applications*
α-Amylase	*Bacillus subtilis* *Bacillus licheniformis* *Aspergillus oryzae*	Endo-hydrolysis of α-1,4-glucosidic linkages	Starch processing
Glucoamylase	*Aspergillus oryzae* *Aspergillus niger* *Rhizopus oryzae*	Removes glucose from nonreducing end of starch, also splits α-1,6-linkages at branch points but more slowly	Starch processing; brewers' and distillers' mashes
Pullulanase	*Klebsiella aerogenes*	Splits α-1,6-glycosidic linkages in pullulan and amylopectin	Starch processing
Glucose isomerase	*Bacillus coagulans* *Streptomyces albus*	Converts D-glucose to D-fructose. This enzyme is actually a xylose isomerase that converts D-xylose to D-xylulose	Production of high-fructose syrups
β-Glucanase	*Bacillus subtilis* *Aspergillus niger* *Penicillium emersonii*	Degrades β-glucan by cleaving β-1,3 (4)-glucosidic linkages	Brewing
Invertase	*Saccharomyces cerevisiae*	Splits sucrose to glucose and fructose	Confectionery industry; baking
Lactase	*Saccharomyces lactis* *A. oryzae, A. niger,* *Rhizopus oryzae*	Splits lactose to glucose and galactose	Dairy industry (treatment of milk and whey)
Pectinase	*A. oryzae, A. niger,* *Rhizopus oryzae*	Degrades pectin, α-1,4-linked anhydrogalacturonic acid with some of the carboxyl groups esterified as the methyl esters	Clarification of fruit juices and wines
Neutral protease	*Bacillus subtilis* *Aspergillus oryzae*	Hydrolyzes peptide bonds in proteins	Flavoring of meat and cheese; baking
Alkaline protease	*Bacillus licheniformis*	Hydrolyzes peptide bonds in proteins	Laundry detergents
Rennin	*Mucor miehei* spp. Recombinant enzyme produced in *E. coli* and fungi	Hydrolyzes a specific bond in κ-casein, leading to coagulation of milk proteins	Cheesemaking
Lipase	*A. oryzae, A. niger,* *Rhizopus oryzae*	Hydrolyzes ester linkages in fats	Dairy industry; detergents

Glucose Isomerase

• D-glucose ketoisomerase: causes the isomerization of glucose to fructose
• since reaction is reversible the ration of glucose and fructose depends on the enzyme and reaction conditions
• high fructose corn syrup fructose 2x sweeter than sucrose

Chymosin

• site-specific proteolysis by chymosin detaches hydrophilic "tails" of κ-casein resulting in coagulation (curlding)
• calf chymosin (prochymosin) cloned and expressed in E. coli (first genetically engineered protein approved for human consumption, 1990)

Proteases

• used in laundry detergents

4.7. Single cell protein production

• microbial protein for use as human food or animal feed
• source of low-cost protein?

Advantages:

1. rapid growth rate and high productivity
2. high protein content (30-80% of dw)
3. ability to utilize a wide range of cheap carbon sources methane, methanol, molasses, whey, lignocellulose waste, etc.
4. relatively easy selection of cells
5. little land area required
6. production independent of season and climate

Table 8 - Microorganisms used for SCP production using various carbon sources

Carbon substrate	Microorganism
Carbon dioxide	*Spirulina* species *Chlorella* species
Liquid hydrocarbons (*n*-alkanes)	*Saccharomycopsis lipolytica* *Candida tropicalis*
Methane	*Methylomonas methanica* *Methylococcus capsulatus*
Methanol	*Methylophilus methylotrophus* *Hyphomicrobium* species *Candida boidinii* *Pichia angusta*
Ethanol	*Candida utilis*
Glucose (hydrolysed starch)	*Fusarium venenatum*
Inulin (a polyfructan)	*Candida* species *Kluyveromyces* species
Molasses	*Candida utilis* *Saccharomyces cerevisiae*
Spent sulphite waste liquor	*Paecilomyces variotii*
Whey	*Kluyveromyces marxianus* *Kluyveromyces lactis* *Penicillium cyclopium*
Lignocellulosic wastes (solid substrate)	*Chaetomium* species *Agaricus bisporus* *Cellulomonas* species

• protein content and quality largely dependent on the specific microbe utilized and on the fermentation process
• fast growing aerobic microorganisms

Some problems:
• high nucleic acid content
• high protein content (elevated RNA levels - ribosomes)
- digestion of nucleic acids results in elevated levels of uric acid
- treatment with RNAses
• sensitivity or allergic reactions

Single Cell Protein

Mushrooms
Pekilo prossess
• filamentous fungus **Paecilomyces variotii**
• use of waste from wood processing (monosaccharides + acetate)
• use as animal feed

Pruteen
• methanol (from methane - natural gas) as C1 carbon source
• methylotrophic bacteria (*Methylophilus methylotrophus*)
• feed protein
Quorn
• fungal mycelium, *Fusarium* (mycoprotein) for human consumption
• processing to resemble meat

Single Cell Protein - SCP - SCP is the name given to a variety of microbial products, that are produced by fermentation. When properly produced, this materials make satisfactory proteinaceous ingredients for animal feed or human food. The production of protein from hydrocarbon wastes of the petroleum industry is the most recent microbiological industry.

Yeast, fungi, bacteria, and algae are grown on hydrocarbon wastes, and cells are harvested as sources of protein. It has been calculated that 100 lbs of yeast will produce 250 tons of proteins in 24 hours, whereas a 1000 lbs steer will synthesize only 1 lb of protein 24 hours and this after consuming 12 to 20 lbs of plant proteins. Similar, algae grown in ponds can produce 20 tons (dry weight) of protein, per acre, per year.

This yield is 10 to 15 times higher than soybean and 25 to 50 times higher than corn. There are both advantages and disadvantages in using microorganisms for animal or human consumption. Bacteria are usually high in protein (50 to 80 percent) and have a rapid growth rate. The principal disadvantages are as follows:

1. Bacterial cells have small size and low density, which makes harvesting from the fermented medium difficult and costly.
2. Bacterial cells have high nucleic acid content relative to yeast and fungi This can be detrimental to human beings, tending to increase the uric acid level in blood. This may cause uric acid poising or gout. To decrease the nucleic acid level additional processing step has to be introduced, and this increases the cost.
3. The general public thinking is that all bacteria are harmful and produce disease. An extensive education programme is1required to remove this misconception and to make the public accept bacterial protein.

Yeasts have as advantages their larger size (easier to harvest), lower nucleic add content, high lysine content and ability to grow at acid pH. However the most important advantage is familiarity and acceptability because of the long history of its use in traditional fermentations. Disadvantages include lower growth rates, lower protein content (45 to 65 per cent), and lower methionine content than in bacteria.

Filamentous fungi have advantages .in ease of harvesting, but have their limitations in lower growth rates, lower protein content, and acceptability. Algae have disadvantages of having cellulosic cell walls which are not digested by human beings. Secondly, they also concentrate heavy metals.

Single cell protein basically comprises proteins, fats carbohydrates, ash ingredients, water, and other elements such as phosphorus and Potassium. The composition depends upon the organism and the substrate which it grows. some typical compositions which are compared with soymeal and fish meal. If SCP is to be used successfully, there are five main criteria to be satisfied;

1. The SCP must be safe to eat.
2. The nutritional value dependent on the amino acid composition must be high.
3. It must be acceptable to the general public.
4. It must have the functionality, i.e. characteristics, which are found in common staple foods.
5. The economic viability of the SCP process is extremely complex and is yet to be demonstrated.

4.8. Microbial polymers and production of solvents

- e.g. xanthan gum from *Xanthomonas campestris*

Table 9 - Microbial polymers and production of solvents

Polymer	Application
Xanthan	Emulsion stabilization and suspension agent in foods
	Foam stabilization in foods
	Crystallization inhibitor in foods
	Viscosity control in oil drilling mud and inkjet printing
Bacterial cellulose	Moisture retention in wound dressings
	High strength acoustic diaphragms in sound reproduction
Hyaluronic acid	Hydrating agent in cosmetics and pharmaceuticals
	Replacement for synovial fluid and vitreous humor in biomedicine
Emulsan	Emulsifier and vaccine adjuvant
Curdlan	Gelling agent in foods
Gellan	Gelling agent in foods
Pullulan	Food coatings
Various	Paper coating and water flocculant

- **Biopolymers**
  - exopolysaccharides can be used as stabilizers, etc.
  - microbial plastics:
    - poly-ß-hydroxybutyrate (PHB), which is commonly used by some bacteria as a lipid storage material, can be used as a raw material for plastic based packaging materials
    - the nature of the raw material (and thus the plastic that can be synthesized from it) can be selected by varying the carbon source used to grow the bacteria - using acetate (C2) and butyrate (C4) yields PHB; caproate (C6) yields poly-ß-hydroxycaproate PHC; valerate (C5) yields poly-ß-hydroxyvalerate PHV; mixtures yield co-polymers
- **Biosensors** - bioelectronics utilize the abilities of microbes to measure pollutants and contaminants.

Production of solvents: Acetone-butanol fermentation

Clostridium acetobutylicum

•utilizes EMP pathway for glucose catabolism with the formation of C3 and C4 products

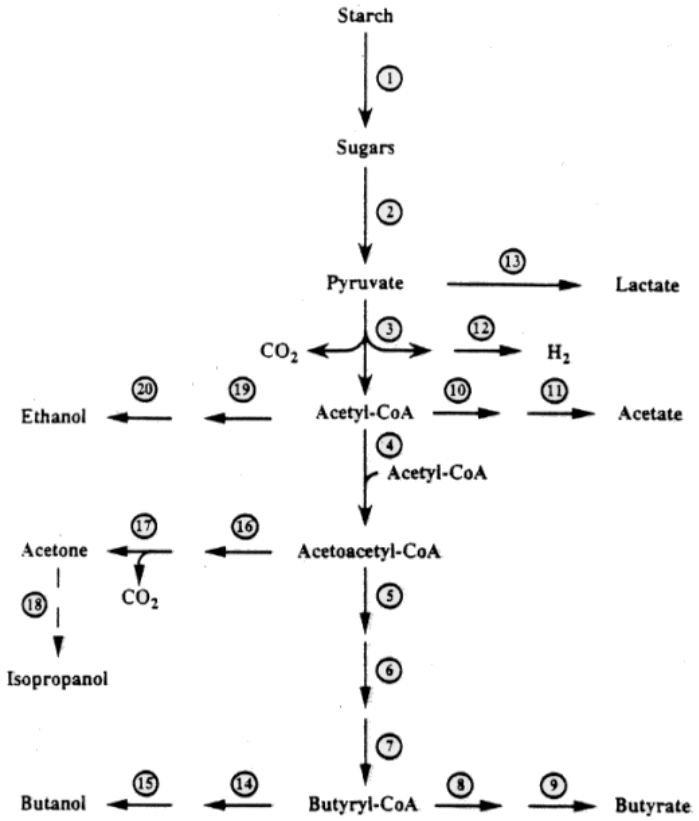


Fig.21 – Acetone-butanol Production

•biphasic fermentation: during growth acetate and butyrate are formed (acidogenic phase)

•as pH drops the culture enters stationary phase and there is a metabolic shift to solvent phase (solvetogenic phase)

•acetyl-CoA as central intermediate, which can be:

a) reduced to butyrate and butanol

b) cleaved via decarboxylation to acetone

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. These fermentations were out of favour till very recently because of the availability of acetone and butanol from the petroleum industry.

Today there is considerable amount of interest in these fermentations. However, the concentration of end products in these fermentations is quite small and the fermentations are a type of mixed fermentation yielding a mixture of compounds such as butyric acid, butanol, acetone etc. Attempts to increase yeilds by use of genetidally altered strains or change in fermentation conditions have been partially successful.

5. Biodegradation and Bioremediation

Bioremediation is also called Biodegradation Enhancement and includes any purposful use of microbes to degrade unwanted substances in the environment

- **natural products**
  - **petroleum** - certain bacteria (some cyanobacteria, pseudomonads, corynebacteria, mycobacteria), green algae and fungi (several molds and yeasts) oxidize hydrocarbons at aerobic water/oil interfaces (with optimal conditions, up to 80% removal within 1 year after a spill)
  - **biodegradable plastics**
    - **photobiodegradable** - structure of polymer altered by UV light in sunlight so that it is now amenable to biodegradation
    - **biochemically biodegradable starch-linked polymers** - starch-digesting bacteria in soil attack the starch, releasing polymer fragments which are degraded by other microbes
- **xenobiotics** - chemically synthesized compounds not found in nature (pesticides, synthetic polymers, etc.) and thus would seem unlikely to be degradable by naturally existing microorganisms; these products tend to be persistent in nature, and many nations are working to ban the use of many of them; **microbes that can degrade xenobiotics are rather diverse and typically include both bacteria and fungi**
  - **PCBs** - certain *Pseudomonas* species have been engineered to accelerate breakdown of polychlorinated biphenyls (formerly used by electric industry as transformer insulation)
  - **PAHs** (poly aromatic hydrocarbons) can be difficult to degrade, but there are microbes in the environment that can accomplish this task, especially when working together
  - **pesticides** - herbicides, insecticides and fungicides
    - these are typically rather complex molecules

        - some xenobiotics are good carbon sources and electron donors for soil microbes, so they are more readily degraded than others
        - other xenobiotics, such as chlorinated insecticides, are recalcitrant to degradation; thus they have rather long persistence times in the environment
            - lindane - 3 years for 75-100% disappearance
            - DDT - 4 years for 75-100% disappearance
            - chlordane - 5 years for 75-100% disappearance
        - to degrade these xenobiotics, microbes may employ co-metabolism, in which an organic material other than the xenobiotic is used as the primary energy source and the xenobiotic is degraded as a secondary process
- **Genetically engineered microbes in bioremediation**
    - **Microbes can be "engineered"** to carry out the biochemical processes needed for bioremediation
        - **concerns about long-term the effects** of genetically engineered microbes on the environment are manifold
        - **fate of genetically engineered microbes** is similar to that of other allochthonous organisms, but even more extreme because they are typically engineered to require nutrients, etc. not naturally present in the environments into which they may be introduced ... so they will die out when the material they were engineered to degrade has been removed from the area.

Waste-water and sewage treatment

Objectives of wastewater treatment:

• reduce the organic content of wastewater
• BOD
• trace (toxic) organics that are recalcitrant to biodegradation
• removal / reduction of nutrients to reduce pollution of receiving waters
• nitrogen, phosphorous
• removal or inactivation of pathogenic microorganisms and parasites

Treatment methods:

• various types of bioreactors to produce effluent that can be discharged into the natural environment without adverse effects
• combinations of physical & chemical treatment and aerobic / anaerobic biological biodegradation

Biodeterioration Management

- **Jet fuel** - growth of Cladosporium resinal at water/fuel interface is controlled with fungicides

- **Paper production** - microbes and slime produced by them controlled with biocides (environmental problems)
- **Electronics** - microbes damage computer chips by growing on trace contaminants at junctions (must use ultrapure water - no microbes, no organics - to prevent this)
- **Paints** - growth of fungi, etc. in paints (esp. latex-based) is controlled by use of quaternary ammonium salts, barium salts and chlorinated phenolics (mercury compounds no longer used due to toxicity for humans)
- **Textiles and leather goods** - fungal growth is controlled with phenolics in textiles and copper compounds in leathers
- **Metals and concrete** - microbial metabolites (e.g., sulfuric acid produced by Thiobacilli) are frequently responsible for corrosion of concrete sidewalks, highways, bridges and building as well as sewer pipes

Activated Sludge Process

1. Preliminary treatment
• removal of debris and coarse materials that may clog equipment

2. **Primary** treatment
• screens, settling tanks and skimmers to remove suspended solids
• physical separation

3. **Secondary** treatment
• aerobic microbiological process: trickling filters, activated sludge
• effluent from primary treatment aerated, aerobic bacteria growing in flocs degrade organic material
• an important characteristic of the process is the recycling of a large proportion of the biomass
• removal of BOD and nutrients:
1. oxidation of biodegradable organic matter
(soluble organic matter converted to new cell mass)
2. flocculation, separation of newly formed biomass from effluent

4. **Tertiary or advanced** treatment
• removal of ammonia and phosphate
• oxidation of ammonia to nitrate followed by denitrification
• accumulation of polyphosphate granules
• chemical treatments

5. **Disinfection** (when needed)
• chlorination
Microbiological mining - Sulfur and Iron-Oxidizing Bacteria
• *Thiobacillus*, *Acidithiobacillus*, *Beggiatoa*, and others

Thiobacillus thiooxidans (Jaffe and Waksman 1922)
• scattered in the Proteobacteria: α,β, γ subdivisions
• acidophiles
• chemolithotrophs: energy from oxidation of reduced sulfur compounds or iron
• used in bioleaching of ores
• problems with acid mine drainage

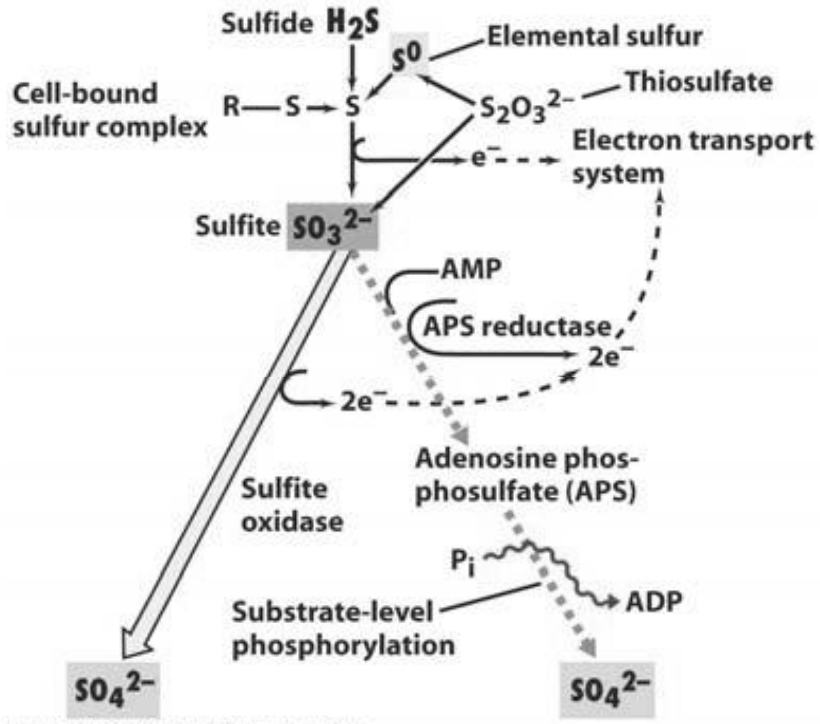


Fig.22– Microbiological mining by Sulfur Bacteria

Bioleaching - Enhanced Recovery of Metals. Since high grade ore deposits are easily accessible, these become rapidly depleted. It thus 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 who could do it efficiently. This potential of microbes could only be realised 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 two important natural resources - metals and petroleum.

It was in 1957 that a relationship between the presence of *Thiobacillus ferrooxidans* and the dissolution of metals in copper leaching operation was recognised by American microbiologists. *T. ferrooxidans* and *T. thiooxidans* are thermoacidophilic archaebacteria.

They are autotrophs and grow in acidic and hot environments. It has been demonstrated that these *Thiobacillus* spp. can be used for extraction of copper and uranium from insoluble minerals. This implication of microbial activity in weathering, leaching and deposition of mineral ores could develop into a recent field of biotechnology - biohydrometallurgy. Biomineralisation is the deposition of metals as insoluble oxides and sulphides due to microbial activity.

Microbial mining is the process of bioleaching recovers metals from ores that are not suitable for direct smelting due to their low metal content. Bioleaching uses microbes to alter the physical or chemical properties of a metallic ore so that the metal can be extracted. Metals can be extracted economically from low grade sulphide or sulphide containing ore by exploiting metabolic activities of *Thiobacilli*, particularly *T. ferrooxidans.*

Under optimal conditions in the laboratory, as much as 97% of the copper in low-grade ores has been recovered by bioleaching, but such high yields are not achieved in actual mining operations. The process is at present commercially used for recovery of copper and uranium from low-grade ores.

Laboratory experiments could show that recovery of other metals such as Ni, Zn, Co, Sn, Cd, Mb, Pb, Sb, As and Se from their low- grade sulphide-containing ores is also possible through bioleaching. The leaching process can also be used to separate the insoluble lead sulphate ($PbSO_4$) from other metals that occur in the same ore.

The general process carried out by *T. ferrooxidans* (TF) and related species can be shown by the following equation. MS + SO_2 - MSO_4 where M is divalent metal. Because metal sulphide is insoluble and metal sulphate usually water soluble, this transformation produces a readily leachable form of the metal. T.f., a chemolithotroph derives energy through oxidation of either a reduced, sulphur compound or ferrous iron.

It exerts its bioleaching action by oxidising the metal sulphide being recovered either directly converting S_2- Jo SO_4 and or indirectly by oxidising the ferrous iron content of the ore to ferric ion. The ferric ion, in turn, chemically oxidises the metal to be recovered to a soluble form that can be leached from the ore. It is possible to leach the ore in situ without first mining it, if the ore formation is porous and overlays a water-impermeable stratum.

A pattern of boreholes is established with some of the holes used for injecting the leaching liquour and others for the recovery of leachate. More frequently, however, this bioleaching process is used after the ore is mined, broken up and piled in heaps on a water-impermeable formation or on a specially constructed apron. Water is then pumped to the top of ore heap and trickles down through the ore to the apron.

A continuous reactor leaching operation for recovery of copper from its low-grade sulphideore. The leaching water and ore usually supply enough dissolved mineral nutrients required by TF, but in some cases NH_3 and PO_4 may be added. The leached metal is extracted with an organic solvent and then removed from solvent by stripping. Both the leaching liquor and the solvent are recycled.

Dictionary of terms

Absorption - Movement of ions and water into as organism as a result of metabolic processes, frequently against an electrochemical potential gradient (active) or as a result of diffusion along an activity gradient (passive).
***Acetobacter aceti* -** A bacterium which can "spoil" alochol-containing beverages by turning the ethanol into vinegar (acetic acid). Discovered by Louis Pasteur, during the 1800s.
Acetogenic bacterium- Prokaryotic organism that uses carbonate as a terminal electron acceptor and produces acetic acid as a waste product.
Acidophile- Organism that grows best under acid conditions (down to a pH of 1).
Actinomycete - Nontaxonomic term applied to a group of high G + C base composition, Gram-positive bacteria that have a superficial resemblance to fungi. Includes many but not all organisms belonging to the order Actinomycetales.
Adsorption - Process by which atoms, molecules, or ions are taken up and retained on the surfaces of solids by chemical or physical binding.
Aerobic - Needing oxygen for growth, growing in the absence of oxygen.
Having molecular oxygen as a part of the environment. (ii) Growing only in the presence of molecular oxygen, as in aerobic organisms. (iii) Occurring only in the presence of molecular oxygen, as in certain chemical or biochemical processes such as aerobic respiration.
Aerotolerant anaerobes- Microbes that grow under both aerobic and anaerobic conditions, but do not shift from one mode of metabolism to another as conditions change. They obtain energy exclusively by fermentation.
Agar - Complex polysaccharide derived from certain marine algae that is a gelling agent for solid or semisolid microbiological media. Agar consists of about 70% agarose and 30% agaropectin. Agar can be melted at temperature above 100ºC; gelling temperature is 40-50ºC.
Agarose - Nonsulfated linear polymer consisting of alternating residues of D-galactose and 3,6-anhydro-L-galactose. Agarose is extracted from seaweed, and agarose gels are often used as the resolving medium in electrophoresis.
Alkalophile- Organism that grows best under alkaline conditions (up to a pH of 10.5).
Anabolism- Metabolic processes involved in the synthesis of cell constituents from simpler molecules. An anabolic process usually requires energy.
Anaerobic- (i) Absence of molecular oxygen. (ii) Growing in the absence of molecular oxygen, such as anaerobic bacteria. (iii) Occurring in the absence of molecular oxygen, as a biochemical process.
Anaerobic respiration- Metabolic process whereby electrons are transferred from an organic, or in some cases, inorganic compounds to an inorganic acceptor molecule other than oxygen. The most common acceptors are nitrate, sulfate, and carbonate.
Antagonist- Biological agent that reduces the number or disease-producing activities of a pathogen.
Antibiotic 1)Chemical substance formed as a metabolic byproduct in bacteria or fungi and used to treat bacterial infections. Antibiotics can be produced naturally, using

microorganisms, or synthetically. 2)Organic substance produced by one species of organism that in low concentrations will kill or inhibit growth of certain other organisms.

Coined by Selman Waksman during the 1940s, this term refers to organic compounds that are naturally formed and secreted by various species of microorganisms and/or plants. It has a defensive function and is often toxic to other species (e.g., penicillin, originally produced by bread mold, is toxic to numerous human pathogens). Antibiotics generally act by inhibiting protein synthesis, DNA replication, synthesis of cell wall (cytoskeleton) constituents, inhibition of required cell (e.g., bacteria) metabolic processes, and nucleic acid (DNA and RNA) biosynthesis; hence killing the (targeted bacteria) cells involved. Inorganic (e.g., certain metals) molecules may also have antibiotic properties.

Antibiotic Resistance

A property of a cell (e.g.. pathogenic bacteria) that enables it to avoid the effect of an antibiotic that had formerly killed or inhibited that cell. Ways this can occur include:

- changing the structure of the cell wall (plasma membrane).
- synthesis (manufacture) of enzymes to inactivate the antibiotic (e.g., penicillinases, which inactivate penicillin).
- synthesis of enzymes to prevent antibiotic entering cell.
- active removal of the antibiotic from the cell. For example, the **membrane transporter protein** molecules known as **ABC transporters** are sometimes able to help pathogenic bacteria resist certain antibiotics by transporting-out the antibiotic before it can kill the bacteria. The ABC transporter is a V-shaped molecule embedded in the (bacteria) cell's plasma membrane, with the 'open end' of the "V" pointed toward the interior of the cell. When molecules of certain antibiotics (inside the cell) contact the ABC transporter molecule, the two "arms" of the ABC transporter **close around the antibiotic molecule, the ABC transporter flips-over, and thereby sends the antibiotic molecule out through the exterior of the cell's plasma membrane**.

replacing some critical cell metabolic processes, with (new) metabolic processes that bypass the antibiotic's (former) effect.

Antiseptic- Agent that kills or inhibits microbial growth but is not harmful to human tissue.

Archaea- 1)Evolutionarily distinct group (domain) of prokaryotes consisting of the methanogens, most extreme halophiles and hyperthermophiles, and *Thermoplasma*. 2) Single-celled life forms that can live at extreme ocean depths (i.e., high pressure) and in the absence of oxygen. *Archaea* were delineated/named by Carl Woese. Enzymes robust (i.e., sturdy) enough for industrial process utilization have been isolated by scientists from some strains of *Archaea*

Archaebacteria- Older term for the Archaea.

Aseptic technique- Manipulating sterile instruments or culture media in such a way as to maintain sterility.

Autotroph- Organism which uses carbon dioxide as the sole carbon source.

Bacillus - Bacterium with an elongated, rod shape.

Bacillus subtilis - A bacterium commonly used as a host in recombinant DNA experiments. Important because of its ability to secrete proteins.

Bacillus subtilis GBO3 is a bacterium that is used as a fungicide on flower and ornamental seeds, and on agricultural seeds including seeds for cotton, vegetables, peanuts, and soybeans. The bacterium colonizes the developing root system of the plant and thus competes with certain fungal disease organisms.

Bacillus subtilis MBI 600 is a bacterium which is used in fungicide products as a treatment for seeds of cotton, beans, barley, wheat, corn, peas, peanuts and soybeans. Products containing this active ingredient are used for the suppression of root diseases and seedling diseases caused by certain fungi.

Bacillus subtilis strain QST 713 is a naturally occurring widespread bacterium that can be used to control plant diseases and fungal pathogens including: blight, scab, gray mold, and several types of mildew.

Bacillus subtilis var. *amyloliquefaciens* strain FZB24 is a naturally occurring bacterium that can be used to control various fungal diseases in non-food crops, and to enhance plant growth.

Bacillus thuringiensis (Bt) - Naturally occurring soil bacterium that generates a protein toxic to a variety of lepidoptera, such as corn borers, but is harmless to people and animals.

Bacillus thuringiensis subspecies *israelensis* strain EG2215 is a manufacturing use product designed for production of end-use products for mosquito control. The end-use sites are for outdoor use only and include irrigation ditches, roadside ditches, flood water, standing ponds, woodland pools, snow melt pools, pastures, catch basins, storm water retention areas, tidal water, salt marshes, and rice fields.

B.t. kurstaki - One of the approximately 30 subspecies groupings within the approximately 20,000 different strains of the soil bacteria known (collectively) as *Bacillus thuringiensis (B.t.)*. When eaten (e.g., as part of a genetically engineered plant), the protoxin proteins produced by *B.t. kurstaki* are toxic to certain caterpillars (Lepidoptera larvae), such as the European corn borer (pyralis).

Bacteria - All prokaryotes that are not members of the domain Archaea.

Bacteriochlorophyll- Light-absorbing pigment found in green sulfur and purple sulfur bacteria.

Bacteriocin- Agent produced by certain bacteria that inhibits or kills closely related isolates and species.

Bacteriophage 1)Virus that lives in and kills bacteria. Also called phage. 2) Virus that infects bacteria, often with destruction or lysis of the host cell.

Bacterium Any of a large group of microscopic organisms with a very simple cell structure. Some manufacture their own food, some live as parasites on other organisms, and some live on decaying matter.

Beauveria bassiana ATCC 74040 is a fungus that is used as a pesticide for controlling many kinds of insects. This active ingredient is present in several products that can be used in greenhouses and outdoors on all food crops and many non-food crops. Residues of the fungus are not expected to remain on treated food or feed.

Beauveria bassiana strain GHA is a fungus that is used as a pesticide for controlling many kinds of insects. The active ingredient can be used on all food crops and many non-food crops at various outdoor and indoor sites.

Bifidus - A "family" of bacteria species that live within the digestive systems of certain animals (e.g., humans, swine, etc.). Examples include *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobactrium adolescentis*, and *Bifidobacterium acidophilus*. In general, *Bifidus bacteria help to promote good health of the host animals, by several means:*

- *they produce organic acids (e.g., propionic, acetic, lactic), which make the host animal's digestive system more acidic. Because most pathogens (i.e., disease-causing microorganisms) grow best at a neutral pH (i.e., neither acidic nor base/caustic), the growth rates of pathogens are thereby inhibited.*
- **Biocatalysis**—Biocatalysis is the use of isolated enzymes and/or micro-organisms as biocatalysts to conduct chemical reactions.
- **Biocatalyst** In bioprocessing, an enzyme that activates or speeds up a biochemical reaction. According to the American Heritage Dictionary, a biocatalyst is "A substance, especially an enzyme, that initiates or modifies the rate of a chemical reaction[, often] in a living body." Micro-organisms, including bacteria and fungi (e.g., yeasts), can also be used as biocatalysts.
- **Biocide -** Any chemical or chemical compound that is toxic to living things (systems). Literally "biokiller" or killer of biological systems. Includes insecticides, bactericides, fungicides, etc. Most bactericides accomplish their task (i.e., killing bacteria) via massive lysis (disintegration) of bacteria cell walls (membranes). However, one (i.e., triclosan) kills bacteria by inhibiting enoyl-acyl protein reductase; a crucial enzyme utilized by bacteria in their synthesis of fatty acids.
- **Biofilm**- Microbial cells encased in an adhesive, usually a polysaccharide material, and attached to a surface.

Bioremediation – 1)The use of microorganisms to remedy environmental problems, rendering hazardous wastes nonhazardous. 2)Use of microorganisms to remove or detoxify toxic or unwanted chemicals from an environment. **Biotransformation** - The use of enzymes in chemical synthesis to produce chemical compounds of a desired stereochemistry.

Blastocyst (Blastula) -The 4- to 5-day-old ball of undifferentiated cells from which a prospective embryo develops. In mammals it consists of two distinct parts: the inner cell mass and the trophoblast.

Broth - A fluid culture medium (for growing microorganisms).

Bt crops: Crops that are genetically engineered to carry a gene from the soil bacterium *Bacillus thuringiensis* (Bt). The bacterium produces proteins that are toxic to some pests but non-toxic to humans and other mammals. Crops containing the Bt gene are able to produce this toxin, thereby providing protection for the plant. Bt corn and Bt cotton are examples of commercially available Bt crops.

Budding- Asexual reproduction (usually for yeast) beginning as a protuberance from the parent cell that grows and detaches to form a smaller, daughter cell.

Catabolism- Biochemical processes involved in the breakdown of organic compounds, usually leading to the production of energy.
Catabolite repression- Transcription-level inhibition of a variety of inducible enzymes by glucose or other readily used carbon source.
Catalyst – 1)An agent (such as an enzyme or a metallic complex) that facilitates a reaction but is not itself changed during the reaction. 2)Substance that promotes a chemical reaction by lowering the activation energy without itself being changed in the end. Enzymes are a type of catalyst.
Cell culture - Growth of cells under laboratory conditions.
Chemoautotroph- Organism that obtains energy from the oxidation of reduced inorganic compounds or elements and obtains carbon from carbon dioxide.
Chemolithotroph- Organism that obtains energy from the oxidation of inorganic compounds and uses inorganic compounds as electron donors.
Chemoheterotroph- Organism that obtains energy and carbon from the oxidation of organic compounds.
Chemoorganotroph- Organism that obtains energy and electrons (reducing power) from the oxidation of organic compounds.
Chemostat - Continuous culture device usually controlled by the concentration of limiting nutrient and dilution rate.
Cometabolism- Transformation of a substrate by a microorganism without deriving energy, carbon, or nutrients from the substrate. The organism can transform the substrate into intermediate degradation products but fails to multiply at its expense.
Commensalism- Interaction between organisms where one organism benefits from the association while the second organism remains unaffected.
Culture -1)As a noun, cultivation of living organisms in prepared medium; as a verb, to grow in prepared medium. 2)Population of microorganisms cultivated in an artificial growth medium. A pure culture is grown from a single cell; a mixed culture consists of two or more microbial species or strains growing together.
Culture medium - Any nutrient system for the artificial cultivation of bacteria or other cells; usually a complex mixture of organic and inorganic materials.
Dextran - A polysaccharide produced by yeasts and bacteria as an energy storage reservoir (analogous to fat in humans). Consists of glucose residues, joined almost exclusively by alpha-1,6 linkages. Occasional branches (in the molecule) are formed by alpha 1,2, alpha 1,3, or alpha 1,4 linkages. Which linkage is used depends on the species of yeast or bacteria producing the dextran.
Diazotroph- Organism that can use dinitrogen as its sole nitrogen source, i.e. capable of N_2 fixation.
Differential medium- Cultural medium with an indicator, such as a dye, which allows various chemical reactions or microbial genera to be distinguished during growth.
Dilution plate count method- Method for estimating the viable numbers of microorganisms in a sample. The sample is diluted serially and then transferred to agar plates to permit growth and quantification of colony-forming units.
Direct count- Method of estimating the total number of microorganisms in a given mass of soil by direct microscopic examination.

Domain- Highest level of biological classification, superseding kingdoms. The three domains of biological organisms are the Bacteria, the Archaea, and the Eukarya.
Doubling time- Time needed for a population to double in number or biomass.
Enrichment culture- Technique in which environmental (including nutritional) conditions are controlled to favor the development of a specific organism or group of organisms.
Enzyme – 1)A protein catalyst that facilitates specific chemical or metabolic reactions necessary for cell growth and reproduction. 2)Protein within or derived from a living organism that functions as a catalyst to promote specific reactions.3) Biologically-derived, biodegradable proteins that speed up chemical reactions. For example, in a biorefinery producing cellulosic ethanol and other chemicals, a group of enzymes called cellulases is needed to breakdown cellulose into sugars that can be fermented to produce the desired products.
Eubacteria-Old term for the Bacteria.
Eukarya- Phylogenetic domain containing all eukaryotic organisms.
Eukaryote – 1)A cell or organism containing a true nucleus, with a well-defined membrane surrounding the nucleus. All organisms except bacteria, viruses and cyanobacteria are eukaryotic. Compare Prokaryote. 2)Organism having a unit membrane-bound nucleus and usually other organelles.
Exponential growth- 1)Period of sustained growth of a microorganism in which the cell number constantly doubles within a fixed time period. 2)Period during the growth cycle of a population in which growth increases at an exponential rate. As referred to as logarithmic phase.
Extracellular- Outside the cell.
Extremophiles Microorganisms that live at extreme levels of pH, temperature, pressure and salinity.
Facultative organism- Organism that can carry out both options of a mutually exclusive process (e.g., aerobic and anaerobic metabolism).
Feedstock - The raw material used for chemical or biological processes.
Fermentation -1)The process of growing microorganisms for the production of various chemical or pharmaceutical compounds. Microbes are normally incubated under specific conditions in the presence of nutrients in large tanks called fermentors.
2)Metabolic process in which organic compounds serve as both electron donors and electron acceptors.
3) A term first used with regard to the foaming that occurs during the manufacture of wine and beer. The process dates back to at least 6,000 B.C. when the Egyptians made wine and beer by fermentation. From the Latin word *fermentare*, "to cause to rise." The term "fermentation" is now used to refer to so many different processes that fermentation is no longer accepted for use in most scientific publications. Three typical definitions are given below:

1. A process in which chemical changes are brought about in an organic substrate through the actions of enzymes elaborated (produced) by microrganisms.
2. The enzyme-catalyzed, energy-yielded pathway in cells by which "fuel" molecules such as glucose are broken down anaerobically (in the absence of

oxygen). One product of the pathway is always the energy-rich compound adenosine triphosphate (ATP). The other products are of many types: alcohol, glycerol, and carbon dioxide from yeast fermentation of various sugars; butyl alcohol, acetone, lactic acid, and acetic acid from various bacteria; citric acid, gluconic acid, antibiotics, vitamin B_{12} and B_2 from mold fermentation. The Japanese utilize a bacterial fermentation process to make the amino acid, L-glutamic acid, a derivative of which is widely used as a flavoring agent.

3. An enzymatic transformation of organic substrates (feedstocks), especially carbohydrates, generally accompanied by the evolution of gas. A physiological counterpart of oxidation, permitting certain organisms to live and grow in the absence of air; used in various Industrial processes for the manufacture of products such as alcohols, acids, and cheese by the action of yeasts, molds, and bacteria. Alcoholic fermentation is the best known example. Also known as zymosis. The leavening of bread depends on the alcoholic fermentation of sugars. The dough rises due to production of carbon dioxide gas that remains trapped within the viscous dough.

Wine fermentation is the critical conversion of a grape's sugar content into alcohol by active yeast. The higher the sugar content in the grape the higher the alcohol content in the wine, if there is not vintner intervention. The common form of sugars that reside in a grape's juice are the fairly familiar glucose and fructose.

Technically speaking in alcohol fermentation, sugar + yeast = alcohol, CO_2 and heat

Genome - 1)The total hereditary material of a cell, comprising the entire chromosomal set found in each nucleus of a given species. 2) Complete set of genes present in an organism.

Genomics -1)The study of genes and their function. Recent advances in genomics are bringing about a revolution in our understanding of the molecular mechanisms of disease, including the complex interplay of genetic and environmental factors. Genomics is also stimulating the discovery of breakthrough health-care products by revealing thousands of new biological targets for the development of drugs and by giving scientists innovative ways to design new drugs, vaccines and DNA diagnostics. Genomic-based therapeutics may include "traditional" small chemical drugs, as well as protein drugs and gene therapy. 2) The study of the molecular organization of genomes, their information content, and the gene products they encode.

Genotype – 1) Genetic makeup of an individual or group. Compare Phenotype. 2) Precise genetic constitution of an organism.

Genus (plural, genera)- The first name of the scientific name (binomial); the taxon between family and species.

Good Laboratory Practice for Nonclinical Studies (GLPNC) - The Good Laboratory Practice (GLP) that is required by the U.S. Food and Drug Administration (FDA) for studies of the safety and toxicological effects of new drugs for livestock.

Good Manufacturing Practices (GMP) - The set of general methodologies, practices, and procedures mandated by the U.S. Food and Drug Administration (FDA) which is to be followed in the testing and manufacture of pharmaceuticals. The purpose of GMPs is essentially to provide for record keeping and in a wider context to protect the public.

GMP guidelines exist instead of specific regulations due to the newness of the technology, and may later be superceded (modified) due to further advances in technology and understanding.

Growth- In microbiology, an increase in both cell number and cellular constituents.

Growth Curve - The change in the number of cells in a growing culture as a function of time.

Growth factors -1)Naturally occurring proteins that stimulate the growth and reproduction of specific cell types. Growth factors are essential to regenerative medicine and tissue engineering. 2) Organic compound necessary for growth because it is an essential cell component or precursor of such components and cannot be synthesized by the organism itself. Usually required in trace amounts.

Growth rate- The rate at which growth occurs, usually expressed as the generation time.

Growth rate constant- Slope of the log of the number of cells per unit volume plotted against time.

Growth yield coefficient-Quantity of biomass carbon formed per unit of substrate carbon consumed.

Heterotroph- Organism capable of deriving carbon and energy for growth and cell synthesis from organic compounds; generally also obtain energy and reducing power equivalents from organic compounds.

Industrial biotechnology (or white biotechnology)—Distinct from medical (red biotechnology) and agricultural biotechnology (green biotechnology), industrial biotechnology "is the application of modern biotechnology for the industrial production of chemical substances and bioenergy, using living cells and their enzymes, resulting in inherently clean processes with minimum waste generation and energy use." (Royal Belgian Academy Council of Applied Science, "Industrial Biotechnology and Sustainable
Chemistry," January 2004, 10.

The Commission's definition of industrial biotechnology is: the manufacture of liquid fuels and chemical products using enzymes, micro-organisms, fermentation, or biocatalysis at any stage of production, regardless of the type of raw materials used (e.g., biomass, fossil fuelbased, or inorganic substances), or the manufacture of liquid fuels and chemical products from renewable resources regardless of the type of processing technology used.

Industrial Biotechnology Association (IBA)

An American trade association of companies involved in biotechnology. Formed in 1981, the IBA tended to consist of the larger firms involved in biotechnology. In 1993, the Industrial Biotechnology Association (IBA) was merged with the Association of Biotechnology Companies (ABC) to form the Biotechnology Industry Organization (BIO).

Inoculum- Material used to introduce a microorganism into a suitable situation for growth.

K-strategy- Ecological strategy where organisms rely on physiological adaptations to environmental resources for continued survival with the community. K strategists are usually stable and permanent members of the community.
Koch's Postulates-Set of laws formulated by Robert Koch to prove that an organism is the causal agent of disease.
Lag phase-Period after inoculation of fresh growth medium during which population numbers do not increase.
Medium (plural, media)- 1)Any liquid or solid material prepared for the growth, maintenance, or storage of microorganisms.2)A substance containing nutrients needed for cell growth.
Mesophile- Organism whose optimum temperature for growth falls in an intermediate range of approximately 15 to 40ºC.
Metabolism- 1)All biochemical reactions in a cell, both anabolic and catabolic. 2)A firm structure of layered microorganisms with complementary physiological activities.
Microaerophile- Organism that require or prefer a low concentration of oxygen for growth. Sometimes indicates an organism that will carry out its metabolic activities under aerobic conditions but will grow much better under anaerobic conditions.
Microbial population- Total number of living microorganisms in a given volume or mass of soil.
Metabolism All biochemical activities carried out by an organism to maintain life.
Microbial herbicides and pesticides Microorganisms that are toxic to specific plants or insects. Because of their narrow host range and limited toxicity, these microorganisms may be preferable to their chemical counterparts for certain pest-control applications.
Micrometer- One-millionth of a meter, or 10^{-6} meter, the unit usually used for measuring microorganisms.
Mutagen -1)A substance that induces mutations. 2)Substance that causes the mutation of genes.
Mutant -1)A cell that manifests new characteristics due to a change in its DNA. 2)Organism, population, gene, or chromosome that differs from the corresponding wild type by one or more base pairs.
Mutation 1)A change in the genetic material of a cell.2) Heritable change in the base sequence of the DNA of an organism.
Mutualism- Interaction between organisms where both organisms benefit from the association.
Mycelium (plural, mycelia)- Mass of hyphae that form the vegetative body of many fungal organisms.
Neutralism- Lack of interaction between two organisms in the same habitat.
Obligate- (i) Adjective referring to an environmental factor (for example, oxygen) that is always required for growth. (ii) Organism that can grow and reproduce only by obtaining carbon and other nutrients from a living host, such as obligate symbiont.
Organotroph- Organism that obtains reducing equivalents (stored electrons) and carbon from organic substrates.

Pasteurization- Process using mild heat to reduce microbial numbers in heat-sensitive materials.
Photoautotroph- Organism able to use light as its sole source of energy and carbon dioxide as sole carbon source.
Photoheterotroph- Organism able to use light as a source of energy and organic materials as carbon source.
Plant growth-promoting rhizobacteria (PGPR)-Broad group of soil bacteria that exert beneficial effects on plant growth usually as root colonizers. Many are members of the genus *Pseudomonas.*
Plate count- Number of colonies formed on a solid culture medium when uniformly inoculated with a known amount of soil, generally as a dilute soil suspension. The technique estimates the number of certain organisms present in the soil sample.
Pour plate- Method for performing a plate count of microorganisms. A known amount of a serial dilution is placed in a sterile Petri dish and then a melted agar medium is added and the inoculum mixed well by gently swirling. After growth the number of colony forming units is counted.
Prebiotics - Chemical compounds or microorganisms (e.g., yeasts)-- administered alone or in combination (e.g., in the feed rations of animals)-- that (generally) act to stimulate growth of beneficial types of bacteria within the digestive system of animals (e.g., livestock). Those compounds can include some organic acids (e.g., propionic acid, malic acid, etc.). For example, adding certin strains of yeast (culture) and malate (malic acid) to cattle feed rations has been shown to stimulate *Selenomonas ruminantium* bacteria (growth) in the rumen (i.e., the "first stomach" in cattle). *Selenomonas ruminantium* tend to constitute 22-51% of the total bacteria in a typical rumen, and are important for optimal digestion (e.g., of the grass eaten by that animal).
Pure culture -1)In vitro growth of only one type of microorganism. 2)Population of microorganisms composed of a single strain. Such cultures are obtained through selective laboratory procedures and are rarely found in a natural environment.
r-strategy- Ecological strategy where organisms rely on high reproductive rates for continued survival within the community. Populations of r-strategists are subject to extreme fluctuations.
Rhizoplane- Plant root surfaces and usually strongly adhering soil particles.
Rhizosphere- Zone of soil under the influence of plant roots in which the kinds, numbers, or activities of microorganisms differ from that of the bulk soil.
Rhizosphere competence- Ability of an organism to colonize the rhizosphere.
Scale-up Transition from small-scale production to production of large industrial quantities.
Secondary metabolite- Product of intermediary metabolism released from a cell, such as an antibiotic.
Selective medium -1)Nutrient material constituted such that it will support the growth of specific organisms while inhibiting the growth of others. 2)Medium that allows the growth of certain types of microorganisms in preference to others. For example, an antibiotic-containing medium allows the growth of only those microorganisms resistant to the antibiotic.

Serial dilution- Series of stepwise dilutions (usually in sterile water) performed to reduce the populations of microorganisms in a sample to manageable numbers.
Species- In microbiology, a collection of closely related strains sufficiently different from all other strains to be recognized as a distinct unit.
Spread plate- Method for performing a plate count of microorganisms. A known amount of a serial dilution is spread over the surface of an agar plate. After growth the number of colony-forming units is counted.
Stationary phase- Period during the growth cycle of a population in which growth rate equals the death rate.
Sterilization- Rendering an object or substance free of viable microbes.
Strain - A group or organisms of the same species that possesses distinctive genetic characteristics that set it apart from others within the same species, but which differences are not "severe" enough for it to be considered a different breed or variety (of that species). The basic taxonomic unit of microbiology. Can also be used to designate a population of cells derived from a single cell.
Symbiosis- Living together in intimate association of two dissimilar organisms. The interactions between the organisms can be commensal or mutualistic.
Synergism- Association between organisms that is mutually beneficial. Both populations, however, are capable of surviving in their natural environment on their own.
Thermophile- Organism whose optimum temperature for growth is between 45 and 85ºC.
Viable- Alive; able to reproduce.
Viable but nonculturable- Organisms that are alive but cannot be cultured on laboratory media.
viable count- Measurement of the number of live cells in a microbial population.
Wild type-Strain of microorganism isolated from nature. The usual or native form of a gene or organism.
Xenobiotics -1)Synthetic chemicals believed to be resistant to environmental degradation. A branch of biotechnology called bioremediation is seeking to develop biological methods to degrade such compounds. 2)Compound foreign to biological systems. Often refers to human-made compounds that are resistant or recalcitrant to biodegradation and decomposition.
Yeast A general term for single-celled fungi that reproduce by budding. Some yeasts can ferment carbohydrates (starches and sugars) and thus are important in brewing and baking. Fungus whose thallus consists of single cells that multiply by budding or fission.

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