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المرحلة الرابعة – الدراستين الصباحية والمسائية

الفصل الدراسي الثاني

تدريسي المادة:

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Lecture 1: Introduction to Biotechnology

- Biotechnology is not a single technology; it is a group of technologies.
- Biotechnology is based on biology, which is the study of life. The basic unit of life is the cell.
- Biologists study the structure and functions of cells—what cells do and how they do it. Biotechnologists use this information to develop products.

Definition of Biotechnology

Some definitions of Biotechnology:

- Using organisms or their products for commercial purposes.
- A collection of technologies that use living cells, systems, organisms and/or biological molecules to solve problems and develop or make useful products.
- Using biological processes and technology to solve problems or making useful products.
- **Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.**

History of Biotechnology:

- The origins of biotechnology date back nearly 10,000 years ago when people were collecting plant seeds for planting the next year. There is evidence that Babylonians, Egyptians and Romans used these same selective breeding practices for improving livestock.
- By 6000 B.C., beer, wine and bread were produced by fermentation.
- By 4000 B.C., the Chinese used lactic acid bacteria to make yogurt, molds for making cheese and acetic acid bacteria to make vinegar.
- Louis Pasteur is considered the father of biotechnology by discovering that fermentation is performed by microorganisms.
- Karl Ereky (1919) was the first to give the term biotechnology for describing processes using living organisms to make a product or run a process such as industrial fermentations.

Historical development of biotechnology (Fig. 1.1):

1) Ancient Biotechnology (before 1885)

- Discovering of microorganisms
- Traditional microbial industries (bread, cheese, beer and wine)

2) Classical Biotechnology (1885-1975)

- The fermentation theory of Pasteur
- Production of single cell protein (SCP), antibiotics, enzymes, vitamins, gibberellins, amino acids, nucleotides, steroids, chemicals like acetone, butanol, ethanol and organic acids
- Tissue cultures techniques

3) Modern Biotechnology (1975-until now)

- Enhancement of microorganisms' productivity by genetic engineering techniques
- Production of therapeutic proteins (insulin, interferon, etc)
- Production of new sources of energy (Biogas and biodiesel)
- Production of vaccines by plants
- Production of genetically modified foods(GMF)
- Production of artificial chromosomes

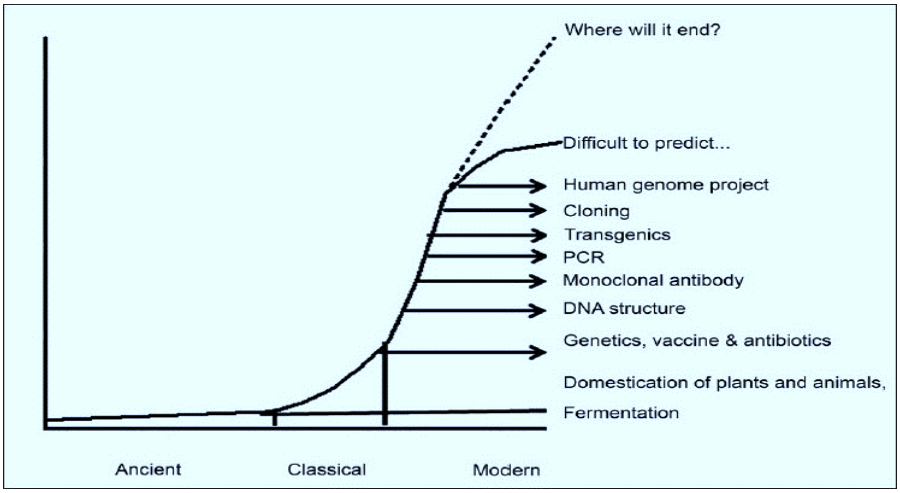


Fig.1.1: History of the development of biotechnology

Areas of biotechnology:

Term	Applications
Green biotechnology	Agriculture and Environment
Red biotechnology	Health, Medical and Diagnostics
White biotechnology	Industry
Blue biotechnology	Aquaculture, Coastal and Marine
Yellow biotechnology	Food and nutrition sciences
Brown biotechnology	Desert
Gold biotechnology	Bioinformatics and Nanotechnology
Dark biotechnology	Biowarfare and Bioterrorism
Purple biotechnology	Patents, Publications and Inventions

Biotechnology also can be named according to the organisms that used such as;

- Microbial biotechnology (also called “microbial technology” or “industrial microbiology”)
- Plant biotechnology
- Animal biotechnology

Biotechnological Process:

Any biotechnological process can be separated into the following 5 major steps or operations (Fig. 1.2):
(1) Strain (or culture) choice and improvement
(2) Mass culture (large-scale culture)
(3) Optimization of cell responses
(4) Process operation
(5) Product recovery or downstream processing

1. Strain Choice:

The first step in such a biotechnological process is the identification of a biological agent (microorganism/animal cell/plant cell) capable of producing the desired compound. This would generally involve the isolation of such a micro-organism from an appropriate habitat and its improvement through suitable strain development strategies.

2. Mass Culture:

It is necessary to culture the strain on a large scale, once a suitable strain has been developed; it needs to be maintained for as long as it is needed. Such strains can be used either to produce the biomass, (for example; SCP) or to recover some compounds from the biomass or the medium.

3. Optimization of Cell Responses:

In general, the conditions favouring rapid cell growth and biomass production are different from those of producing a compound of interest, e.g., antibiotics. Therefore, to optimize the biochemical yields, the culture conditions have to be precisely regulated.

4. Process Operations:

The steps of a biotechnological process need to be fully optimized for safety, reproducibility, control and efficiency at all the scales of operation. In major

part, this is the function of process engineering design developed with a full understanding of the biological, chemical and socioeconomic factors.

5. Product Recovery:

The goal of any biotechnological process is to recover (obtain) the needed product(s) in a useful form. The efficiency of product recovery is directly reflected in the product cost. The mode of this operation also determines the environmental friendliness of the process.

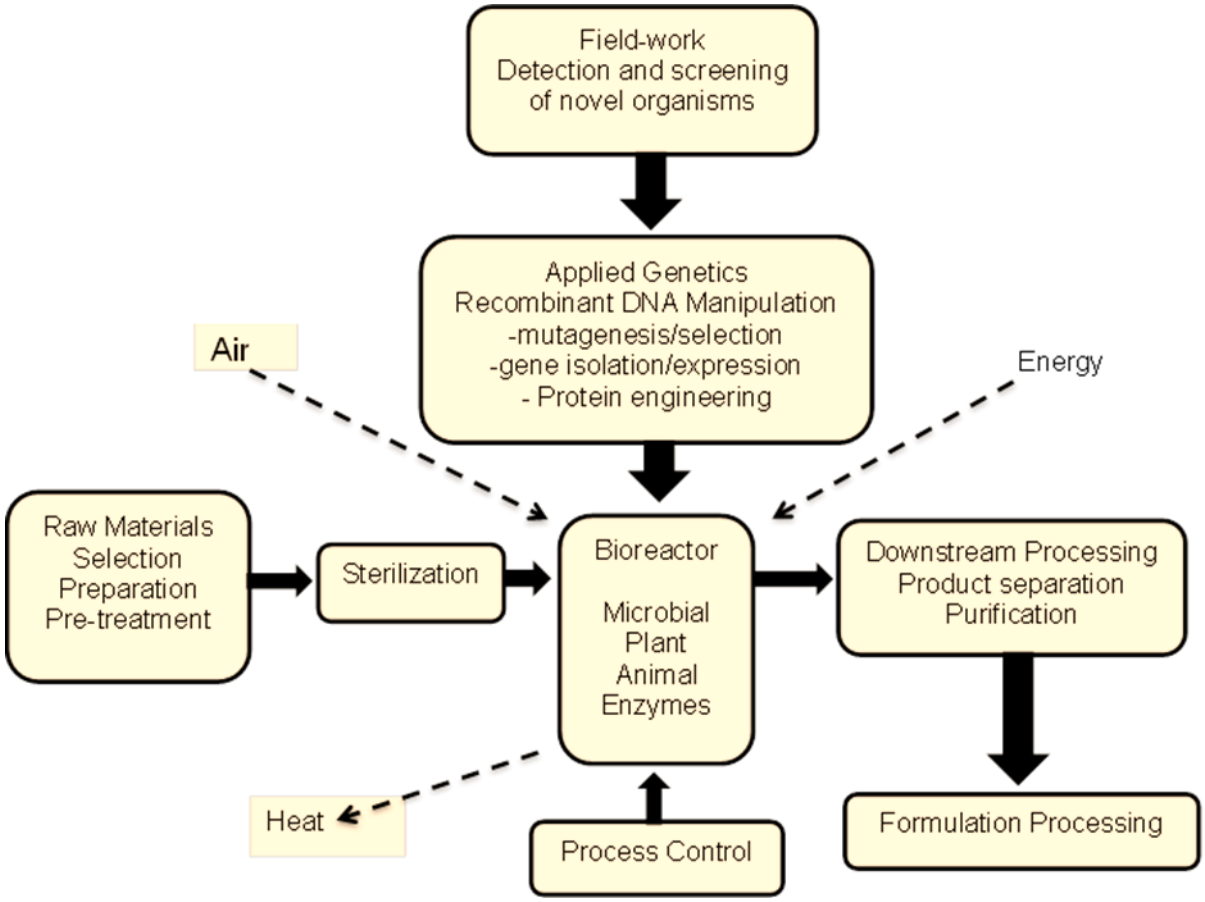


Fig. 1.2: The stages of a biotechnological process

Some examples of applications of Biotechnology (outputs):

1- Medical applications:

- The treatment of certain diseases such as cancer.
- The production of vaccines and immunizations.
- Diagnosis of diseases.
- Gene therapy.
- Stem cell research.
- Production of proteins and genes.

Biotechnology has created more than 200 new biotherapeutics and vaccines, including products to treat cancer, diabetes, HIV/AIDS and autoimmune disorders. The majority of these products are therapeutic proteins.

2- Agricultural applications:

- Food production, such as genetically modified foods
- Increased nutritional value
- Resistance to herbicides, pesticides
- Plants not required the addition of fertilizer.
- Stress – resistant plant (alkalinity, acidity, frost, drought)
- A plant used to produce the vaccine and medical products

3- Industrial applications:

- The enzymes are the most important outputs in this area and there are currently more than 450 enzymes work as a catalyst in various industrial applications, such as: carbohydrases (e.g.amylases), proteases, peptidases, lipases, oxireductases and transferases.
- Energy production.

4- Environmental applications:

- The major environmental use is cleaning through bioremediation.
- **Bioremediation** is the use of biotechnology to process or degrade a variety of natural and manmade products, especially those contributing to pollution.

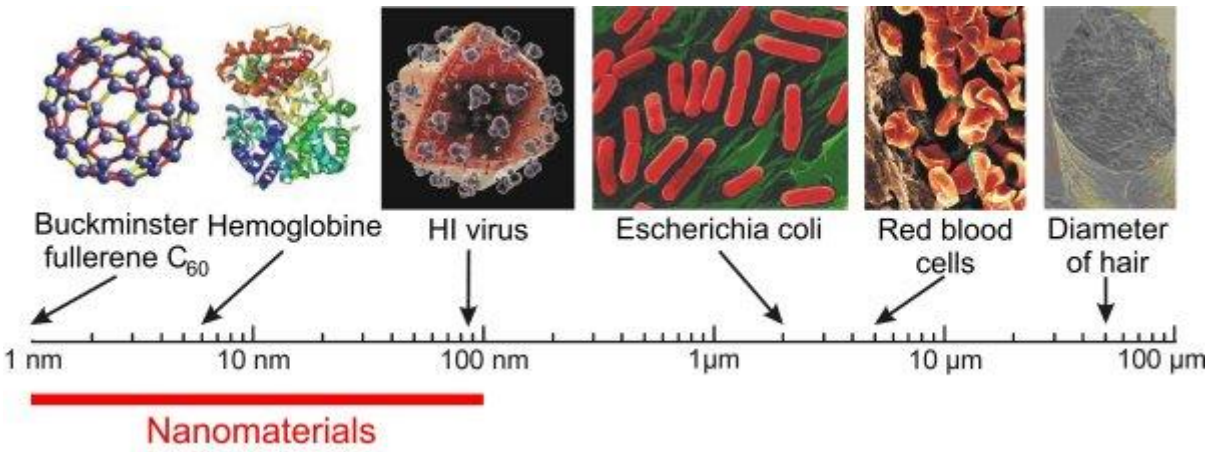
Lecture 2: Gold biotechnology

1- Nanotechnology

Nanotechnology, shortened to "nanotech", is the study of the controlling of matter on an atomic and molecular scale. Generally, nanotechnology deals with structures sized 100 nanometres or smaller in at least one dimension and involve developing materials or devices within that size. One nanometer (nm) is one billionth, or 10^{-9}, of a meter.

- The word nano is from the Greek word ‘Nanos’ meaning Dwarf.
- The term "nano-technology" was first used by Norio Taniguchi in 1974, though it was not widely known.
- The concepts that seeded nanotechnology were first discussed in 1959 by renowned physicist Richard Feynman.

Because of quantum size effects and large surface area to volume ratio, nanomaterials have unique and different properties compared with their larger counterparts, enabling unique applications.



Comparison of Nanomaterials Sizes

Nanobiotechnology is the creation of functional materials, devices and systems, through the understanding and control of matter at dimensions in the nanometer scale length (1-100 nm), where new functionalities and properties of matter are observed and harnessed for a broad range of applications.

Nanotechnology + Biotechnology = Nanobiotechnology

Some Applications of Nanobiotechnology

Medical applications

- Biological imaging for medical diagnostics.
- Advanced drug delivery systems.
- Biosensors for airborne chemicals or other toxins.

Targeted drug delivery

1. Nanoparticles containing drugs are coated with targeting agents (e.g. conjugated antibodies).
2. The nanoparticles circulate through the blood vessels and reach the target cells.
3. Drugs are released directly into the targeted cells.

Thermal ablation of cancer cells

1. Nanoshells have a metallic outer layer and silica core.
2. Selectively attracted to cancer cells either through a phenomenon called enhanced permeation retention or due to some molecules coated on the shells.
3. The nanoshells are heated with an external energy source killing the cancer cells.

Environmental applications

Green nanotechnology refers to the use of nanotechnology to enhance the environmental sustainability of processes producing negative externalities. It also refers to the use of the products of nanotechnology to enhance sustainability.

Green nanotechnology has two goals:

1. Producing nanomaterials and products without harming the environment or human health.
2. Producing nano-products that provide solutions to environmental problems.

Food industry applications

Nanotechnology can be applied in the production, processing, safety and packaging of food. A nanocomposite coating process could improve food packaging by placing anti-microbial agents directly on the surface of the coated film.

New foods called nano-foods are among the nanotechnology-created consumer products coming onto the market, there are more than 609 known or claimed nano-products.

Nanotoxicology

It is the study of the toxicity of nanomaterials. It addresses the toxicology of nanoparticles that appear to have toxicity effects that are unusual and not seen with larger particles. Nanotoxicological studies are intended to determine whether and to what extent these properties may pose a threat to the environment and human beings.

Nanopollution is a generic name for all waste generated by nanodevices or during the nanomaterials manufacturing process. This kind of waste may be very dangerous because of its size. It can float in the air and might easily penetrate animal and plant cells causing unknown effects.

2- Bioinformatics (Biocomputing)

- The marriage of biology and computer science has created a new field called 'Bioinformatics'.
- The term "bioinformatics" is short for "biological informatics".
- 1978: the term Bioinformatics first used

What is Bioinformatics?

- No standard definition
- Bioinformatics is the field of science in which biology, computer science, and information technology merge into a single discipline.

Aims of Bioinformatics:

The aims of bioinformatics are threefold:

1- Organizing Data in the correct manner
2- Proper Analysis of the Data
3- Interpreting the data in a biologically meaningful manner

Bioinformatics is being used in the following fields:

- Microbial genome applications
- Gene therapy
- Drug development
- Antibiotic resistance
- Evolutionary studies
- Waste cleanup Biotechnology
- Crop improvement
- Forensic analysis
- Bio-weapon creation
- Insect resistance
- Improve nutritional quality

Red biotechnology

Red or medical biotechnology is the applications of biotechnology in the medical fields and health care.

Gene therapy

Gene therapy is an experimental technique that uses genes to treat or prevent disease. The most common approach for correcting faulty genes is to insert a “normal” gene into the genome to replace an “abnormal” disease-causing gene.
Although gene therapy is a promising treatment option for many diseases, the technique remains risky and is still under study to make sure that it will be safe and effective.

Types of gene therapy

There are 2 types of gene therapy:

- **Germ line gene therapy**: where germ cells (sperm or egg) are modified by the introduction of functional genes, which are integrated into their genome. Therefore changes due to therapy would be heritable and would be passed on to later generation.
- **Somatic gene therapy**: where therapeutic genes are transferred into the somatic cells of a patient. Any modifications and effects will be restricted to the individual patient only and will not be inherited by the patient's offspring or any later generation.

Gene delivery

Vectors used in gene therapy are:

- **Viral Vectors;** One of the most promising vectors currently being used is harmless viruses.

- **Non-Viral Vectors;** Simplest method of non-viral transfection is direct DNA injection.

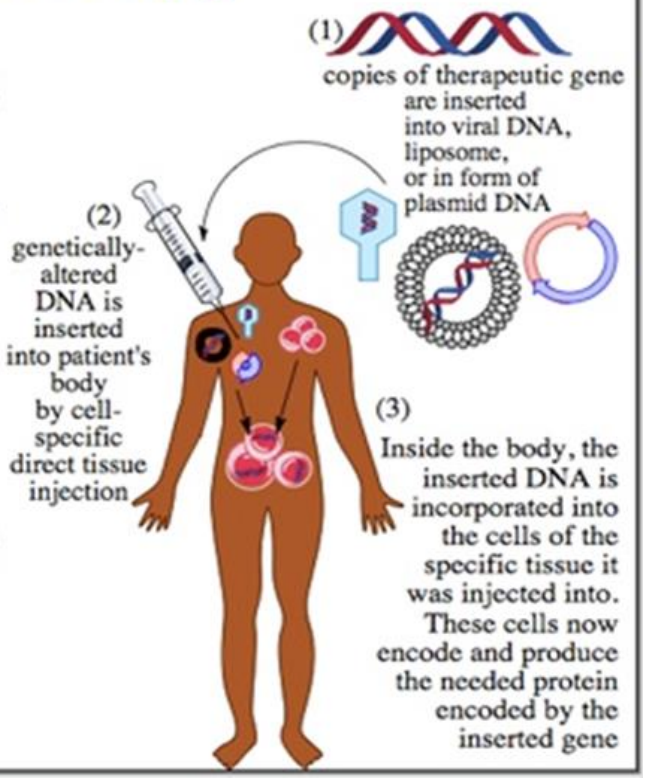
Two techniques have been used to deliver vectors;

1. **In in vivo gene therapy**; the vector can be injected or given intravenously (by IV) directly into a specific tissue in the body, where it is taken up by individual cells.
2. **In Ex vivo gene therapy**; a sample of the patient’s cells can be removed and exposed to the vector in a laboratory setting. The cells containing the vector are then returned to the patient.

If the treatment is successful, the new gene delivered by the vector will make a functioning protein.

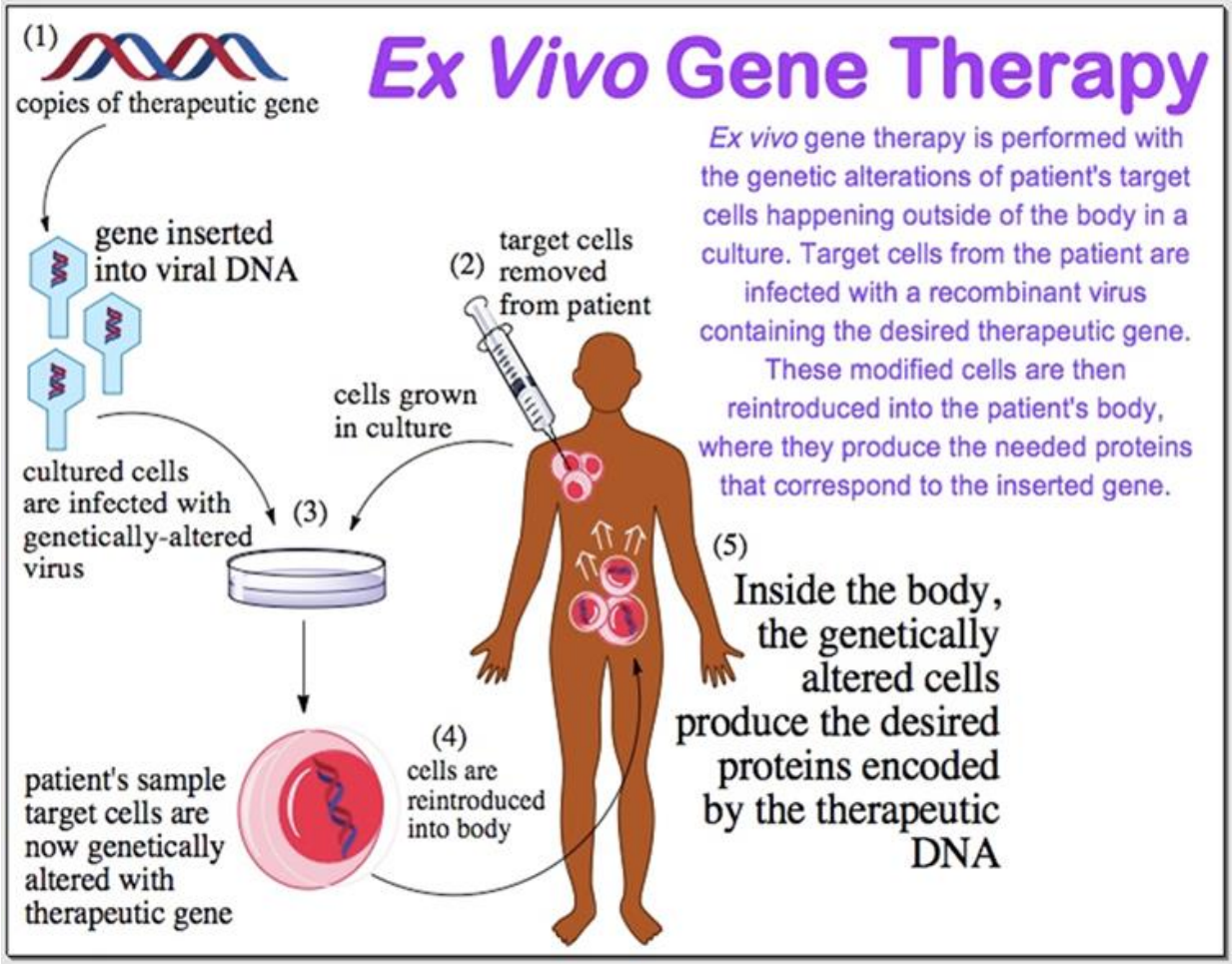
In Vivo Gene Therapy

In vivo gene therapy involves introduction of therapeutic DNA directly into the patient's body. The DNA is introduced by cell-specific direct injection into tissue in need. DNA in the form of a plasmid vector is introduced by a dermal vaccination. Modified liposomes are not currently used for gene therapy, but they will likely be the next advancement in therapeutic gene delivery as cell-specific receptor-mediated DNA carriers. Once inside the body and in contact with the specifically targeted cells, the inserted DNA is incorporated into the tissue's cells where it encodes the production of the needed protein.



Ex Vivo Gene Therapy

Ex vivo gene therapy is performed with the genetic alterations of patient's target cells happening outside of the body in a culture. Target cells from the patient are infected with a recombinant virus containing the desired therapeutic gene. These modified cells are then reintroduced into the patient's body, where they produce the needed proteins that correspond to the inserted gene.



Stem cell therapy

Stem-cell therapy is the use of stem cells to treat or prevent a disease or condition.

Stem cells are precursor cells that can divide to produce either more identical stem cells or many other different cell types in the body. This capability has stimulated enormous interest in the potential of stem cells to replace defective or damaged cells that cause disease.

Two broad categories of stem cells exist:

- **Embryonic stem cells**
- **Adult stem cells**

In a developing embryo, stem cells can differentiate into all the specialized embryonic tissue. In adults, stem cells act as a repair system for the body replacing specialized damaged cells.

Stem cell therapy provides hope for a cure for patients of incurable afflictions such as Parkinson's disease and Alzheimer's disease, and also for people suffering from paralysis resulting from spinal cord injuries.

The combination of stem cells with gene therapy might allow the rebuilding of new body parts to substitute for old and defective ones.
With the use of stem cells to regenerate healthy bone marrow cells, a permanent cure is expected, as healthy cells can grow and divide continuously.

Lecture 3: Fermentation by microorganisms

- **Fermentation** is a process where the microbial, plant and animal cells are used to carry out enzyme-catalyzed transformations of organic matter.
- Fermentation is considered the first application in biotechnology.
- Fermentation Technology could be defined simply as the study of the fermentation process, techniques and its application.
- In general, the fermentation process is divided into two parts i.e. Up Stream Processing (USP) and Down Stream Processing (DSP).

The reasons for using microorganisms in fermentation:

1. The ratio of surface area to volume is high so that the nutrients in the medium consumed quickly forced the metabolic reactions.
2. Adaptation for different ecological conditions, so that it very easy to transfer M.Os. from their natural habitat to the lab. They can grow on cheap carbon and nitrogen sources to produce compounds with high economic value.
3. The ability to achieve huge chemical reactions.
4. It is very easy to deal with microorganisms genetically and design genetically modified organisms, which produced higher amounts of product.

Requirements of fermentation:

1- Specific strain or microbial enzymes.
2- Raw material substrate (Fermentation medium).
3- Controlled favourable environment.

Specific strain or microbial enzymes

Microorganisms hold the key to the success or failure of a fermentation process. It is therefore important to select the most suitable microorganisms to carry out the desired industrial process. The most important factor for the success of any fermentation industry is a production strain. The M.Os.that isolated from nature have low production efficiency, therefore; there are two ways to enhance productivity; ecological ways and genetic ways.

Fermentation medium (raw material)

- The growth medium (liquid or solid) in which microbes grow and multiply is called the fermentation medium.
- The selected microbe should be able to utilize and grow on cheap sources of carbon and nitrogen. Usually, these sources are waste

products of the industrial process e.g. molasses, whey, corn steep liquor etc. Care is taken to avoid the use of such microbes which require expensive nutrients for their growth.

- Fermentation media must satisfy all the nutritional requirements of the microorganism and fulfill the technical objectives of the process. All microorganisms require water, sources of energy, carbon, nitrogen, mineral elements and possibly vitamins plus oxygen if aerobic. The nutrients should be formulated to promote the synthesis of the target product, either cell biomass or a specific metabolite.

The main factors that affect the final choice of individual raw materials are as follows:

1. Cost and availability: ideally, materials should be inexpensive and of consistent quality and year-round availability.
2. Ease of handling in solid or liquid forms, along with associated transport and storage costs, e.g., requirements for temperature control.
3. Sterilization requirements and any potential denaturation problems.
4. Formulation, mixing, complexity and viscosity characteristics may influence agitation, aeration and foaming during fermentation and downstream processing stages.
5. The concentration of target product to be attained, its rate of formation and yield per gram of substrate utilized.
6. The levels and range of impurities and the potential for generating further undesired products during the process.
7. Overall health and safety implications.

Controlled favourable environment

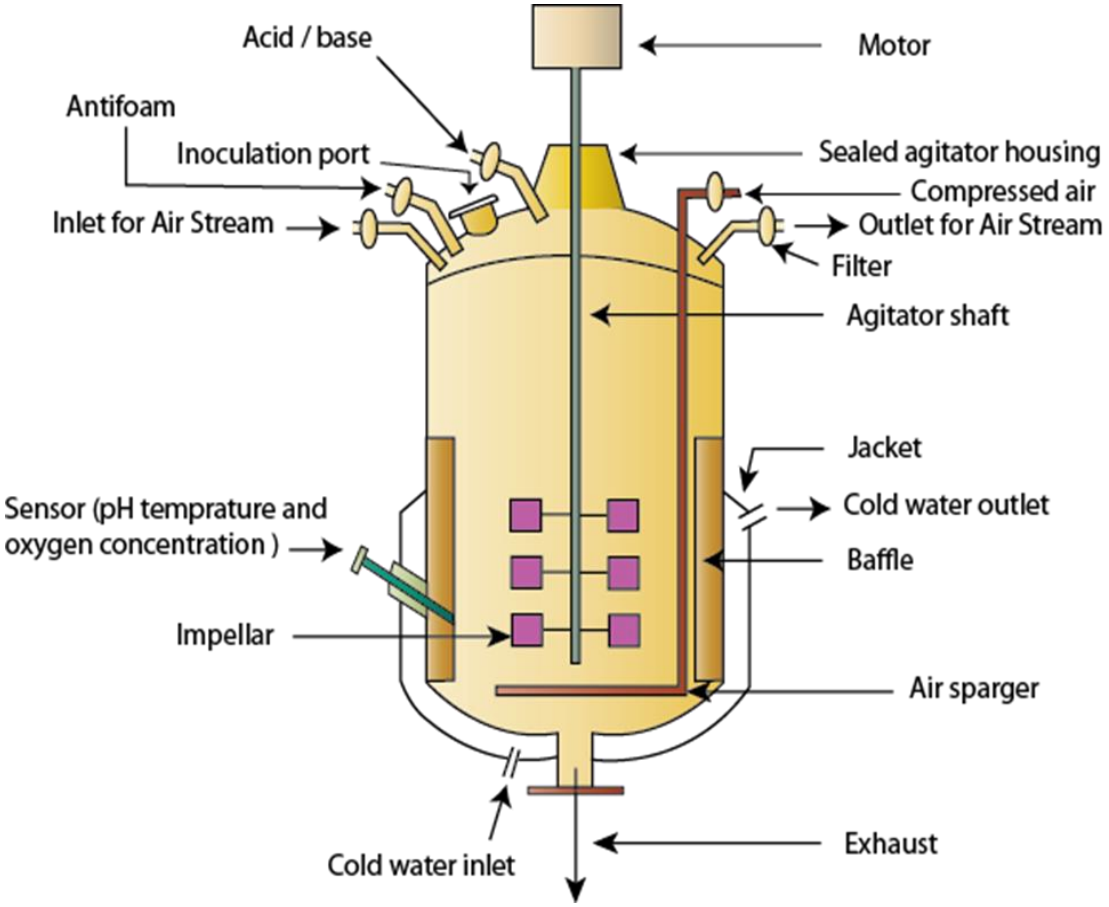
For the production of a desired microbial product, it is of utmost importance to optimize the physical (temperature, aeration etc.) and chemical (carbon, nitrogen, mineral sources etc.) composition of the fermentation medium. To maintain these stringent conditions, microbes are grown in containers called as ferment-ers/ors or bioreactors. The capacity of bioreactors may vary from 10 liters to 100,000 liters depending on the product. Fermentor is known as the heart of the fermentation process.

- ❖ **A fermentor**, also called a bioreactor, is a vessel in which a particular microbe is grown under controlled conditions to produce a desired byproduct or biomass.

The fermentors aim to provide a stabilized condition for the growth of cells and better production of the desired byproduct.

In designing and constructing a fermenter several points must be considered:

1- The vessel should be capable of being operated aseptically for many days and should be reliable in long-term operation.
2- Adequate aeration and agitation should be provided to meet the metabolic requirements of the M.O. However the mixing should not cause damage to the organism.
3- Power consumption should be as low as possible.
4- A system of temperature control should be provided.
5- A system of pH control should be provided.
6- The vessel should be designed to require the minimal use of labour in operation, harvesting, cleaning and maintenance.
7- The vessel should be suitable for a range of processes.
8- The vessel should be constructed to ensure a smooth internal surface.
9- The cheapest materials which enable satisfactory results to be achieved should be used.
10- There should be adequate service positions for individual plants.



Fermenter Instrumentation & Control

1- Aeration & Agitation

- **The Aeration System (Sparger)** should be provided M.O in submerged culture with sufficient oxygen for metabolic requirements
- **The agitation system (Agitator or impeller and baffles)** should ensure that a uniform suspension of microbial cells is achieved in a homogenous nutrient medium.

The impeller has two main functions:

1- To diminish the size of air bubbles to give a bigger interfacial area for oxygen transfer & decrease the diffusion path.
2- To maintain a uniform environment throughout the vessel contents.

Baffles are metal strips roughly one-tenth of the vessel diameter and attached radially to the wall. They are normally incorporated into agitated vessels of all size to prevent a vortex & to improve aeration efficiency.

2- Temperature

The temperature in a vessel is the most important parameter to monitor & control in any process. It may be measured by mercury in a glass thermometer, bimetallic thermometer, pressure bulb thermometer, thermocouples, metal-resistance thermometer or thermistors.

3- Foam Sensing & Control

The formation of foam is a difficulty in many types of microbial fermentation which can create serious problems if not controlled. It is common practice to add an antifoam to a fermenter when the culture starts foaming above a certain predetermined level.

Important material uses as antifoaming agents are: Castor Oil, Fatty acids, Fatty Acids Esters, Fatty Acids Sulfate, Sulphonate, Olive Oil, Mono & DiGlyceride, Silicones Oil (best one)

4- pH Measurement & Control

5- Flow Measurement & control of both gases & liquids

6- Carbon Dioxide Electrodes

7- On-line Analysis of Chemical Factors.

Characteristics of large scale fermentations

- Fermantations = any large-scale microbial production
- Fermentors = tank use for fermentation
- Fermenters = microorganisms responsible for the production

Inoculum Development

The process adopted to produce a culture volume sufficient for inoculation in the fermentation medium is called inoculum development.

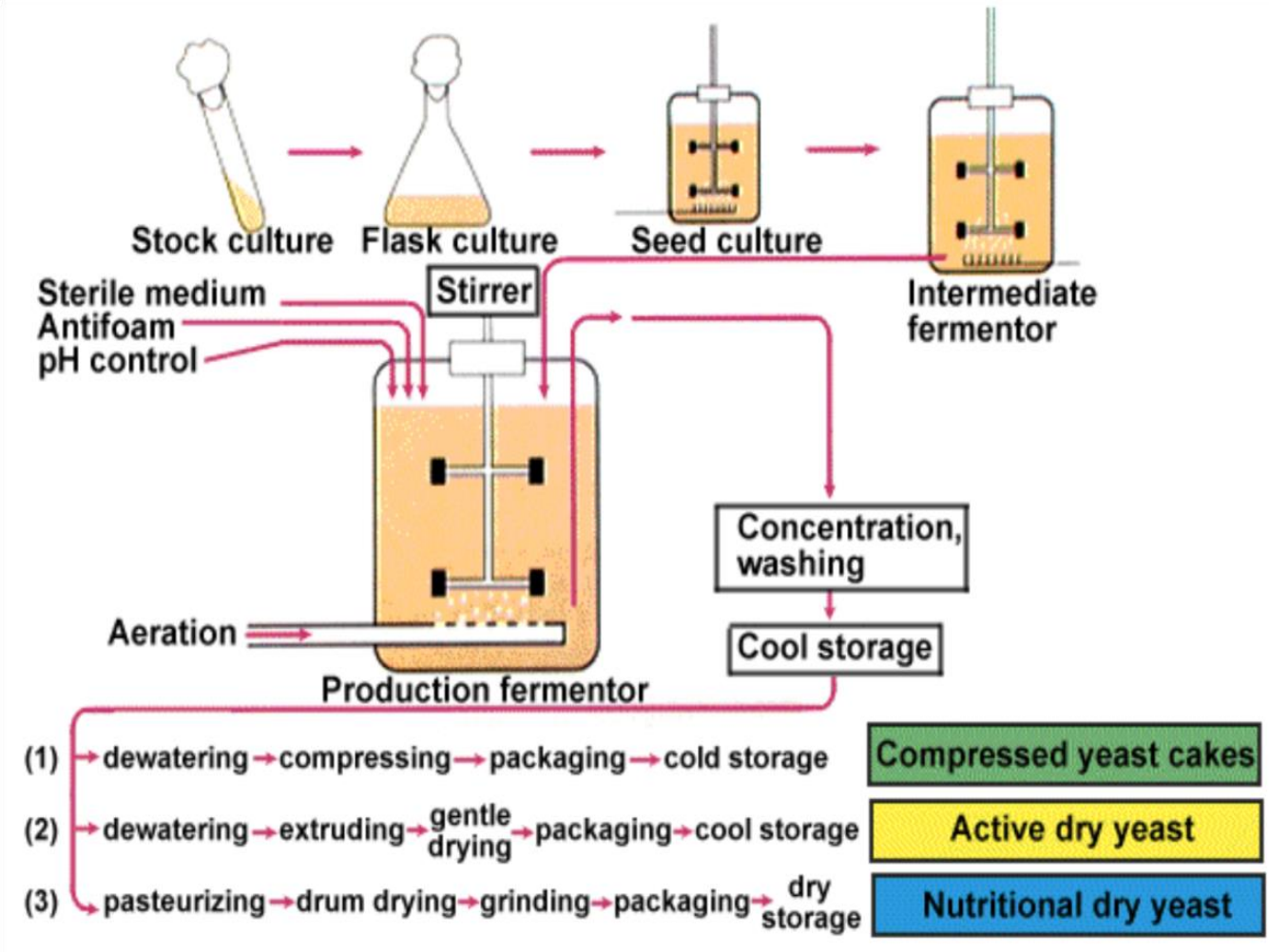
To achieve a maximal yield of the product via fermentation, the culture inoculum should have the following characteristics: it should be

- Metabolically highly active.
- Easy to prepare in large volume.
- Of suitable morphological form.
- Free from microbial contamination.

In industry, the size of the fermentation medium can be very high e.g. 100,000 liters, which means that the minimal inoculum size will be 2000 liters. To prepare an inoculum of this magnitude is not an easy task. Starting from a stock culture, which may be in lyophilized form or on a slant-agar, the inoculum is built up in a number of stages:

1. A small amount of culture is inoculated in a shake-flask and incubated.
2. It is transferred to a larger flask and incubated.
3. It is then transferred to a small laboratory fermentor.
4. The size of the culture inoculum is further increased by transferring this culture into a pilot scale fermentor.

Example of fermentation process:
Industrial production of yeast cells



Lecture 4: Types of Fermentation

I/ Liquid Fermentation

May be carried out as:

1- Batch culture.
2- Continuous culture.
3- Fed-Batch culture.

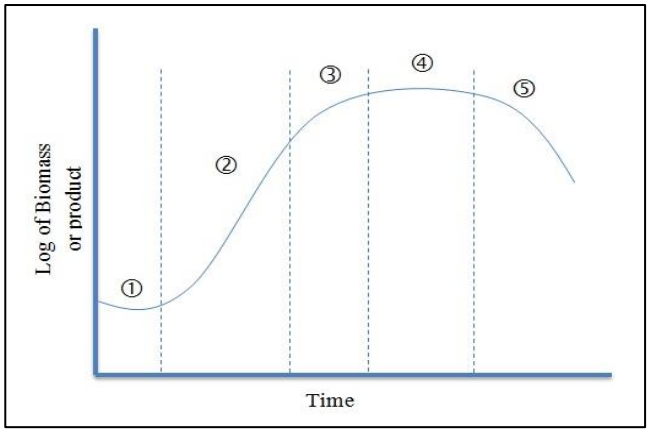
Reactions can occur in static or agitated cultures. In the presence or absence of oxygen, and aqueous or low moisture condition (Solid Substrate Fermentation). The growth of organisms may be considered as the increase of cell material expressed in terms of mass or cell number. **Optimal expression of growth** will be dependent on the transport of necessary nutrients to cell surfaces and on environmental parameters such as temperature and pH. The quantity of cell material (X) can be determined by (dry weight, wet weight, DNA or protein) or numerically by the number of cells.

Doubling time (td): relates to the period required for the doubling in weight of biomass.
Generating time (g): relates to the period necessary for the doubling of cell numbers.

During balanced or exponential growth, when growth is controlled only by intrinsic cellular activities, g=td provided every cell of the population is able to divide. Average doubling time increase with increasing cell size e.g. bacteria 0.25 to 1, yeast 1.15 to 2, mold and fungi 2 to 6.9 and plant cells 20-40 hrs.

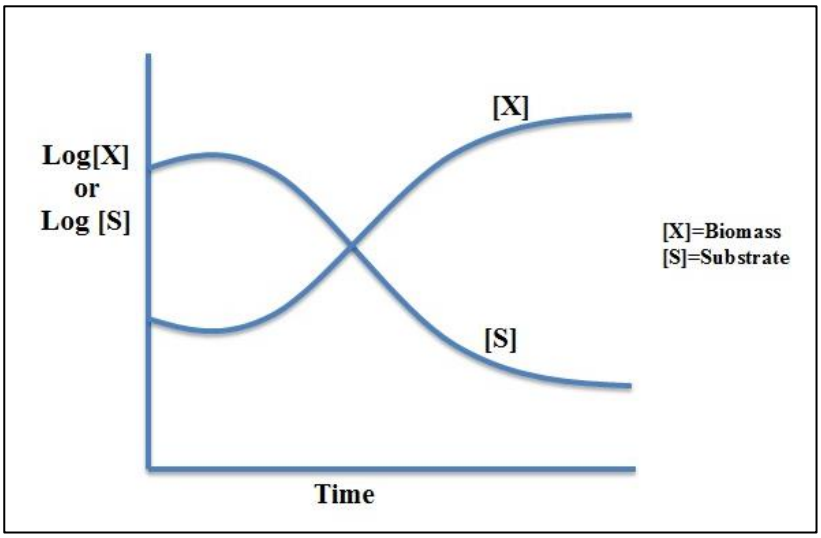
Batch Culture

Is an example of a closed culture system which contains and initiate, a limited amount of nutrient. The inoculated culture will pass through some phases



1-Lag 2-Log 3-Deceleration 4-Stationary phase 5-Death phase

After inoculation, there is a period during which no growth appears to take place, this period (Lag phase). In the commercial process, the length of the Lag phase should be reduced. Following a period during which the growth rate of the cells gradually increases, the cells grow at a constant maximum rate and the period is known Log phase. The growth will continue indefinitely. However, growth results in the consumption of nutrients and the excretion of microbial products. After a certain time, the growth rate of the culture decreases until growth ceases. The cessation of growth may be due to the depletion of some essential nutrient in the medium (substrate limitation), the accumulation of some autotoxic product of the organism in the medium.



Growth characteristics in a Batch Culture of M.O.

The decrease in growth rate and the cessation of growth, due to the depletion of the substrate, may be described by the relationship between μ and the residual growth-limiting substrate.

$$\mu = \frac{\mu_{max}\,[S]}{K_s + [S]}$$

[S]= residual substrate concentration
Ks= saturation constant
μ = specific growth rate
μ_{max} = maximum specific growth rate

Ks: numerically equal to substrate concentration when μ is half μ_{max}.
Ks measure the affinity of the organism for its substrate. Low Ks means the organism has a very high affinity for the limiting substrate and high Ks means the organism has a low affinity for the substrate.
A simple relationship exists between growth rate and utilization of substrate. In simple systems (batch) growth rate is a constant fraction (Y) of the substrate utilization rate:

$$\frac{dx}{dt} = Y \frac{ds}{dt}$$

$\frac{dx}{dt}$= rate of increase of conc. of organism

Y: Yield constant and over any finite period of growth
ثابت الانتاج خلال مدة النمو

$$Y = \frac{Weight\ of\ cells\ formed}{Weight\ of\ substrate\ used}$$

Knowing the value of the three growth constants μmax, Ks, and Y can give a complete quantitative description of the growth cycle of the batch culture.

Advantages of Batch Fermentation

It used to optimize organism or biomass production and then to carry out specific chemical transformation such as end-product formation (antibiotics, organic acids) or decomposition of substance (sewage treatment). Many important products are optimally formed during the stationary phase of the growth cycle in batch culture. (produce biomass, primary metabolites and secondary metabolites).

Continuous Culture (Opened Culture)

In contrast to batch culture, in continuous cultivation, the addition of nutrients and the removal of an equal fraction of the total culture volume occur continuously. Continuous methods of cultivation will permit the organism to grow under steady-state conditions, that is growth occurs at a constant rate and in a constant environment. Factor such as pH and concentration of nutrients and metabolic products which inevitably change during the growth cycle of a batch culture can be held constant in continuous culture.

These parameters can be independently controlled allowing the experimenter to obtain realistic information on the role of each to the growth of the organism.

In a completely mixed continuous culture system sterile medium is fed into the bioreactor at a steady flow-rate (f) and culture broth emerges from it at the same rate keeping the volume of culture in the vessel (V) constant.

$$\mathbf{D=\frac{f}{V}}$$

F= flow rate

V= the volume

D=number of complete volume changes per hour (dilution rate)

When:

$\mu > D \longrightarrow \frac{dx}{dt}$ is positive and cell concentration will increase.

$\mu < D \longrightarrow \frac{dx}{dt}$ is negative and cell wash out with occur.

$\mu = D \longrightarrow \frac{dx}{dt}$ =0 and X is constant.

In this case, the steady-state has been achieved where the concentration of the organism will not change with time.

Applications of continuous culture:

1- Industry: Used in the production of therapeutic Pharmaceuticals, antibiotics, ethanol, and fermented foods such as cheese.
2- Research: Used to collect data to be used in the creation of a mathematical model of growth for specific cells or organisms, analysis of biological processes in micro-organisms, and study biofilm formation in *Pseudomonas aeruginosa*.
3- Biological waste treatment.

Continuous industrial microbial processes are much less common than batch processes.

Fed – Batch Culture (Semi continuous)

It is a form of cultivation that involves continuous or sequential addition of medium or substrate to the initial batch without removal of culture fluid. Product yield from such systems can well excess conventional batch culture. This approach is widely practiced in industry for example in the production of baker's yeast. The use of fed-batch by fermentation industry takes advantage

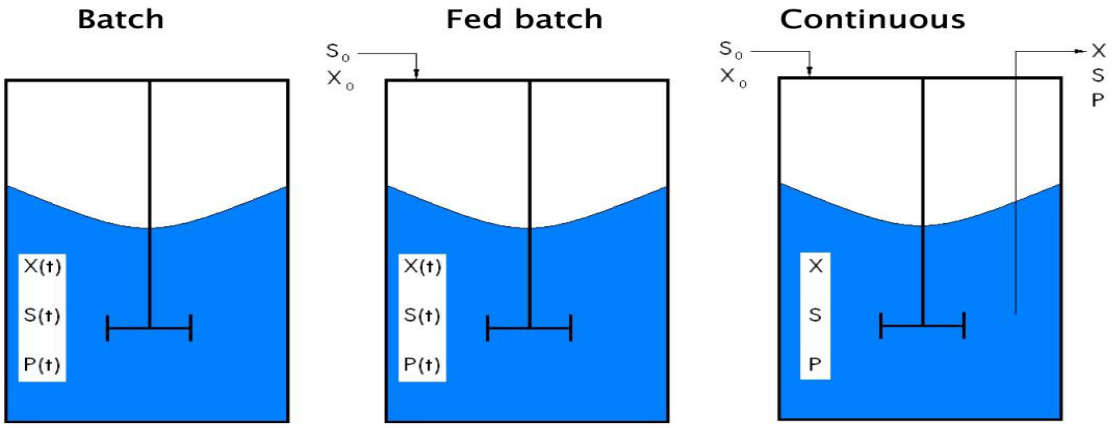
of the act that residual substrate concentration may be maintained at a very low level which advantageous in:

1- Removing the repressive effect of rapidly utilized carbon sources and maintaining conditions in the culture within the aeration capacity of the fermenter
2- Avoiding the toxic effects of a medium component

Applications of Fed-Batch Cultures

- **The yeast cell production:** The oldest and first well-known industrial application of a fed-batch operation was introduced after the end of World War I. It was the yeast cell production in which sugar (glucose) was added incrementally during fermentation to maintain a low sugar concentration to suppress alcohol formation.
- **Penicillin production:** In which the energy source (e.g., glucose) and precursors (e.g., phenyl acetic acid) were added incrementally during fermentation to improve penicillin production.

Fed-batch cultures have been tested for the production of various products such as yeasts; antibiotics; amino acids; organic acids; enzymes; alcoholic solvents; recombinant DNA products; proteins; and others.



x: biomass, s: substrate, p: product, t: time

II/ Solid Substrate Fermentation SSF

It concerned with the growth of M.O on solid materials in the absence or near absence of free water. Biological activity ceases when the moisture content of the substrate is about 12%.

The most common substrate used in solid substrate fermentation are cereal grains, legume seeds, wheat bran, lingo cellulosic materials such as wood and straw and a variety of other plant and animal matter. The compounds are polymeric molecules, cheap, easily obtainable and represent a concentrated source of nutrients. The type of M.O that grow well under the condition of

solid substrate fermentation are certain filamentous fungi and few yeasts can grow at a value between a_w = 0.6-0.7, more than bacteria a_w =1.

Steps of SSF:

1- The grains are moistened with water and ground to form a paste. Additional supplements like salts etc. may be added to the solids before sterilization.
2- The solid material is then transferred to shallow metallic containers and is steam sterilized.
3- This is followed by the spraying of culture inoculum on to the surface of the sterilized medium and incubation is carried out under controlled conditions of temperature, air and humidity.

SSF processes can be classified based on the seed culture for fermentation into:

1- **Pure culture**, such as lactic acid production from wheat bran using *Lactobacillus amylophilus*.
2- **Mixed culture**, such as cellulase production using *Trichoderma reesei* with *Aspergillus* spp.

Advantage:

1- Simple media with cheaper nature rather than a costly component.
2- Low moisture content of materials gives economy of bioreactor space, low liquid effluent treatment, less microbial contamination, often no need to sterilize, easier downstream processing.
3- Aeration requirement can be met by simple gas diffusion or by aeration intermittently, rather than continuously yields of products can be high.

Disadvantages:

1- Processes limited mainly to molds that tolerate low moisture level.
2- Metabolic heat production in large-scale operation creates problems.
3- Process monitoring e.g. moisture levels, biomass, O_2 and CO_2 levels, is difficult to achieve accurately.
4- The slower growth rate of M.O.

Some examples of solid substrate fermentation

Example	Substrate	Microorganism
Mushroom production	Straw	*Agaricus*
Soy sauce	Soya bean	*Aspergillus*
Cheese	Milk crud	*Penicillium*
Enzymes	Wheat bran	*Aspergillus*
Organic acid	Molasses	*Aspergillus*

Lecture 5: Products of Fermentation

Major Groups of Commercial Fermentation Products:

1- Microbial biomass or cells.
2- Microbial enzymes.
3- Microbial metabolites.
4- Bioconversion or Biotransformation.

Microbial Biomass or cells

Microbial biomass or cells may be subdivided into two major processes:

a) Production of baker's yeast by *Saccharomyces cerevisiae*.

b) Production of microbial cells used as food for human or animal (Single-cell protein/SCP) which are in fact either: (i) whole cells of *Spirullina* (as algae), (ii) *Candida* or *Saccharomyces* (as yeast) and (iii) *Lactobacillus* (as bacteria).

Microbial Enzymes

Enzymes have been produced commercially from the plant, animal and microbial sources. However, microbial enzymes have the enormous advantage of being able to be produced in large quantities by establishing fermentation techniques.

Table below contains microbial enzymes used in the production of commercial fermentation industries.

Industry	Enzyme	Source (Genus)
Baking, Flavours	Amylase	*Aspergillus* , *Bacillus*
Beer, Laundry detergents	Protease	*Aspergillus*, *Bacillus*
	Lipase	*Aspergillus, Rhizopus*, *Bacillus*
Dairy	Catalase	*Aspergillus,, Corynebacterium, Micrococcus*
	Lactase (β-galactosidase)	*Aspergillus*
Pharmaceutical & Clinical	Amylase	*Bacillus*
	Streptokinase	Heamolytic Streptococci
Fruit Juice	Pectinase	*Aspergillus , Penicillium*

Microbial Metabolites

Metabolites are the intermediates and products of metabolism.
Metabolism is the sum of all the biochemical reactions carried out by an organism.

Metabolism involves two pathways:

a) Primary metabolic pathways (PMPs). Their products are called **primary or central metabolites**

b) Secondary metabolic pathways (SMPs). Their products are called **Secondary metabolites**

1. **Primary or central metabolites:**
   - They are microbial products made during the trophophase (exponential phase) of growth whose synthesis is an integral part of the normal growth process.
   - They include intermediates and end products of anabolic metabolism, which are used by the cell as building blocks for essential macromolecules (e.g., amino acids, nucleotides) or are converted to coenzymes (e.g., vitamins). Other primary metabolites (e.g., citric acid, acetic acid and ethanol) result from catabolic metabolism.
   - Industrially, the most important primary metabolites are amino acids, nucleotides, vitamins, solvents and organic acids.

2. **Secondary metabolites**:
   - They do not play a role in the growth and are formed during the end or near the idiophase (stationary phase) of growth.
   - Usually has an important ecological function.
   - Many Secondary metabolites have antimicrobial activity, others are specific enzyme inhibitors, some are growth promoters and many have pharmacological properties.
   - Examples; antibiotics, pesticides, pigments, toxins.

Examples of Primary Metabolites

Organic Acid Production

- Organic acids can be used both as:

1. Additives in the food industry.
2. Chemical feedstock.

- Except for the production of citric acid which is manufactured entirely by fermentation, there is frequently great competition between microbiological & chemical processes for the production of the various organic acids.

The citric acid ($C_6H_8O_7$)

- Citric acid has been known as a natural plant substance since the end of the nineteenth century.
- Since 1893 scientists have known that it is produced by filamentous fungi.
- In 1923 the first practical microbial fermentation for the production of this organic acid was started.
- Today over 99% of the world's output of citric acid is microbially produced.
- The strains that are used for citric acid production are:
  Aspergillus niger, A.wentii ,A.clavatus ,Penicillium luteum ,P. citrinum ,Mucor piriformi and *Saccharomycopsis lipolytica*.
- During the last 30 years, the interest of researchers has been attracted by the use of yeasts (mostly Candida spp. and some Rhodotorula spp.) as citric acid producers. *C. lipolytica* has been developed as a microbial cell factory for citric acid production in recent years.
- Compared to *Penicillium* strains, only mutants of *Aspergillus niger* are used for commercial production, Why?

1. Aspergilla produce more citric acid per unite time.
2. Production of the undesirable side products can be suppressed in these mutants.

Uses of Citric acid:

1. As a food additive/preservative found in many different processed foods and soft drinks.
2. As an ingredient in cosmetic products to balance the pH levels, small amounts of citric acid can be found in shampoos, body wash, face cleansers, nail polish, hand soap and other cosmetics products.
3. As a powerful cleaning agent, it works well as both a cleaner and a deodorizer.
4. As a powerful water softener, it an ideal all-natural choice for treating hard water.
5. Citric acid is widely used as an acidulent in creams, gels, and liquids of all kinds.

Amino Acid Production

- Taste – enhancing properties of glutamic acid were discovered in 1908 in Japan.
- Commercial production of sodium glutamate from acid hydrolysates of wheat & soy protein began soon after.

- In 1957, L-glutamic acid was discovered as a product in the spent medium of *Corynebacterium glutamicum* & this organism subsequently became the major source of sodium glutamate.

Commercial Uses of Amino Acids:

1. Food Industry:

Amino acids are used either alone or in combination:

- as flavor enhancers.
- as an antioxidant for the preservation of fruit juices.
- as a low-calorie artificial sweetener in the soft-drink industry.
- in the preparation of feed for animals.

2. Pharmaceutical Industry:

The amino acids can be used as medicines. Essential amino acids are useful as ingredients of infusion fluids, for administration to patients in post-operative treatment.

3. Chemical Industry:

Amino acids serve as starting materials for producing several compounds. For example:

- Glycine is used as a precursor for the synthesis of glyphosate (a herbicide).
- Poly-methyl glutamate is utilized for manufacturing synthetic leather.
- Some amino acids are useful for the preparation of cosmetics.
- With all these applications amino acids are on their way into the synthesis of biodegradable polymers.

Microbiological Methods of Production:

There are three approaches to microbiological production:

1- Direct Fermentation of amino acids using different carbon sources, such as glucose, fructose, molasses, starch, hydrolysis …etc.

2- By converting inexpensive intermediate products via biosynthesis for example glycine which is inexpensive can be converted to L-serine.

3- By the use of enzymes or immobilized cells, sometimes in continuous processes involving enzymes-membrane reactors.

Glutamic Acid:

- L-glutamic acid is manufactured predominantly by microbial means.
- Japanese researchers began developing a direct fermentation process because the D,L-glutamic acid which is formed by chemical synthesis is the racemic mixture.
- The most important industrial strains with high excretion of glutamic acid are *Micrococcus glutamicus & Brevibacterium flavum*. There are similar, Gram-positive, non sporulating, non-motile bacteria.

Vitamins

- Vitamins are defined as essential micronutrients that are not synthesized by mammals.
- Most vitamins are essential for the metabolism of all living organisms, and they are synthesized by microorganisms and plants.
- They are 2 types: Water-Soluble and fat -Soluble Vitamins.
- Most vitamins and related compounds are now industrially produced and microorganisms can be successfully used for the commercial production of many of them.
- Vitamins and related compounds are widely used as food or feed additives, medical or therapeutic agents, health aids, cosmetic and so on.

Riboflavin (Vitamin B2)

- It is a water-soluble vitamin, essential for growth and reproduction in man and animals.
- 75% of the current world production of riboflavin is for feed additive and the remaining for food and pharmaceuticals. The crude concentrated form is also used for feed.
- It is produced by both synthetic and fermentation processes.
- Two closely related ascomycete fungi, *Eremothecium ashbyii* and *Ashbya gossypii*, are mainly used for the industrial production.
- Yeasts (*Candida flaeri, C. famata*, etc.) and bacteria can also be used for practical production.

Example on Secondary Metabolites (Antibiotics)

- Antibiotics are secondary metabolites produced by one type of microorganism that in low concentrations act against other organisms.
- Antibiotics are elaborated by bacteria (predominantly by Actinomycetes in the genus *Streptomyces*) as well as fungi. For example, *Penicillium chrysogenum* (a mold) produces penicillins; *Streptomyces* species (bacteria) produce streptomycins and tetracyclines.
- Over 500 distinct antibiotic substances are produced by streptomycetes.

Penicillin

- Penicillin, produced by *Penicillium chrysogenum,* is an excellent example of a fermentation for which careful adjustment of the medium composition is used to achieve maximum yields.
- Rapid production of cells, which can occur when high levels of glucose are used as a carbon source, does not lead to maximum antibiotic yields.

- Provision of the slowly hydrolyzed disaccharide lactose, in combination with limited nitrogen availability, stimulates a greater accumulation of penicillin after growth has stopped.
- The same result can be achieved by using a slow continuous feed of glucose. If particular penicillin is needed, the specific precursor is added to the medium. For example, phenylacetic acid is added to maximize the production of penicillin G, which has a benzyl side chain.
- Using genetic engineering techniques increased penicillin production up to 30-fold.

Bioconversion or Biotransformation or Microbial Transformations

- **Microbial transformation** is defined as the biological process of modifying an organic compound into a reversible product.
- It involves the use of chemically defined enzyme catalyzed reactions in the living cells and it preferred over the chemical transformation in industries.

Bioconversion differs from chemical conversion in:

- highly specificity
- needing to low temperature
- don't need to use the heavy metals
- milder reaction condition

Microbial transformation reactions are mainly categorized into oxidation/ reduction, hydrolysis, condensation & isomerization reactions (Fig. below).

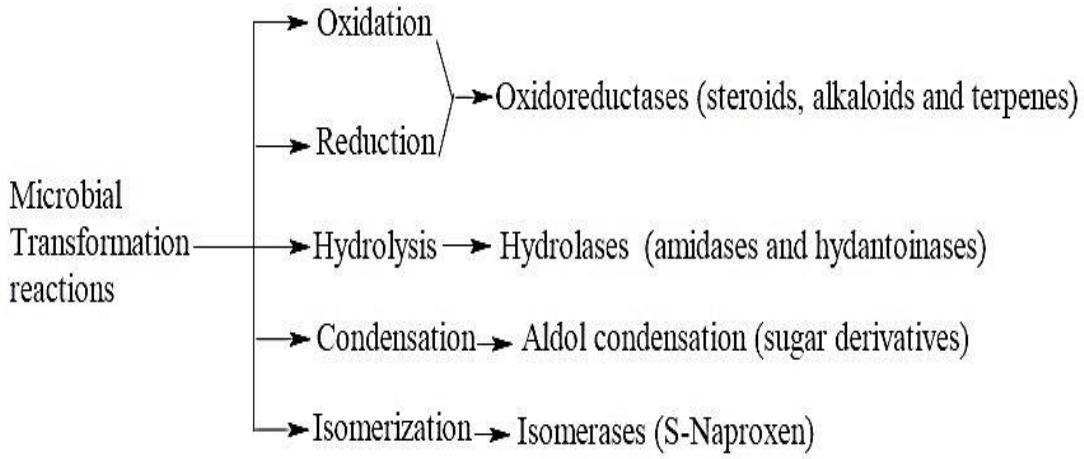


Fig.: Type of transformation reactions catalyse by enzymes

- One of the major applications of microbial transformation is in the production of secondary metabolites.

Examples of biotransformation:

- The industrial production of cortisone. One step is the bioconversion of progesterone to 11-alpha- Hydroxyprogesterone by *Rhizopus nigricans*.
- The conversion of organic materials, such as plant or animal waste, into usable products or energy sources.

Lecture 6: Downstream Processing

- **Downstream processing (DSP) or Product recovery or Bioseparation** is the extraction and purification of a biological product from the fermentation broth.
- DSP is very complex and variable and depending on the type of the product.
- DSP can be divided into five stages (Fig.6.1):
  1. Cell harvesting
  2. Lyses/breakage of cells
  3. Concentration
  4. Purification
  5. Formulation

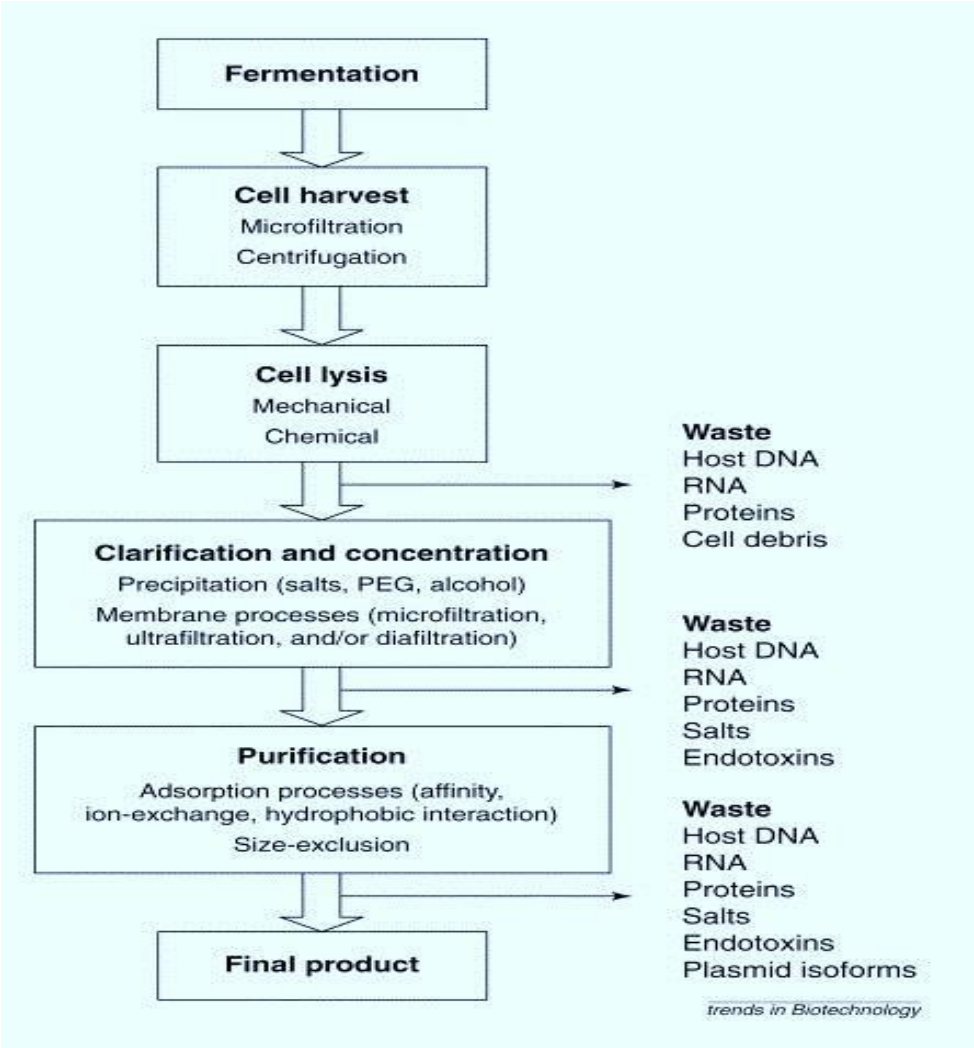


Fig. 6.1: Stages of downstream processing (DSP)

I. Cell harvesting

Once the fermentation is complete, the solid phase (i.e. cell biomass) is separated from the liquid phase by any of the following methods:

1- **Settling:** it depends on size and weight; it descends cells down by gravity and uses in alcohol industry and waste treatment.

2- **Flotation:** A gas is passed through the fermentation broth and then the foam is removed. Sometimes, collector substances (fatty acids) are added which facilitates foam formation.

3- **Flocculation:** At high cell density, some cells (yeast cells) aggregate and thus settle down at the bottom of the fermentors. This process can be accelerated by the addition of flocculating agent like salts, organic polyelectrolyte and mineral hydrocolloid.

4- **Filtration:** It is the most common type of cell separation technique.

Examples on filters:

- **Depth filters:** for the separation of filamentous fungi.
- **Asbestos filters:** for the separation of bacteria.
- **Rotary drum vacuum filters:** for the separation of yeast cells.

5- **Centrifugation:** This is a process of separating cells from the liquid based on the differences in their density.

II. Lyses/breakage of cells

If the desired product is located inside the cell, the cells are first recovered from the fermentor by any of the cell harvesting methods and then the cells must be broken.

Methods for breaking the cells:

- **Physical methods:**
  1. **Ultrasonication:** The cells are disrupted by passing ultra-waves through samples. This technique is ideal in laboratory where sample size is small.

  2. **Osmotic shock:** The cells are suspended in a viscous solution like 20% (w/v) sucrose or glucose. The cell suspension is then transferred to the cold water (4°C) which results in cell lyses.

  3. **Heat shock (Thermolysis):** The cells are exposed to heat which results in disintegration of the cells. It is an economical method but the product has to be heat stable.

4. **Freeze-Thaw method**: It is commonly used to lyse bacterial and mammalian cells. This method of lysis causes cells to swell and ultimately break as ice crystals form during the freezing process and then contract during thawing.

5. **High pressure homogenization:** The cell suspension is forced to pass through a narrow pore at a high pressure which results in breakage of cells.

6. **Grinding with glass beads:** The cell suspension containing glass beads is subjected to a very high speed in a vessel. The cells break as they are forced against the walls of the vessel by the beads.

- **Chemical methods:**

1- **Detergents:** disrupt the structure of cell membranes by solubilizing their phospholipids disrupting lipid:lipid, lipid:protein and protein: protein interactions. These chemicals are mainly used to rupture mammalian cells.

2- **Organic solvents:** mainly act on the cell membrane by solubilizing its phospholipids and by denaturing its proteins.

3- **Acid/Alkali treatment:** It is the easiest and least expensive method available in general lab. The method is fast, reliable and relatively clean way to isolates DNA from cells. It can be used for both laboratory and industrial scale.

- **Enzymatic lysis:** Bacterial cells are lysed by the addition of lysozyme. Fungal cells are lysed by the addition of chitnases, cellulases and mannases.

III. Concentration

Because more than 90% of the cell free supernatant is water and the amount of desired product is very less, the product must be concentrated.

Methods of concentration:

- **Evaporation process**: Water is evaporated by applying heat to the supernatant with/without vacuum.

- **Liquid-liquid extraction:** A desired product (solute) can be concentrated by the transfer of the solute from one liquid to another liquid. This process also results in partial purification of the product.

- **Membrane filtration:** This technique involves the use of semi-permeable membrane.

- **Membrane adsorber:** The membrane contains charged groups or ligands to which a desired product can combine specifically once the aqueous solvent, containing the product, is passed through this. The adsorbed material is then eluted using various buffers and salts.

- **Precipitation:** This is the most commonly used procedure for concentration of compounds especially proteins and polysaccharides. The agents commonly used in the process of precipitation are neutral salts (ammonium sulphate), organic solvents (ethanol, acetone, propanol), non-ionic polymers (PEG) and ionic polymers (polyacrylic acid, polyethylene amine).

IV. Purification by:

- **Chromatography:** It is a procedure for separating molecules based on their sizes, charge, hydrophobicity and specific binding to ligands.

The chromatography techniques:

❖ **Gel Filtration chromatography (size exclusive chromatography):** The matrix is made up of tiny beads having many pores in them. Many types of beads are available having different porosity. Small molecules enter the beads whereas large molecules cannot enter and therefore come out of the column first. By this technique protein of variable sizes can be purified (Fig.6.2).

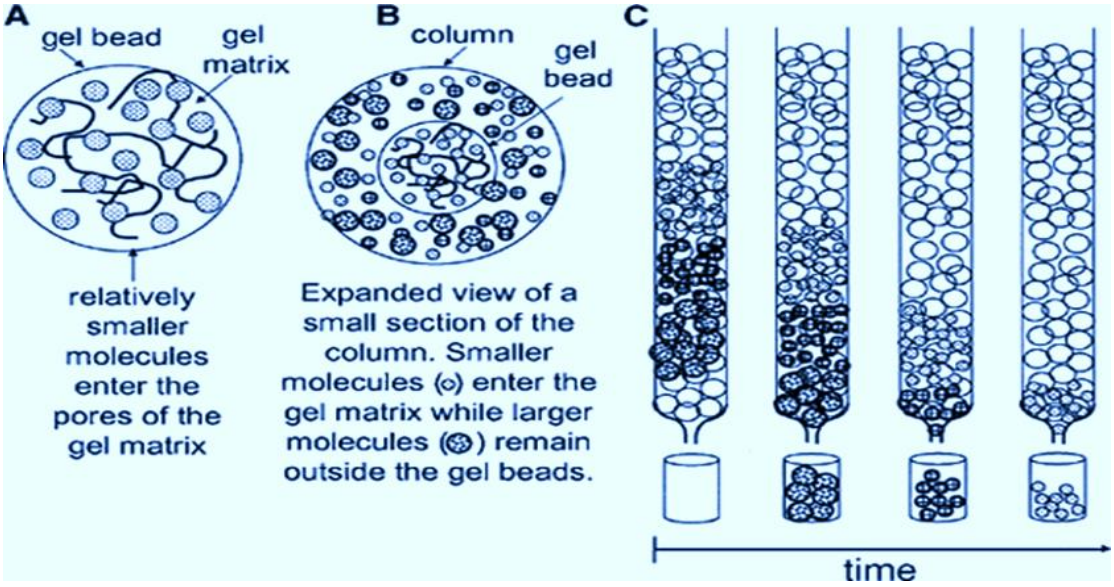


Fig. 6.2: Schematic representation the principle of gel filtration chromatography

❖ **Ion exchange chromatography:** Most of the proteins have a net positive or negative charge. This property of the proteins is exploited for the purification of proteins by passing protein solutions through columns of charged resins. Two types of resins are used in the industry:

- Cation exchangers (carboximethyl cellulose) have negative charged groups.
- Anion exchangers (diethyl aminoethyl) have positive charged groups.

Proteins carrying net positive charge bind to cation exchangers whereas proteins carrying net negative charge bind to anion exchangers (Fig.6.3).

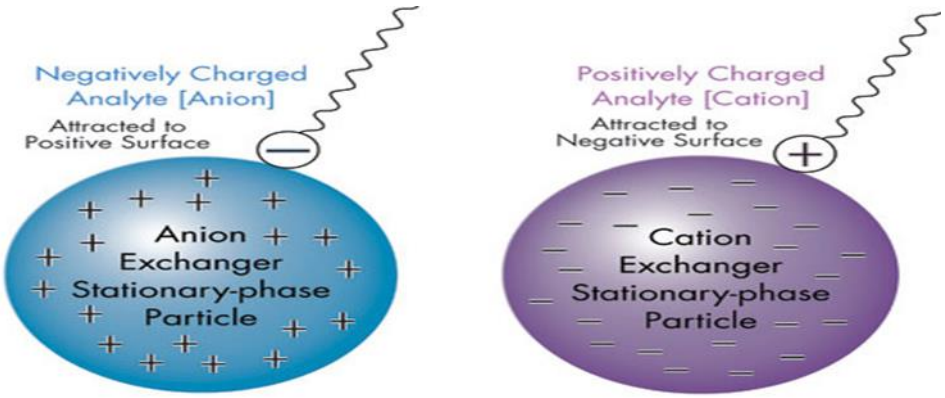


Fig. 6.3: Ion Exchange Chromatography Principle

❖ **Affinity chromatography:** the proteins are separated based on their affinity for a product compound i.e. ligand. Once the protein is bound to the affinity matrix, it is eluted by changing the pH of the eluting buffer or alteration of ionic strength etc (Fig.6.4).

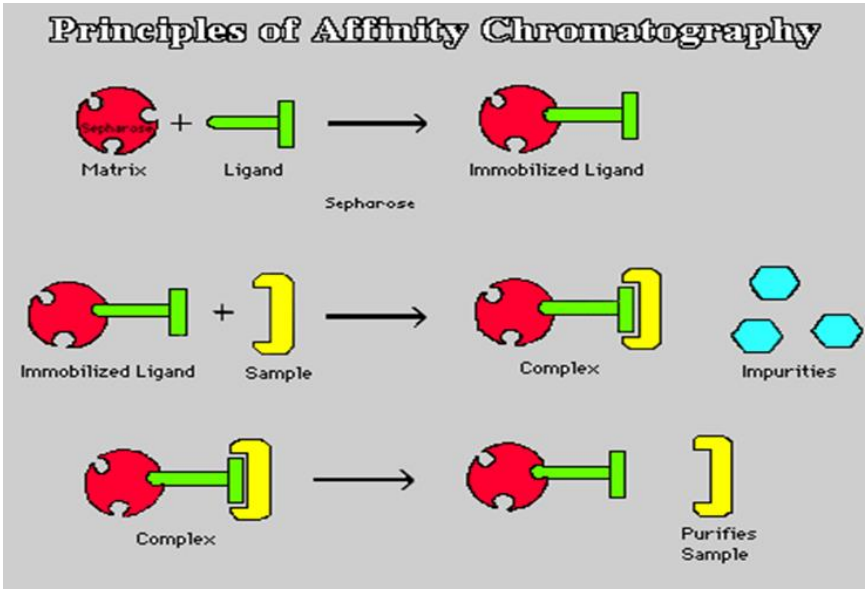


Fig. 6.4: Cartoon illustration of steps for affinity chromatography

- **Crystallization**: It uses mainly for purification of low molecular weight products, such as antibiotics and organic acids.

V. Formulation

It is a common practice to formulate products as dry powders to achieve sufficient stability for the desired shelf life of it. The principle objective for any drying process is the removal of water, which is achieved either by sublimation or by evaporative drying at high temperatures and/or at low vacuum pressures.

Technologies of formulation include:

- Lyophilization
- Spray-drying
- Spray-freeze drying
- Bulk crystallization
- Supercritical fluid technology
- Vacuum drying

All these processes have several limitations.

Lecture 7: Enzyme Technology

- **Enzyme technology** is the use of isolated and purified enzymes as catalysts in the industrial process. Or the study of industrial enzymes and their uses.
- The preferable enzymes used are extracellular with no requirements for complex cofactors. Examples are proteases, cellulases, amylases and lipases gotten from bacterial or yeast cultures.

The advantages and disadvantages of using enzymes are directly related to their properties:

Advantages	Disadvantages
They are specific in their action and are therefore less likely to produce unwanted by-products	They are highly sensitive to changes in physical and chemical conditions surrounding them.
They are biodegradable and therefore cause less environmental pollution	They are easily denatured by even a small increase in temperature and are highly susceptible to poisons and changes in pH. Therefore the conditions in which they work must be tightly controlled.
They work in mild conditions, i.e. low temperatures, neutral pH and normal atmospheric pressure, and therefore are energy saving	The enzyme substrate mixture must be uncontaminated with other substances that might affect the reaction.

The use of microbial cells in fermentation process as catalyses instead of purified enzymes is associated with many disadvantages:

1. High amount of substrate will normally be converted to biomass.
2. Wasteful side-reaction will produce.
3. The condition for growth of organism may not be the same for product.
4. The isolation and purification of the products from the fermentation are bit difficult.

Types of enzymes:

- **Intracellular enzymes**, which are produced inside the cell.
- **Extracellular enzymes,** which are produced outside the cell. Most of enzymes that use in industry are extracellular enzymes.

Table comparing intra- and extra-cellular enzymes:

Extracellular enzymes	Intracellular enzymes
Easier to isolate	More difficult to isolate
No need to break cells – secreted in large amounts into medium surrounding cells	Cells have to be broken apart to release them
Often secreted on their own or with a few other enzymes	Have to be separated out from cell debris and a mixture of many enzymes and other chemicals
More stable	Often stable only in environment inside intact cell
Purification/downstreaming processing is easier/cheaper	Purification/downstreaming processing is difficult/expensive

Uses of enzymes

Depending on the applications of enzymes, they are grouped into four broad categories:

1. Therapeutic uses

Enzyme	source	Application
streptokinase	*Streptococcus pyogenes*	Removal of fibrin clots
L-asparaginase	*E.coli*	Cancer chemotherapy
L-glutaminase	*Achromobacter* spp.	Treatment of leukemia
β-galactosidase	*Lactobacilli* spp.	Treatment of lactose intolerance

2. Analytical uses

Enzyme	source	Application
Glucose oxidase	*Aspergillus niger*	Detection of glucose in blood
urease	Jack beans	Measurement of urea in body fluids

3. Manipulative uses

Enzyme	source	Application
lysozyme	Hen egg white	disrupts mucopeptide of bacterial cell walls
nuclease	bacteria	genetic manipulation

4. Industrial uses

- **The industrial use of enzymes (using the whole microbe)**

Historically, three examples of the industrial use of microbes (and their enzymes) are:

Industry	Microbe
Brewing and baking	*Saccharomyces cerevisiae*
Vinegar production	*Acetobacter*
Yoghurt production	*Lactobacillus*

- **The industrial use of enzymes (not using the whole microbe)**

Enzyme	source	Application
alpha-amylase	*Bacillus* spp.	Conversion of starch to glucose or dextrans in food industry
proteases	*Bacillus* spp.	Laundry aid
Glucose isomerase	*Streptomyces* spp.	Production of high fructose syrups
rennin	bacteria	Cheese making

The detergent industry has been the largest market for industrial enzymes for over 25 years, accounting for 37% of world sales of enzymes. Today more than 90% of detergent enzymes are made from genetically modified organisms (GMOs).

Technology of enzyme production

Many useful enzymes have been derived from plant and animal sources. The moderate enzyme technologies dependent on the microbes to produce the enzymes instead of plants and animals, the reasons for that are:

- High specific activity of produced enzyme.
- Seasonal fluctuation of raw materials and possible shortage due to climatic change do not occur.
- In microbes a wide spectrum of enzyme features such as resistance to high pH and temperature.
- Genetic engineering has greatly increased the possibility for optimizing enzyme yield. Through mutation, induction and selection of growth conditions. Moreover, using the innovative power of gene transfer technology and protein engineering (Fig.7.1). These techniques applied easily in microbes as comparing with plants or animals.

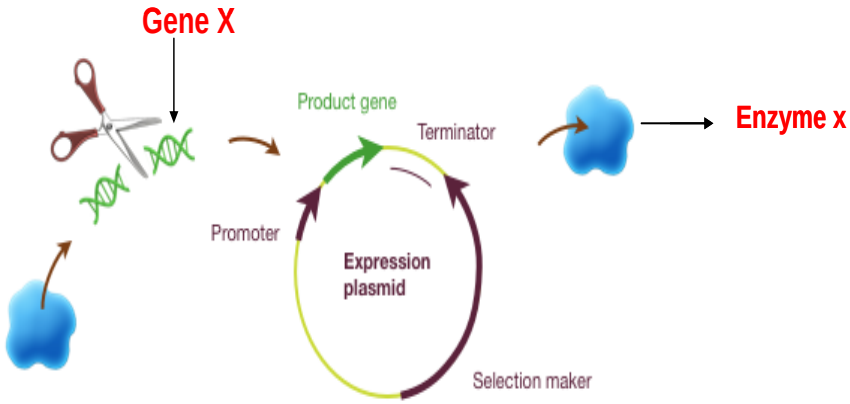


Fig.7.1: Recombinant expression-transferring beneficial enzyme gene to an efficient enzyme-producing organism

In recent years advanced technology has brought about major changes in the technology of enzyme to get the following points:

1. Improving the enzyme activity particularly in extreme environmental conditions.
2. Increasing the enzyme stability.
3. Changing the optimal pH and temperature of enzyme activity.
4. Modifying the specificity of enzymes (catalyses different materials).

Production of enzymes:

- The raw materials are preferable in enzyme production as they are very cheap.
- Industrial enzyme produced from microorganism relies on either;
    - Submerged liquid, is preferable because easier to supply energy and minerals.
    - Solid substrate fermentation.
- At the completion of the fermentation the enzyme may be presented within the microorganism or excreted into the medium. The commercial enzyme preparation for sale will be either solid or liquid form, crude or purified.
- All produced enzymes that use in different field are required to meet toxicity test before sale.
- **The main stages of enzyme production are (Fig.7.2):**

1- Induction
2- Production
3- Extraction
4- Purification
5- Standardization
6- Packaging

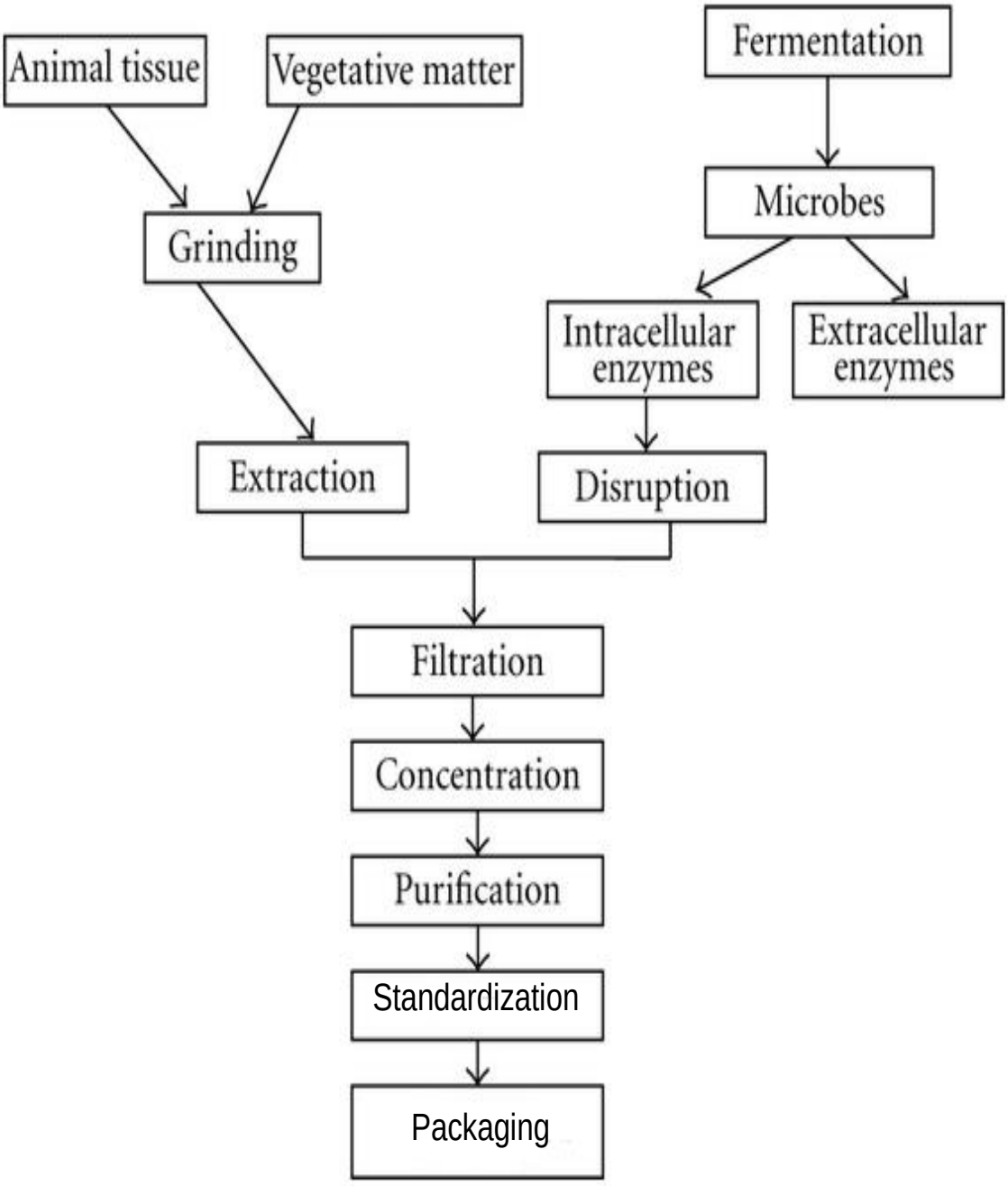


Fig. 7.2: Main stages of enzyme production

Lecture 8: Cell and enzyme immobilization

The immobilization of whole cells or enzymes is the physical localization of intact cells or enzymes to a certain region of space without loss of desired biological activity.

Immobilized enzyme:

An enzyme fixed by physical or chemical means to a solid support to confine a reaction of interest to a particular site.

Advantages and disadvantages of immobilization

Advantages of immobilisation
1. Easier to separate enzyme and products
2. Allows catalysis in unfavourable media
3. Increases stability and can be manipulated easily
4. Allows continuous production/enzyme used for longer
5. Enzyme can be recovered and reused
6. Enzyme does not contaminate product/no purification required

Disadvantages of immobilisation
1. Immobilisation may alter shape of enzyme
2. May alter catalytic ability
3. Enzyme may become detached
4. Expensive

Advantages of using whole cells instead of enzymes:

1. To avoid enzyme extraction and purification steps and their consequences on enzyme activity.
2. High stability and low cost.
3. Wider scope of reactions is possible including multi-step reactions utilizing several enzymes.

Immobilization Methods

The methods of immobilization available are equally applicable to cell and enzyme. Physical and chemical methods are used for enzyme immobilization (EI) (Fig.8.1).

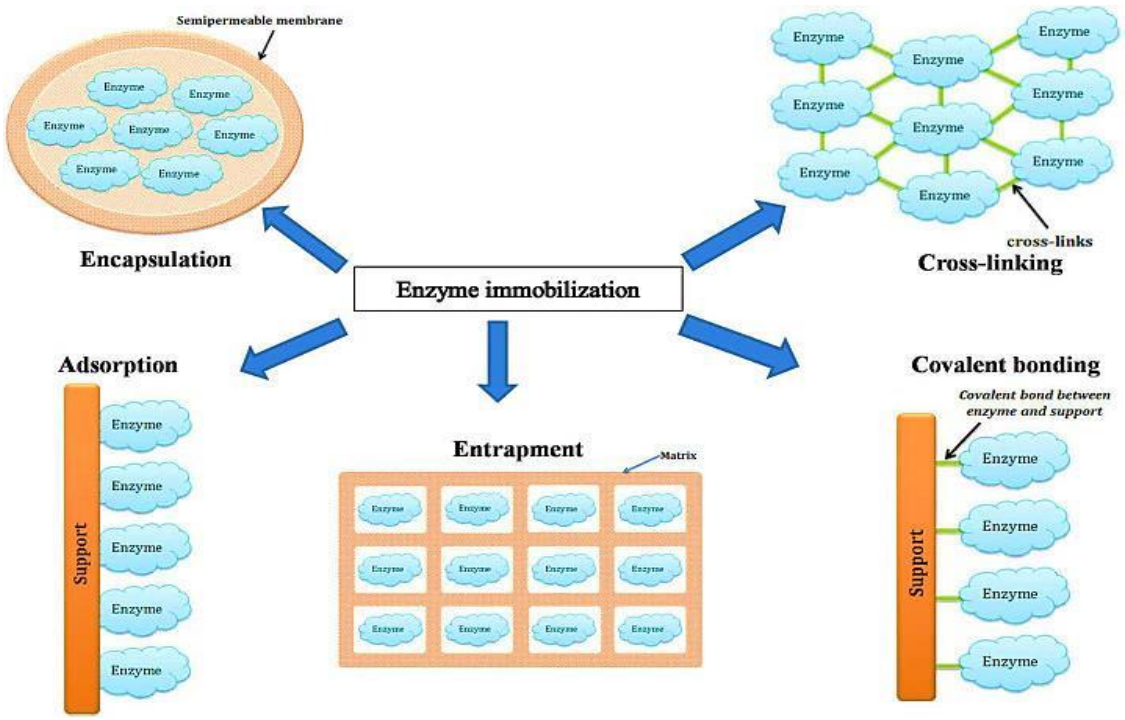


Fig. 8.1: Enzyme immobilization methods

1. Physical methods

Enzyme may be attached onto an insoluble matrix, entrapped within gel or encapsulated within microcapsule or behind a semi-permeable membrane.

- **Adsorption:** The adsorption of cell to organic or inorganic support material is achieved to vander waals forces, ionic interaction, hydrophobic interaction and H-bound (Fig.8.2).

Advantages:

1- Simple and cheap technique
2- Different types of support matrix can be used (DEAE cellulose and carboxy methyl cellulose).

Disadvantages: Desorption of the enzyme resulting from changes in temperature, pH, and ionic strength.

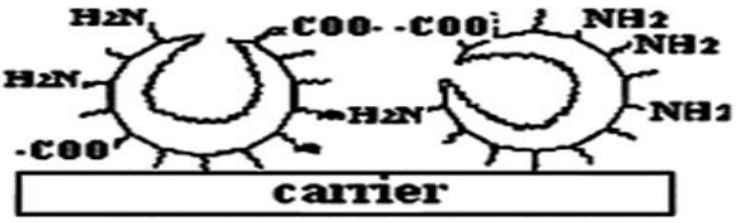


Fig. 8.2: Immobilization of enzyme by adsorption

- **Encapsulation:** Enzyme and cells immobilization in semi-permeable membranes (Fig.8.3), which permits the transport of nutrients from medium to the cells and remove the products. The porosity of the membrane is variable according to the size of products, small pores in case of glucose and large pores in case of antibodies. This immobilization technique is preferable for the animal cells or human cells.

Advantages: do not need to chemical to immobilize the cells.

Disadvantages: bit expensive and required to professional technicians.

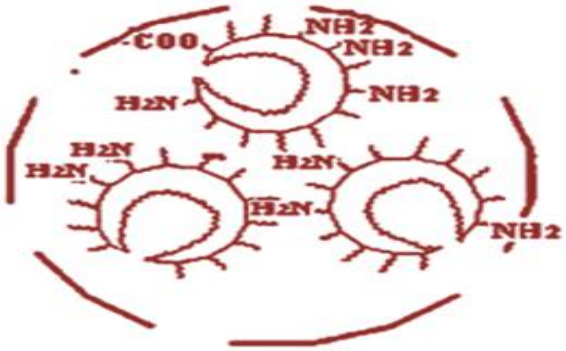


Fig. 8.3: Immobilization of enzyme by microencapsulation

- **Entrapment:** Cell entrapment can be achieved through immobilization in the presence of porous matrix (gel entrapment) or by allowing the cells to move into performed porous matrix (Fig.8.4).

  A wide variety of natural polymers (collagen, gelatin, agar, alginate, agarose and chitin) and synthetic polymers (polyacrylamide) can be gelled into hydrophilic matrices under mild conditions to allow cell entrapment with minimal loss of viability.

Advantages:

- Simple method
- No chemical modification of enzyme will be occurred

Disadvantages:

1- Expensive.
2- Gel structure is easily destroyed by cell growth in the gel matrix and CO_2 production. However, gel can be reinforced i.e. alginate gel was made strongly by the reaction with other molecules like silica.

3- Oxygen limitation in the matrix.
4- Continuous loss of enzyme due to distribution of pore size.

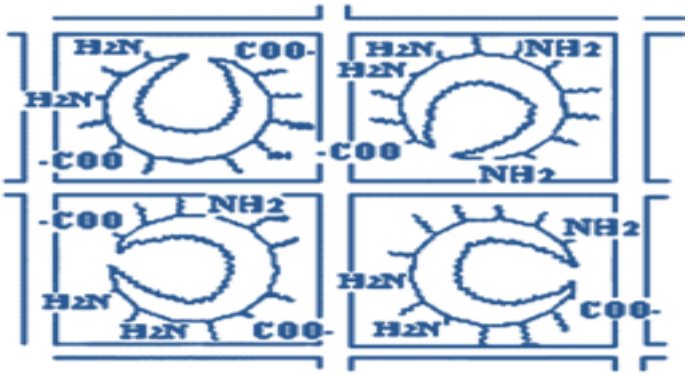


Fig. 8.4: Immobilization of enzyme by entrapment

2. Chemical methods:

Enzymes may be covalently attached to solid supports or cross linked.

- **Covalent attachment :** A large number of chemical reactions have been used for covalent binding of enzyme by way their non-essential functional groups to inorganic carriers (ceramics, glass, iron), natural polymers (sepharose and cellulose) and synthetic polymers (nylon and polyacrylamide) (Fig.8.5).

Advantages:

- Not affected by pH
- The strength of binding is very strong

Disadvantages:

- Active site may be modified.
- Cost process.

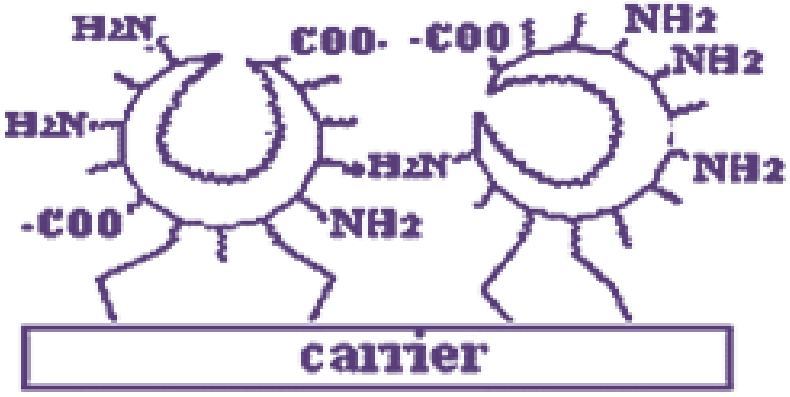


Fig. 8.5: Immobilization of enzyme by covalent attachment

- **Covalent cross-linkage:** Enzyme and microbial cells can be immobilized by cross-linking them with bi or multi-functional reagents such as glutaraldehyde (Fig.8.6).

Advantages: Enzyme strongly bound.

Disadvantages: loss of enzyme activity during preparation.

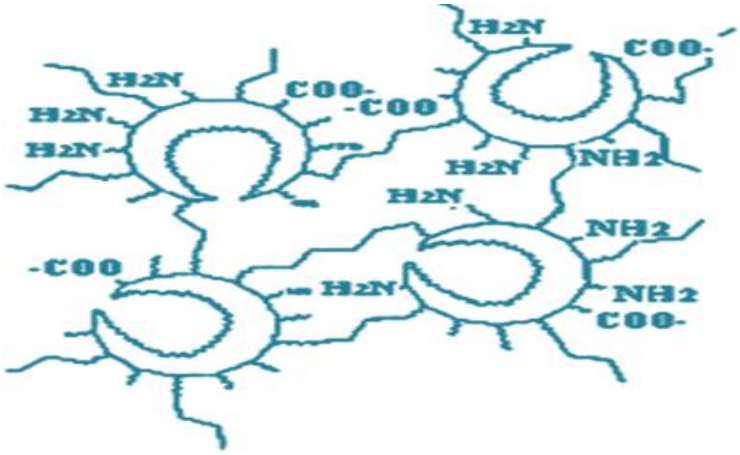


Fig. 8.6: Immobilization of enzyme by covalent cross-linkage

Applications of immobilization technique

Immobilization of enzymes/cells has found varied applications in industry:

- Food industry, the conversion of glucose to high concentration fructose syrups by the enzyme glucose isomerase.
- Pharmaceutical industry, the production of 6-amino penicillanic acid or 7-amino deacetoxy cephalosporanic acid.
- Synthesis and modification of peptides, immobilized proteases.
- Leather industry, immobilized proteases.
- Biosensors, immobilized glucose oxidase and peroxidase enzymes as detection systems for serum glucose levels. Urease for detection of uric acid levels while cholesterol esterase and cholesterol oxidase have been used for detection of serum cholesterol.

Lecture 9: Biosensors

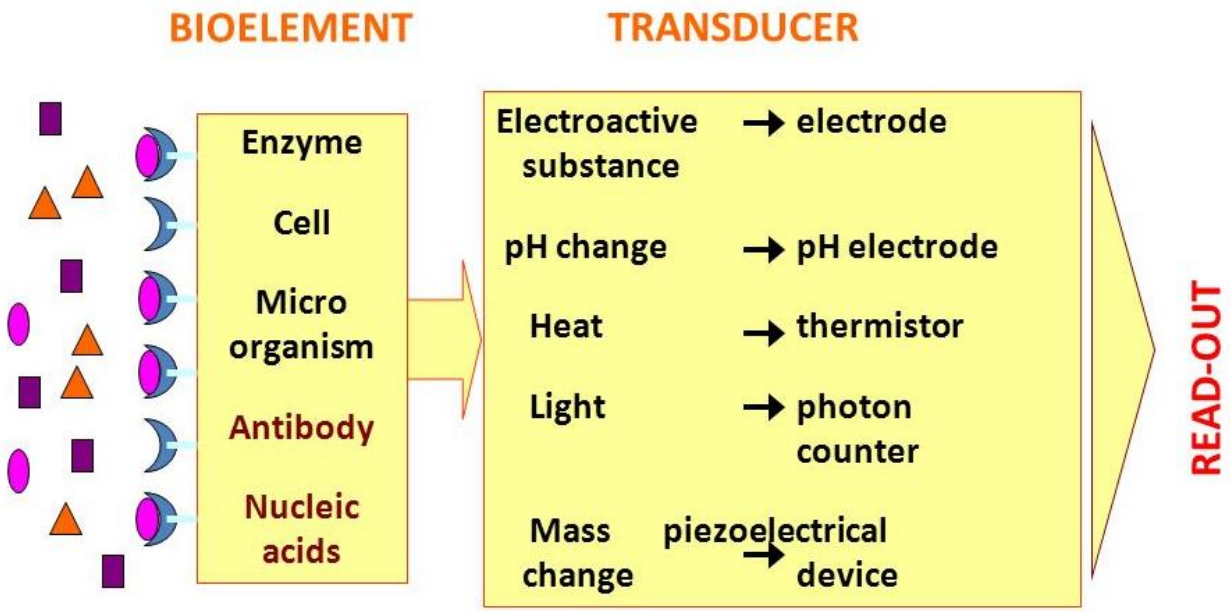
A biosensor is an analytical device for the detection of an analyte that combines a biological component with a physicochemical detector component.

Biosensor consists of 3 parts:

A- The biological recognition elements that differentiate the target molecules in the presence of various chemicals.

B- A transducer that converts the biorecognition event into a measurable signal.

C- A signal processing system that converts the signal into a readable form (figure below).



Biosensors or sensor based on biological material, are now used in a wide variety of disciplines, including medicine, food industry and environmental science.

Biological elements

The main types of recognition (Biological) element used are enzymes and antibodies. In some cases nucleic acid or whole living cell usually bacteria can be used. Also, organelles, cell receptors, and a biologically derived material or biomimic can be used.

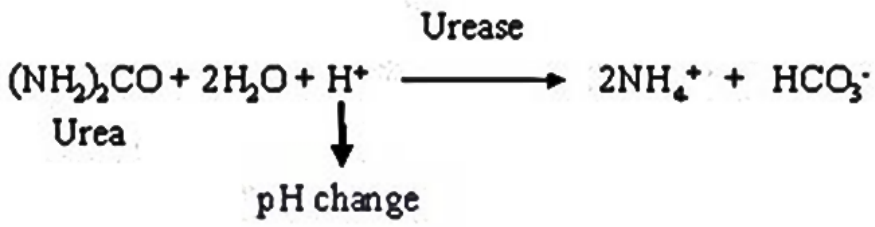
A- Enzyme as biological detection element:

These may be used in a purified form, or may be present in microorganisms. They are biological catalysts for particular reaction and can bind themselves to the specific substrate.

1- Glucose biosensor: are based that the enzyme glucose oxidase (entrapped in polyacrelamid) catalyses the oxidation of glucose to gluconic acid. The consumption of oxygen was followed by electrochemical reduction at platinum electrode, as in oxygen electrode.

The substrate (glucose solution) and O_2 can penetrate the first membrane to reach the enzyme to form product. The concentration of glucose is proportional to the decrease of O_2 concentration.

2- Urease sensors: The hydrolytical breakdown of urea is catalysed by the enzyme urease to give ammonia and carbon dioxide.



B- Tissue materials as biological detection element:

Very simple biosensors can be made using a banana. It was described for the determination of dopamine, an important brain chemical.

Many experiments have been conducted by implanting electrodes in living animal brain to monitor the change in dopamine levels with various activities.

C- Microorganisms as biological detection element:

A microbial biosensor is an analytical device that couples microorganisms with a transducer to enable rapid, accurate and sensitive detection of target analytes in fields as diverse as medicine, environmental monitoring, defense, food processing and safety.

Advantages:

1- They are cheaper source of enzyme than isolated enzyme.
2- They are less sensitive to inhibition by solutes and more tolerant of pH changes and temperature changes.
3- They have longer life time.

Disadvantages:

1- They sometime have longer response time.
2- They have longer recovery times.
3- Like tissues they contain many enzymes and so may have less selectivity.

D- Antibodies as biological detection element (immune-sensors):

Organisms develop antibodies (Abs) which are protein that can bind with an invading antigen (Ag) and remove it from harm.

Advantages:

1- They are very selective.
2- They are ultra-sensitive.
3- They bind very powerfully.

An example is the determination of chorionic acid gonadotropin (HCG) using catalase-labeled HCG.

E- Nucleic acid: Have been much less used so far. They operate selectivity because of their base-pairing characteristic.

Transducers

1. **Electrochemical:** translate a chemical event to an electrical event, such as; Amperometric (most common), Potentiometric, and Conductimetric transducers.

2. **Photochemical (Optical):** translate chemical event to a photochemical event, such as; Colorimetric, Fluorescence, and Reflectance transducers.

3. **Piezoelectric:** translate a mass change from a chemical adsorption event to electrical signal. These are affinity biosensors.

Classes of biosensors

- **Catalytic biosensors:** Biological elements are enzymes (most common), microorganisms, organelles and tissue samples.
- **Affinity biosensors:** Biological elements are antibodies, nucleic acids and hormone receptors.

Ideal Biosensor Characteristics:

1. Sensitivity
2. Simple calibration (with standards)
3. Linear Response
4. Background Signal: low noise, with ability for correction
5. No hysteresis
6. Selectivity
7. Long-term Stability
8. Dynamic Response
9. Biocompatibility

Applications of Biosensor:

1. Clinical diagnosis and bio medicine.
2. Farm, garden.
3. Process control; Fermentation control and analysis.
4. Food and drink production and analysis.
5. Microbiology; Bacterial and viral analysis.
6. Pharmaceutical and drug analysis.
7. Industrial effluent control.
8. Pollution control and monitoring.
9. Mining, industrial and toxic gasses.
10. Military application.

Lecture 10: Plant and Animal biotechnology

Plant tissue culture: is the growth of explant (any plant part) or plant cells *in vitro* (in the laboratory culture media).

- Plant cell culture is based on the unique property of the cell-totipotency.
- Cell-totipotency is the ability of the plant cell to regenerate into whole plant. This property of the plant cells has been exploited to regenerate plant cells under the laboratory conditions using artificial nutrient mediums.
- Gottlieb Haberlandt, the German botanist is regarded as the father of plant tissue culture.

Stages of plant tissue culture

1. **Initiation stage:** A piece of plant tissue (called an explant) is;

(a) cut from the plant
(b) disinfested (removal of surface contaminants)
(c) placed on a medium.

- The objective of this stage is to achieve an aseptic culture. An aseptic culture is one without contaminating bacteria or fungi.

2. **Multiplication stage:** A growing explant can be induced to produce vegetative shoots by including a cytokinin in the medium.

- **A cytokinin** is a plant growth regulator that promotes shoot formation from growing plant cells.

3. **Rooting or preplant stage:** Growing shoots can be induced to produce adventitious roots by including an auxin in the medium.

- **Auxins** are plant growth regulators that promote root formation.

4. **Acclimatization:** A growing, rooted shoot can be removed from tissue culture and placed in soil. When this is done, the humidity must be gradually reduced over time because tissue-cultured plants are extremely susceptible to wilting.

Types of cultures

- Organ Culture
- Explant culture
- Callus culture
- Cell suspension cultures

- Protoplast culture
- Embryo culture
- Anther and Pollen Culture

Some Applications of Cell and Tissue Culture

1. Micropropagation /Clonal Propagation

- Clonal propagation is the process of asexual reproduction by multiplication of genetically identical copies of individual plants.
- Micropropagation is the tissue culture methods of plant propagation.
- The micropropagation is rapid and has been adopted for commercialization of important plants such as banana, apple, and other plants.

2. Production of virus free plants

It has become possible to produce virus free plants through tissue culture at the commercial level. Among the culture techniques, meristem-tip culture is the most reliable method for virus and other pathogen elimination.

3. Production of secondary metabolites

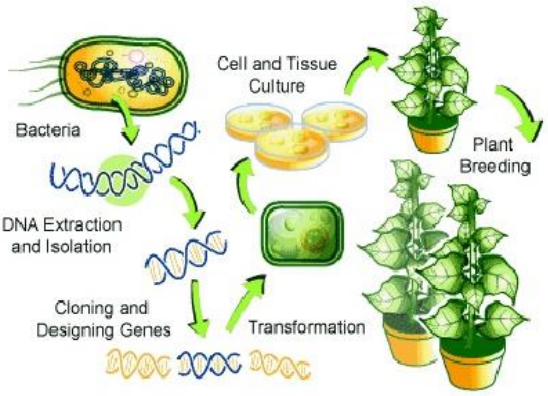
The most important chemicals produced using cell culture is secondary metabolites (Some examples in the table below). These secondary metabolites include alkaloids, glycosides (steroids and phenolics), terpenoids, latex, tannins etc.

Product	Plant source	Uses
Artemisin	*Artemisia spp.*	Antimalarial
Capsaicin	*Capsicum annum*	Cures Rheumatic pain
Taxol	*Taxus spp.*	Anticarcinogenic

Transgenic plants with beneficial traits

- Transgenic plants or transgenic crops are the plants, in which a functional foreign gene has been incorporated by any biotechnological methods that generally are not present in the plant.
- Transgenic plants have many beneficial traits like insect resistance, herbicide tolerance, delayed fruit ripening, improved oil quality, weed control etc.
- The main goal of producing transgenic plants is to increase the productivity.

Making Transgenic Crops



Steps

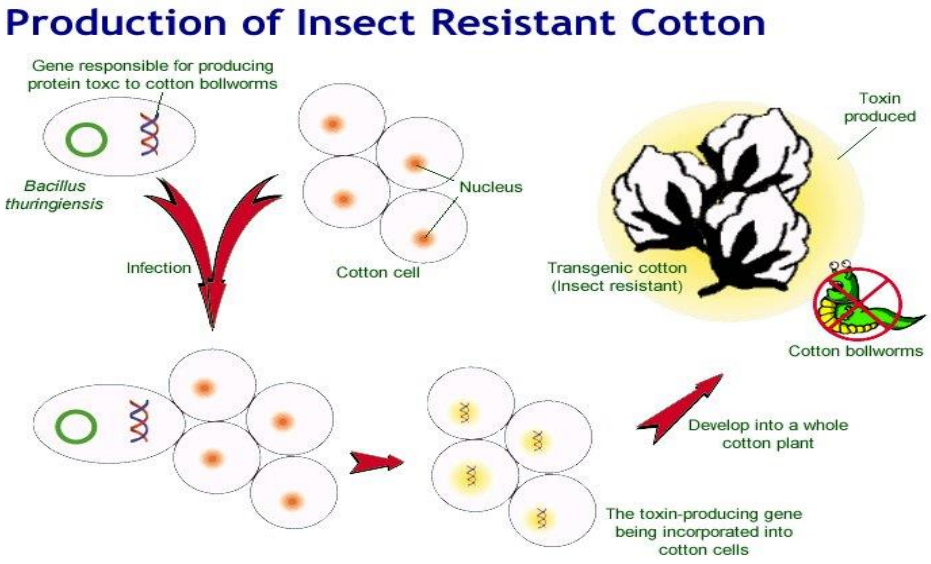
2. extracting DNA
3. cloning a gene of interest
4. designing the gene for plant infiltration
5. transformation
6. plant breeding

- Biotechnology strategies are being developed to overcome problems caused due to biotic stresses (viral, bacterial infections, pests and weeds) and abiotic stresses (physical actors such as temperature, humidity, salinity etc).

Some of the traits introduced in these transgenic plants:

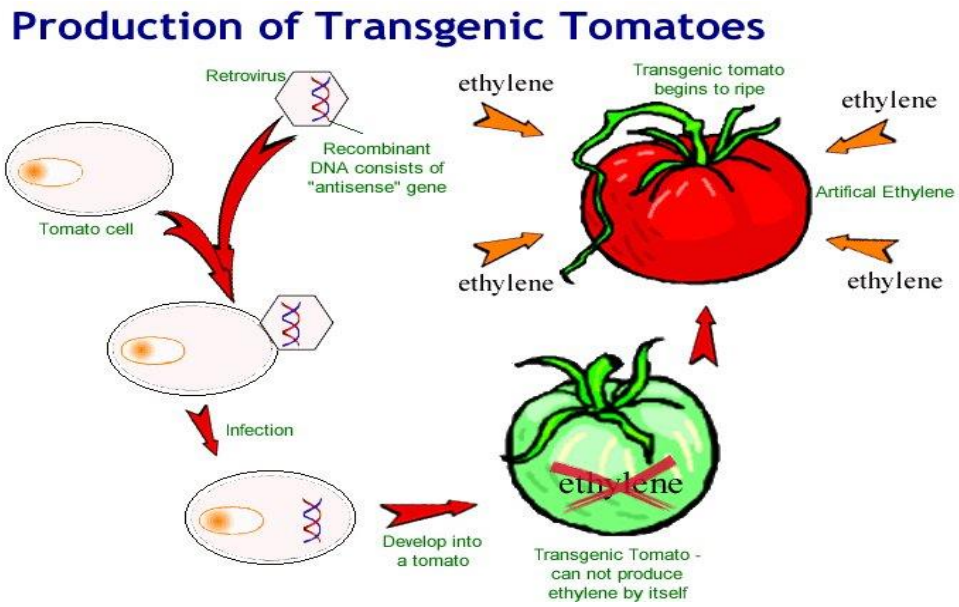
Insect resistance

- The transgenic technology uses an innovative and eco-friendly method to improve pest control management.
- The first genes available for genetic engineering of crop plants for pest resistance were Cry genes (popularly known as Bt genes) from a *bacterium Bacillus thuringiensis*. These are specific to particular group of insect pests, and are not harmful to other useful insects like butter flies and silk worms.



Delayed fruit ripening

The gas hormone, ethylene regulates the ripening of fruits, therefore, ripening can be slowed down by blocking or reducing ethylene production. This can be achieved by introducing ethylene forming gene(s) in a way that will suppress its own expression in the crop plant.



Transgenic plants as bioreactors (molecular farming)

Plants can serve as bioreactors to modified or new compounds. The transgenic plants as bioreactors have some advantages such as:

- The cost of production is low
- There is an unlimited supply
- Safe and environmental friendly
- There is no scare of spread of animal borne diseases

Tobacco is the most preferred plant as a transgenic bioreactor because it can be easily transformed and engineered.

Some of the uses of transgenic plants are:

- Improvement of Nutrient quality
- Improvement of seed protein quality
- Diagnostic and therapeutic proteins
- Edible vaccines
- Biodegradable plastics

Animal tissue culture: is the growth of tissues separate from the animal *in vitro* (in the laboratory culture media).

Types of cell cultures:

A. Primary cell culture

The maintenance of growth of cells dissociated from the parental tissue in culture medium using suitable glass or plastic containers is called Primary Cell Culture. There are two types of it:

1. **Monolayer cultures or Adherent cells (Anchorage Dependent);** Cells shown to require attachment for growth. They are usually derived from tissues of organs such as kidney.
2. **Suspension Culture (Anchorage Independent cells);** Cells which do not require attachment for growth. They are derived from cells of the blood system.

Advantages in propagation of cells by suspension culture method:

- The process of propagation is much faster.
- The frequent replacement of the medium is not required.
- Have a short lag period.
- Treatment with trypsin is not required.
- A homogenous suspension of cells is obtained.
- The maintenance of them is easy and bulk production of the cells is easily achieved.
- Scale-up is also very convenient.

B. Secondary cell cultures or cell line

When a primary culture is sub-cultured, it becomes known as secondary culture or cell line. Subculture (or passage); is the transfer of cells from one culture vessel to another culture vessel.

There are two types of Cell Line or Cell Strain:

Finite cell Lines	Continuous Cell Lines
Have a limited life span	Have unlimited life span, Exhibit heterogeneity
They grow in monolayer form	They grow in monolayer or suspension form
Exhibit the property of contact inhibition	Absence of contact inhibition
The growth rate is slow	The growth rate is rapid
Doubling time is around 24-96 hours	Doubling time is 12-24 hours

Cell line

- Every cell present in the human body is not capable of growing in laboratory, only a few types of cells can grow *in vitro* but they are neither suitable for industrial use nor for scientific purpose, why?

Because many cells die during the course of time releasing toxic substances which inhibit the activity of other live cells.
In order to avoid this problem and to achieve an exponential cell growth, the cells are converted into immortal cells called "cell line".
A tumor tissue represents a transformed cell line. The most famous and the oldest cell line is the Hela cell line.

Culture medium:
Serum is the most economical, easily available and most widely used culture medium for animal cell culture; fetal calf serum is the preferred one.

The major functions of serum as a culture medium are-to provide nutrients, hormones, growth factors, attachment and spreading factors, binding proteins, vitamins, minerals, lipids, protease inhibitors and pH buffer.

Disadvantages of serum:

- Virus, fungi and bacteria may contaminate the serum easily
- Some enzymes presents in serum can convert the cell secretions into toxic compounds

Now there are three types of artificial culture media: Serum –free culture medium, protein- free culture medium, and chemically defined media

Scale up of animal cell culture

Scaling up is the modifying a laboratory procedure, so that it can be used on an industrial scale.

Applications of animal cell culture:

1. They are used as substitute hosts to study the pattern of viral infection.
2. They are used in the manufacture of vaccines, antibodies, hormones, interferon, vitamins, steroids, pharmaceutical drugs…etc.
3. They are good tools for testing the potency of drugs.
4. They are served as models to study the metabolism of various substances.
5. They are used in study of the effects of toxins and contaminants.
6. Cancer research, which requires the study of uncontrolled cell division in cultures.
7. Cell fusion techniques.