Principles of Biotechnology

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Lecture 1:

Concepts of Biotechnology

In this lecture . . .

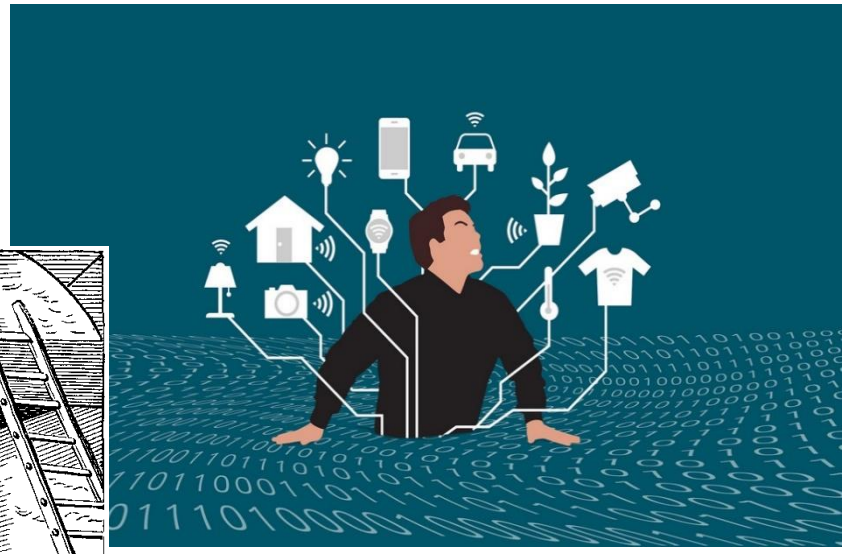
- The Philosophy of Biotechnology
- The Evolution of the Word “Biotechnology”
- Definition of Biotechnology
- Branches of Biotechnology
- The Interdisciplinary Nature of Biotechnology
- Stages of Biotechnology Development

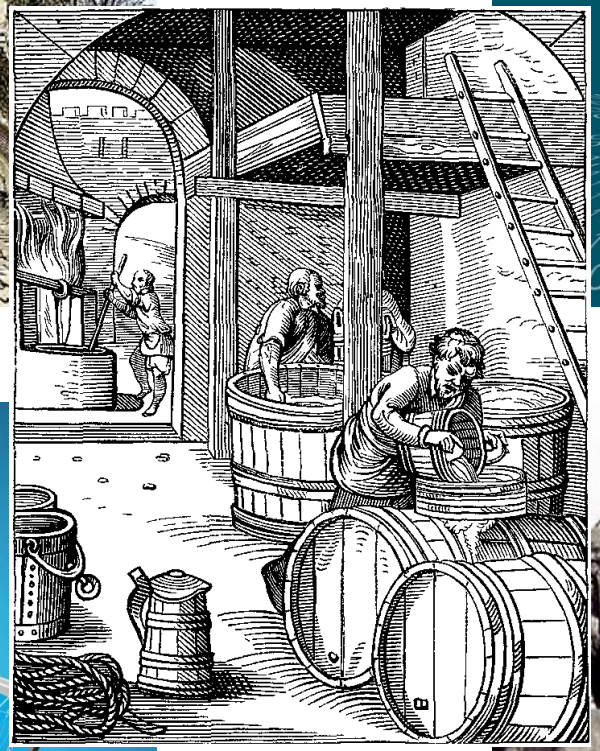
WE ARE

The Philosophy of Biotechnology

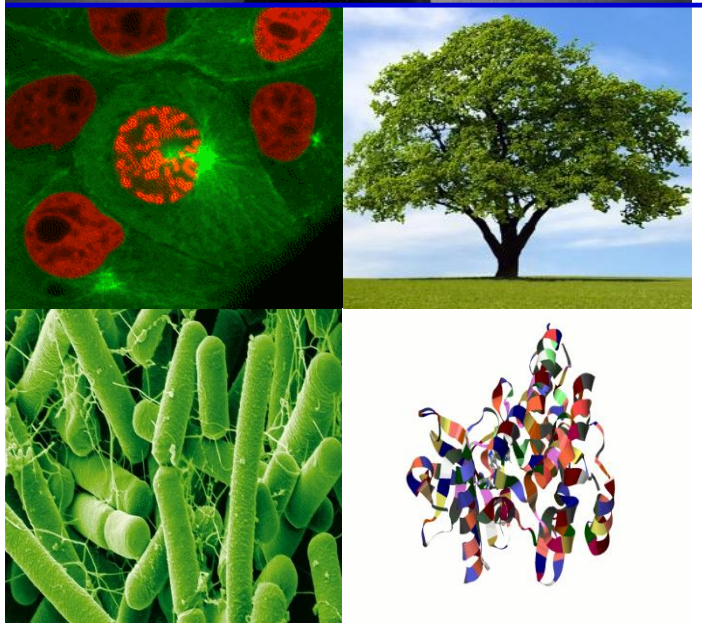
Biotechnologies are at the base of the phenomenon of ***life*** and so they are naturally present in nature. They are not invented by man, they are instead discovered while studying the phenomenon of ***life***. So biotechnologies differ from technologies.

Life™





Technologies are the result of man's intellect and creativity, which are used to invent machines and devices not present in nature, but produced to satisfy man's life needs.



The use of ***biotechnologies*** takes place using ***living*** organisms or ***parts*** of them to make large quantities of products useful to man, and this is one of the areas of high technology in great expansion and from which very interesting results for productive activities have come and are expected.

The Evolution of the Word "Biotechnology"

The word biotechnology has been "**re-developed"** at least four times over the years and its definition changed on each occasion. Robert Bud (Science Museum, London, UK) has **attributed** the first use of the word biotechnology to **Karl Ereky, a Hungarian agricultural economist**.

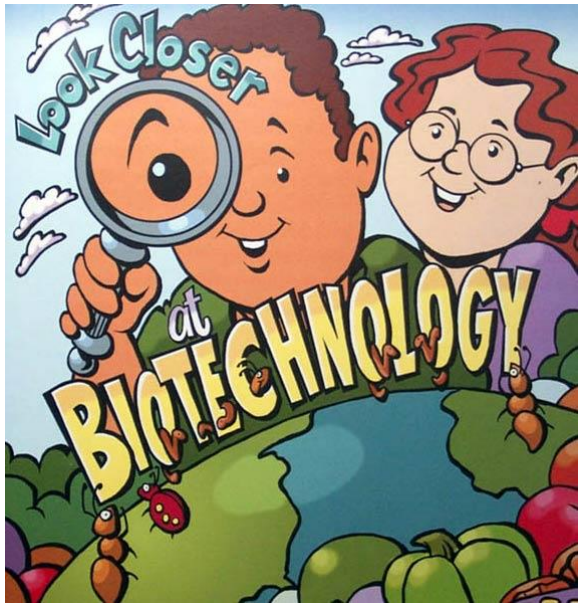
Ereky (German: Karl Ereky; 1878-1952) was a Hungarian agricultural engineer. The term "**biotechnology**" was coined by him in **1919**. He is regarded by some as the "father" of biotechnology. Ereky published in 1919 a manifesto entitled:

"Biotechnology of Meat, Fat and Milk Production in Large Scale Agricultural Enterprise".

Ereky coined his new word to cover the interaction of ***biology*** with ***technology***, connoting all **production by means of biological transformation.**

Károly Ereky (German: Karl Ereky; 1878-1952). Father of Biotechnology.

Definition of Biotechnology



Bio **technology**

means the use of biological processes

means to solve problems or make useful products

What is Biotechnology?

One simple definition is that it is:
“the commercialization of cell and molecular biology”.

Biotechnology can be broadly defined as:
"using organisms or their products for commercial purposes”.

Bake bread

Brew alcoholic beverages

Breed food crops

Domestic animals products

various definitions . . .

According to United States National Science Academy, biotechnology is the:
“Controlled use of biological agents like cells or cellular components for beneficial use”.

It covers both **classical** as well as **modern biotechnology**.

More generally, biotechnology can be defined as:
“The use of living organisms, cells or cellular components for the production of compounds or precise genetic improvement of living things for the benefit of man”.

The European Federation of Biotechnology (EFB) considers biotechnology as:
“The integration of natural sciences and organisms, cells, parts thereof, and molecular analogues for products and services”.

The EFB definition is applicable to both “**traditional** or old” and “**new or modern**” biotechnology.

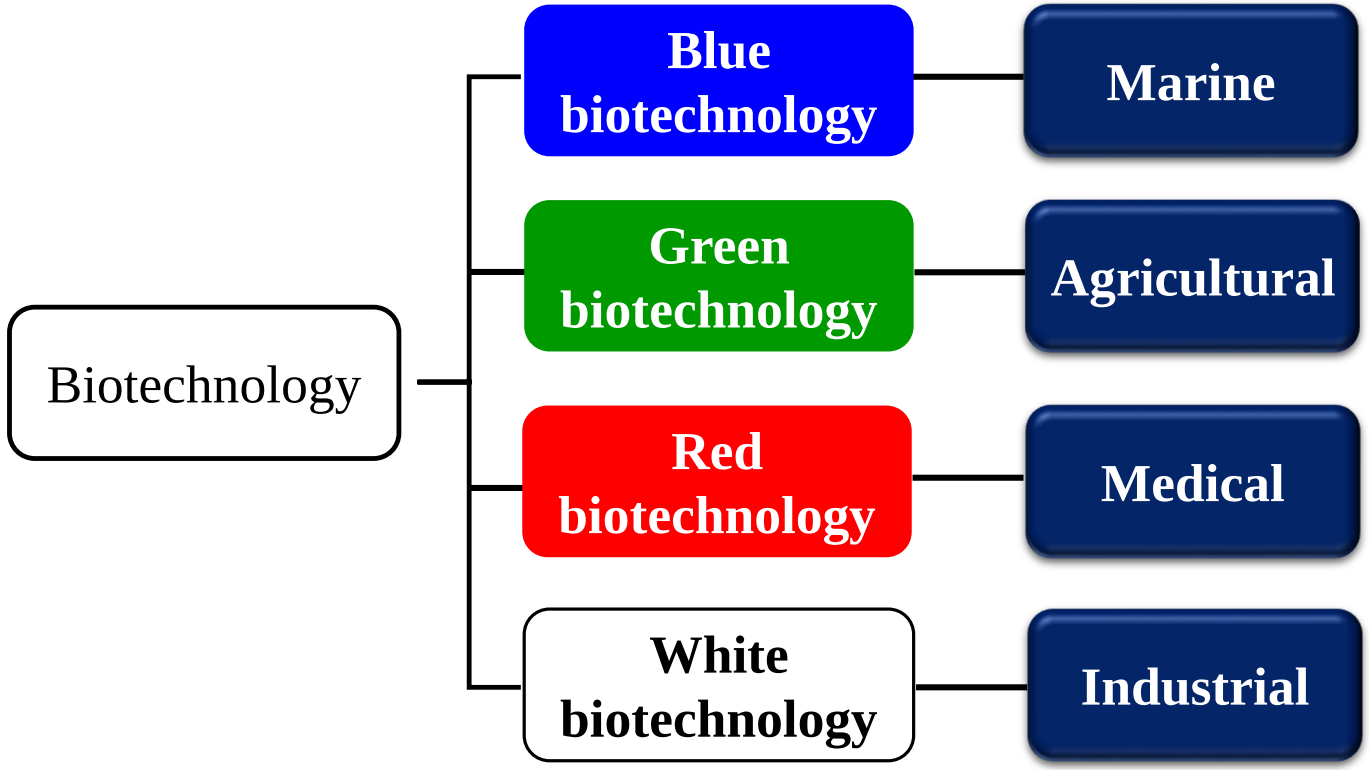
EUROPEAN FEDERATION OF BIOTECHNOLOGY

Another definition given by the **Food and Agricultural Organization (FAO)** is as follows:

1. The use of biological processes or organisms for the production of materials and services of benefit to humankind. Biotechnology includes the use of techniques for the improvement of the characteristics of economically important plants and animals and for the development of microorganisms to act on the environment.
2. The scientific manipulation of living organisms, especially at the molecular genetic level, to produce new products, such as hormones, vaccines or monoclonal antibodies.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

Branches of Biotechnology



Red biotechnology: This area includes medical procedures such as utilizing organisms for the production of novel drugs or employing stem cells to replace/regenerate injured tissues and possibly regenerate whole organs. It could simply be called **medical biotechnology**.

Green biotechnology: Green biotechnology applies to agriculture and involves such processes as the development of pest-resistant grains and the accelerated evolution of disease-resistant animals.

Blue biotechnology: Blue biotechnology, rarely mentioned, encompasses processes in the marine and aquatic environments, such as controlling the proliferation of noxious water-borne organisms.

White biotechnology: White (also called gray) biotechnology involves industrial processes such as the production of new chemicals or the development of new fuels for vehicles.

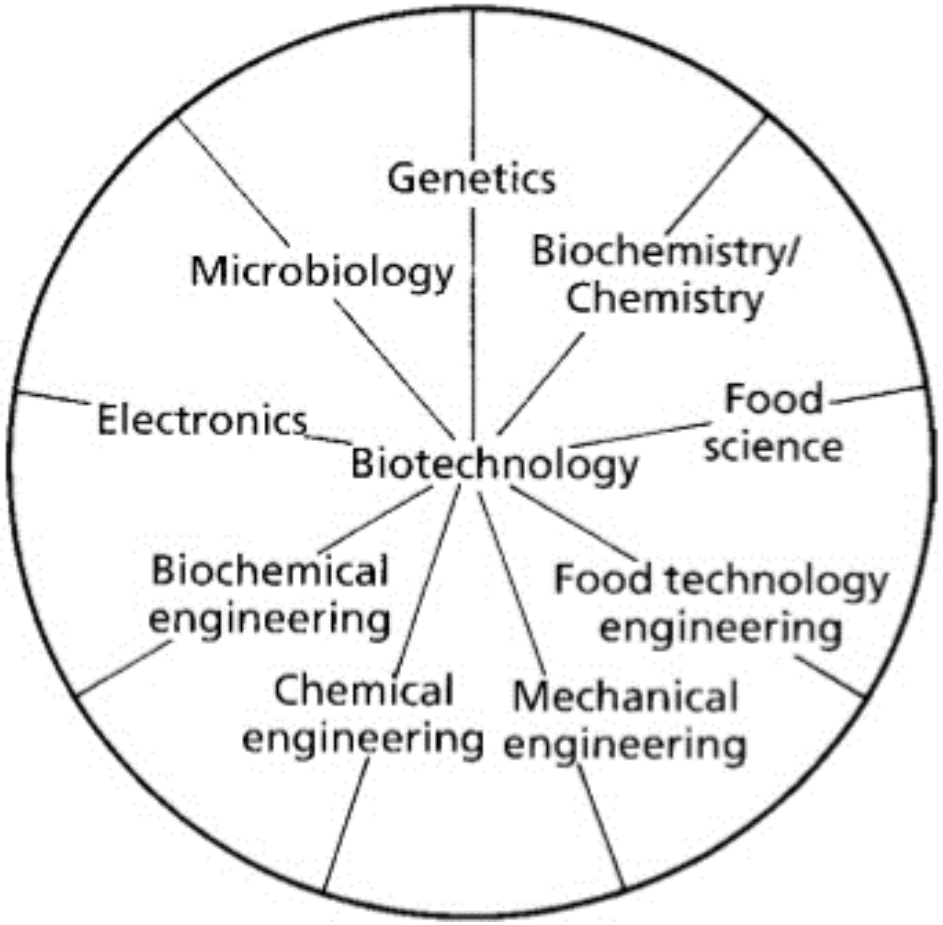
A distinction is made between “non-gene biotechnology” and “gene biotechnology”:

- Non-gene biotechnology: Non-gene biotechnology works with **whole cells, tissues or even individual organisms**. Non-gene biotechnology is the more popular practice, involving plant tissue culture, hybrid seed production, microbial fermentation.

- Gene biotechnology: Gene biotechnology deals with **genes**, the transfer of genes from one organism to another and genetic engineering.

The Interdisciplinary Nature of Biotechnology

Unlike a single scientific discipline, biotechnology can draw upon a wide array of relevant fields, such as **microbiology**, **biochemistry**, **molecular biology**, **cell biology**, **immunology**, **protein engineering**, **enzymology**, **classified breeding techniques**, and the full range of bioprocess technologies.



Stages of Biotechnology Development

Biotechnologies have been traditionally used in productive activities, in agriculture, in zootechnics and in food production in general for a long time. In prehistoric times man prepared drinks and fermented food: the **Sumerians and the Babylonians** produced wine and beer as early as 6000 B.C. and the **Egyptians** produced leavened.

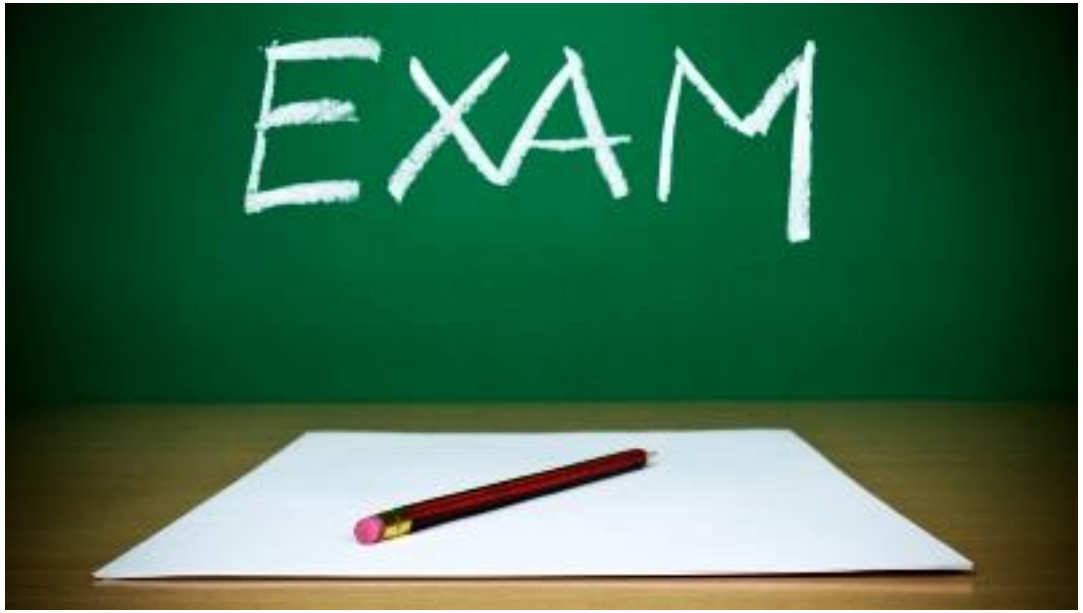
The development of biotechnology can be divided into broad stages or categories, including:

- **Ancient biotechnology** (8000–4000 BC): Early history as related to food and shelter; includes domestication of animals.
- **Classical biotechnology** (2000 BC; 1800–1900 AD): Built on ancient biotechnology; fermentation promotes food production and medicine.
- 1900–1953: Genetics.
- 1953–1976: DNA research, science explodes.
- **Modern biotechnology** (1977): Manipulates genetic information in organisms; genetic engineering; various technologies enable us to improve crop yield and food quality in agriculture and to produce a broader array of products in industries.

Among many, the two core techniques that enabled birth of **modern biotechnology** are:

i. **Genetic engineering:** Techniques to alter the chemistry of genetic material (DNA and RNA), to **introduce** these into host organisms and thus change the **phenotype** of the host organism.

ii. **Maintenance of sterile** (microbial contamination-free) ambience in chemical engineering processes to enable growth of only the desired microbe/eukaryotic cell in large quantities for the manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.



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Lecture 2

Fermentation Technologies:

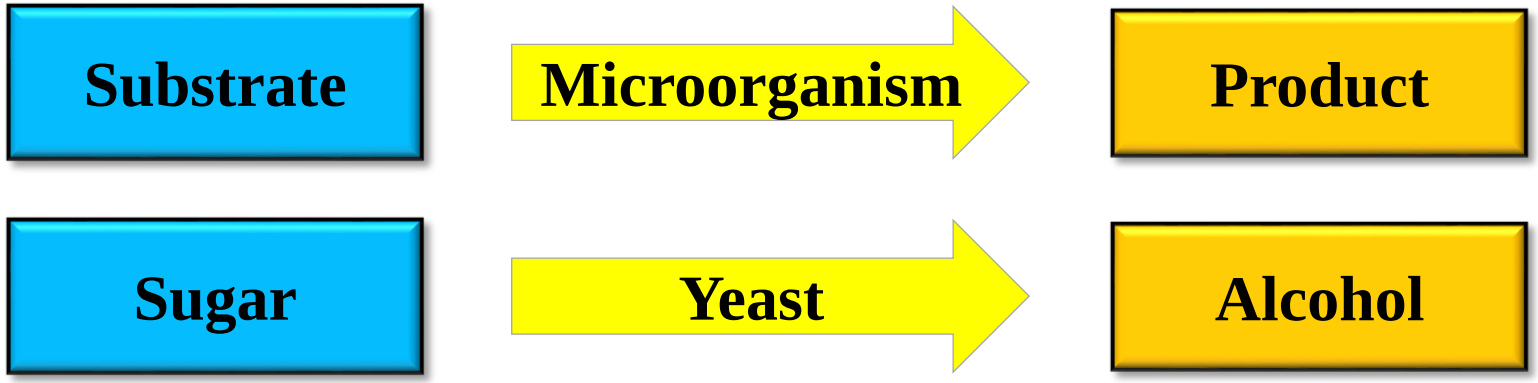
- **Liquid Fermentation**

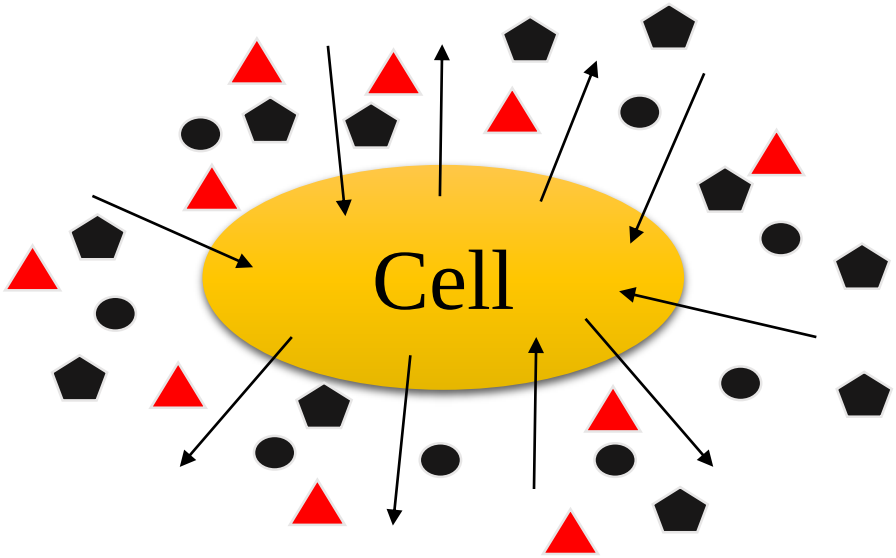
The Concept of Fermentation

Fermentation processes utilize **microorganisms** (e.g. bacteria and fungi) to convert **solid** or **liquid** substrates into various **products**.

The substrates used vary widely, **any material** **that supports microbial growth** being a potential substrate (e.g. starch and corn steep).

Similarly, fermentation-derived products show tremendous variety. Commonly consumed fermented products include bread, chees, sausage, pickled vegetable, cocoa, beers, wine, citric acid, glutamic acid and soy sauce.


Substrate
Microorganism
Product
Sugar
Yeast
Alcohol


Cell


Organic substrates
Microbial enzyme
Product

Definition of Fermentation

- The term “fermentation” comes from a Latin word fermentum (to ferment). The historical definition describes fermentation as:

- the process in which chemical changes in an organic substrate occur as the result of **action of microbial enzymes**.

- Fermentation can be described as respiration **without air**.

History

Fermentation = **zymology**

The first zymologist was **Louis Pasteur**, who as the first made yeast responsible for fermentation.
Louis Pasteur defined fermentation in more simple terms as **"life in the absence of air".**

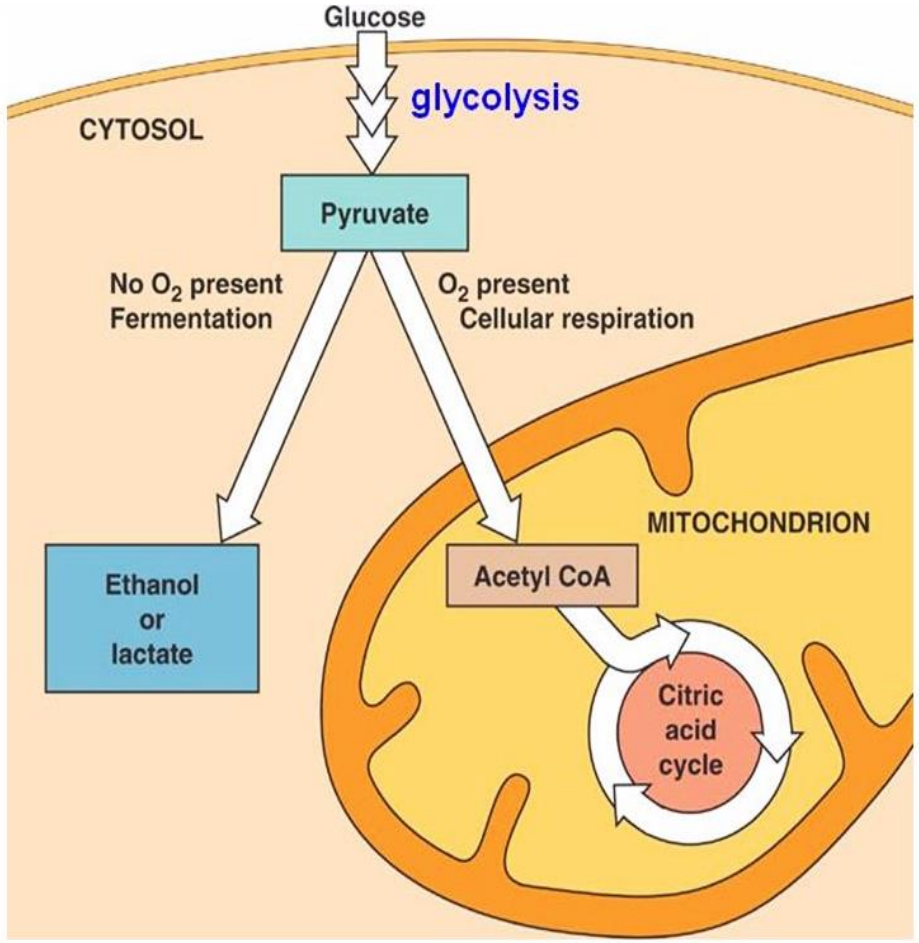
Nowadays, it is a metabolic process in which carbohydrates and related compounds are partially oxidized with the **release of energy** in the absence of any external electron acceptors – organic compounds produced by breakdown of carbohydrates.

During fermentation, incomplete oxidation of organic compounds occurs and for this reason less energy is obtained when compared with aerobic oxidation of the compound.

- In **industrial fermentation** the term of fermentation refers to either **aerobic or anaerobic processes**.

- In biochemical context the term of fermentation a **strictly anaerobic process**, which occurs if pyruvic acid does not enter the Krebs cycle and if electrons from glucose metabolism do not enter electron transport system. In this process, reduced organic compounds are formed, usually acid by-products.

- Industrial fermentation, a term used in chemical engineering, describes the process operations that utilize a chemical change **induced by a living organism or enzyme**, in particular bacteria, yeast, molds or fungi which produce a specified product.



	Aerobic	Anaerobic
Reactants	Glucose and oxygen	Glucose
Products	ATP, water	ATP and lactic acid (animals); or ATP, ethanol (yeast)
Location	Cytoplasm (glycolysis) and mitochondria	Cytoplasm
Stages	Glycolysis (anaerobic), Krebs cycle, oxidative phosphorylation	Glycolysis, fermentation
ATP produced	Large amount (36 ATP)	Small amount (2 ATP)

The Range of Fermentation Processes

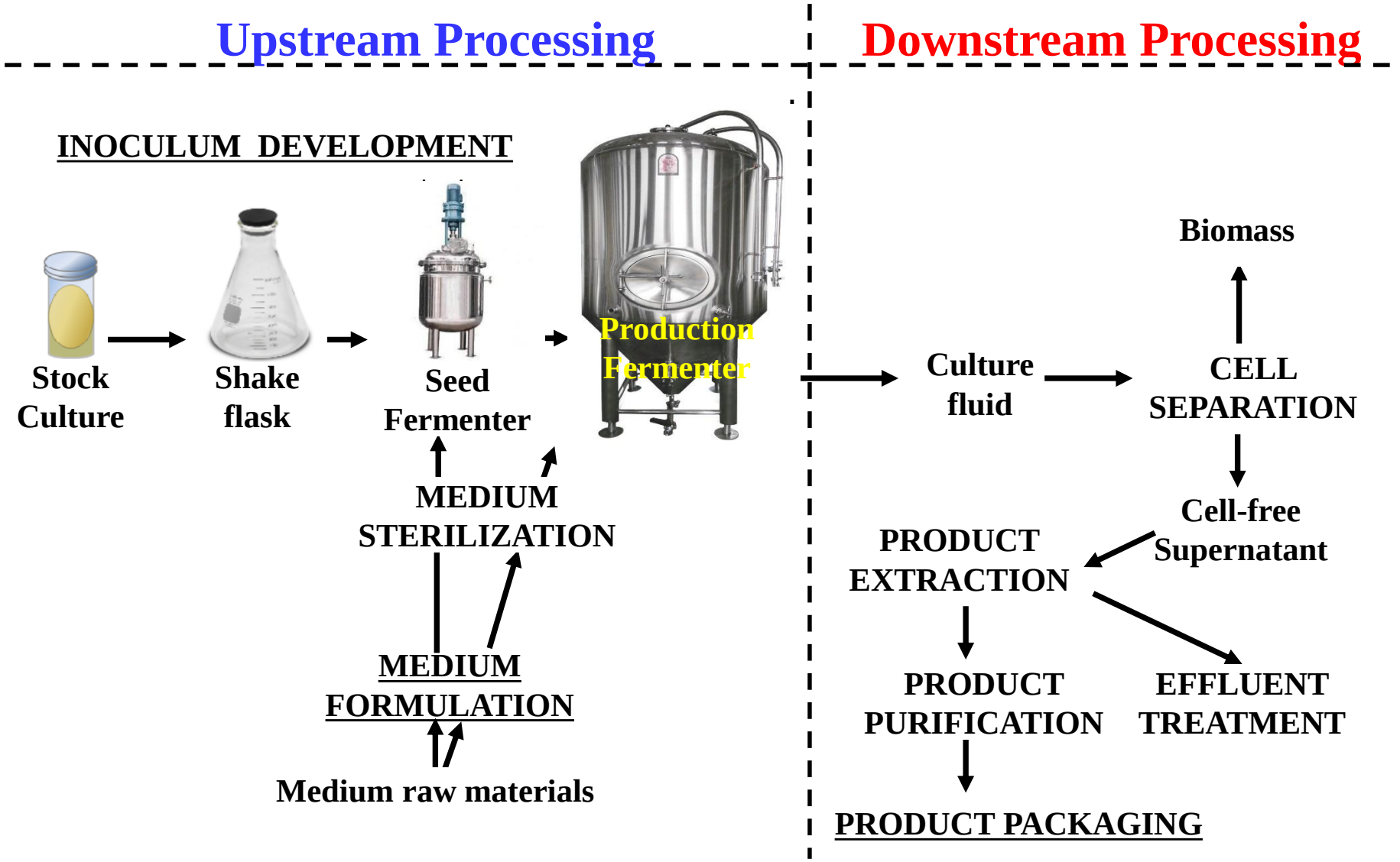
1. Those that produce **microbial cells** (or biomass) as the product.
2. Those that produce **microbial enzymes**.
3. Those that produce **microbial metabolites**.
4. Those that produce **recombinant products**.
5. Those that modify a compound which is added to the fermentation the **transformation process**.

Upstream *vs* Downstream Processing

Upstream involves all factors and processes leading to and including the fermentation and consists of three main areas:

1) The producer organism;
2) 2) The medium and
3) 3) The fermentation process.

Downstream encompasses all processes following the fermentation (Lecture 3, dealing with the Downstream Processing, will follow later).


Upstream Processing
Downstream Processing
INOCULUM DEVELOPMENT
Stock Culture
Shake flask
Seed Fermenter
Production Fermenter
MEDIUM STERILIZATION
MEDIUM FORMULATION
Medium raw materials
Culture fluid
Biomass
CELL SEPARATION
Cell-free Supernatant
PRODUCT EXTRACTION
PRODUCT PURIFICATION
EFFLUENT TREATMENT
PRODUCT PACKAGING

Types of Fermentation Processes

- Types of Fermentation Processes
  - Submerged Fermentation
    - Batch cultivation
    - Fed-batch cultivation
    - Continuous cultivation
  - Solid-State Fermentation

Fundamental difference between SmF and SSF

Factor	SmF	SSF
Substrates	Soluble substrates (sugars)	Insoluble substrates: starch, cellulose, pectin, lignin
Aseptic conditions	Heat sterilization and aseptic control	Vapor treatment, non- sterile conditions
Water	High volumes of water consumed and effluents discarded	Limited consumption of water; low *aw*. No effluent
Metabolic heating	Easy control of temperature	Low heat transfer capacity
Aeration	Limitation by soluble oxygen, high level of air required	Easy aeration and high surface exchange air/substrate
pH control	Easy pH control	Buffered solid substrates
Mechanical agitation	Good homogenization	Static conditions preferred
Inoculation	Easy inoculation, continuous process	Spore inoculation, batch
Contamination	Risks of contamination for single-strain bacteria	Risk of contamination for low-rate growth fungi
Energy consideration	High energy consuming	Low energy consuming
Volume of equipment	High volumes and high cost technology	Low volumes & low costs of equipment
Effluent & pollution	High volumes of polluting effluents	No effluents, less pollution

Submerged fermentation (SmF)

- Submerged fermentation is a process involving the development of microorganisms in a **liquid broth**.
- This liquid broth contains nutrients and it results in the production of industrial enzymes, antibiotics or other products.
- The process involves taking a **specific microorganism** such, as fungi, and placing it in a small closed flask containing the rich nutrient broth. A high volume of oxygen is also required for the process. The production of enzymes then occurs when the fungi interact with the nutrients on the broth resulting in them being broken down.
- At industrial level this production of yeasts has become a major output of microbiological industries as a result of improved fermentation technologies. Fermentation in industries is carried out using **fermenters** which are large vessels which can store huge volumes.

There are three types of industrial fermentation processes based on the methods of fermentation and types of fermenters:

1. Batch fermentation

In bath fermentation, sterile nutrient broth is inoculated with microorganism and cultured in a closed bioreactor for specified time and in specific conditions. **Nothing is added in the mid** of the fermentation process.

2. Fed batch fermentation

In fed batch fermentation, initially **small concentration of substances** are added and then these substances are added in small quantities continuously throughout the whole process of fermentation.

3. Continuous fermentation

In continuous fermentation process, **equal amount of sterile nutrient broth** is added to the open bioreactor and simultaneously equal amount of cultured broth with the products is taken out for purification of products. Depending on the type of fermentation different products such as proteins, enzymes, alcohol, acids, etc. are produced. Some of the industrially valuable fermentation products are discussed below.

Batch **Fed batch** **Continuous**

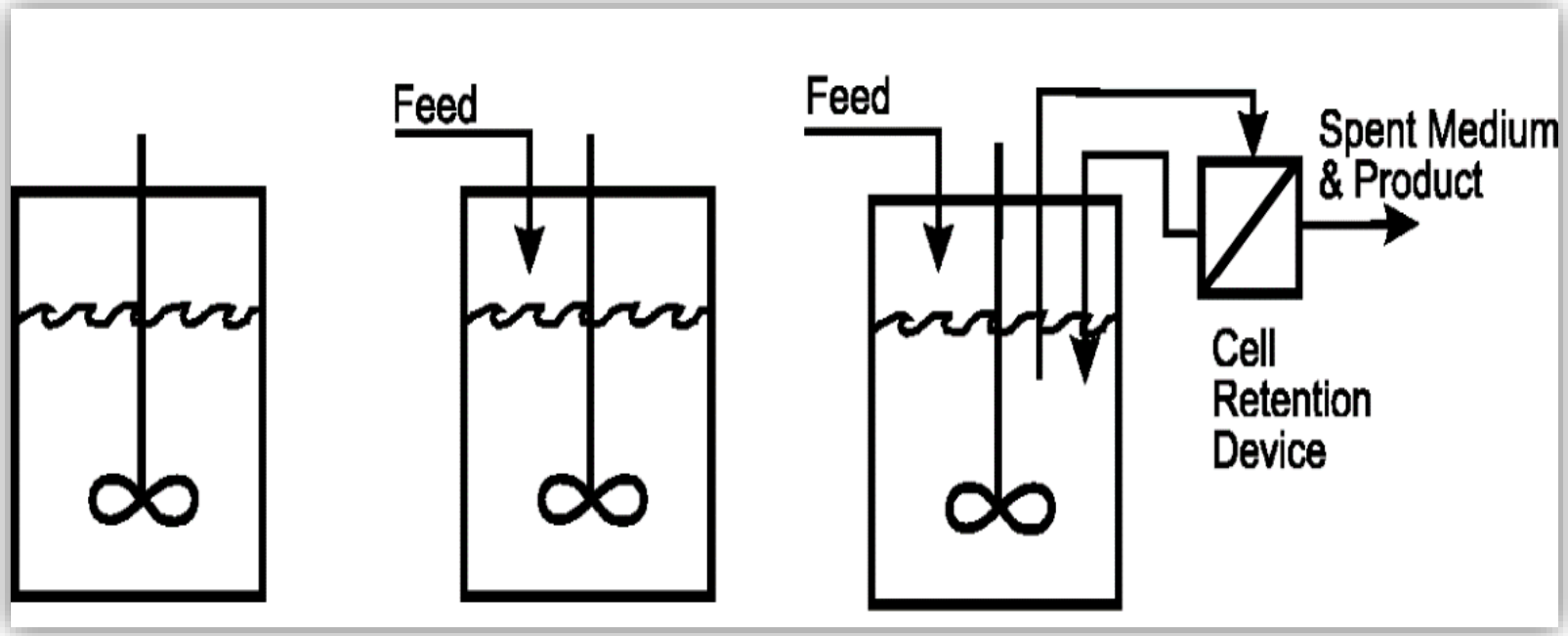


Figure . Types of industrial fermentation processes

(A) Batch Culture (growth in pure culture in a flask)

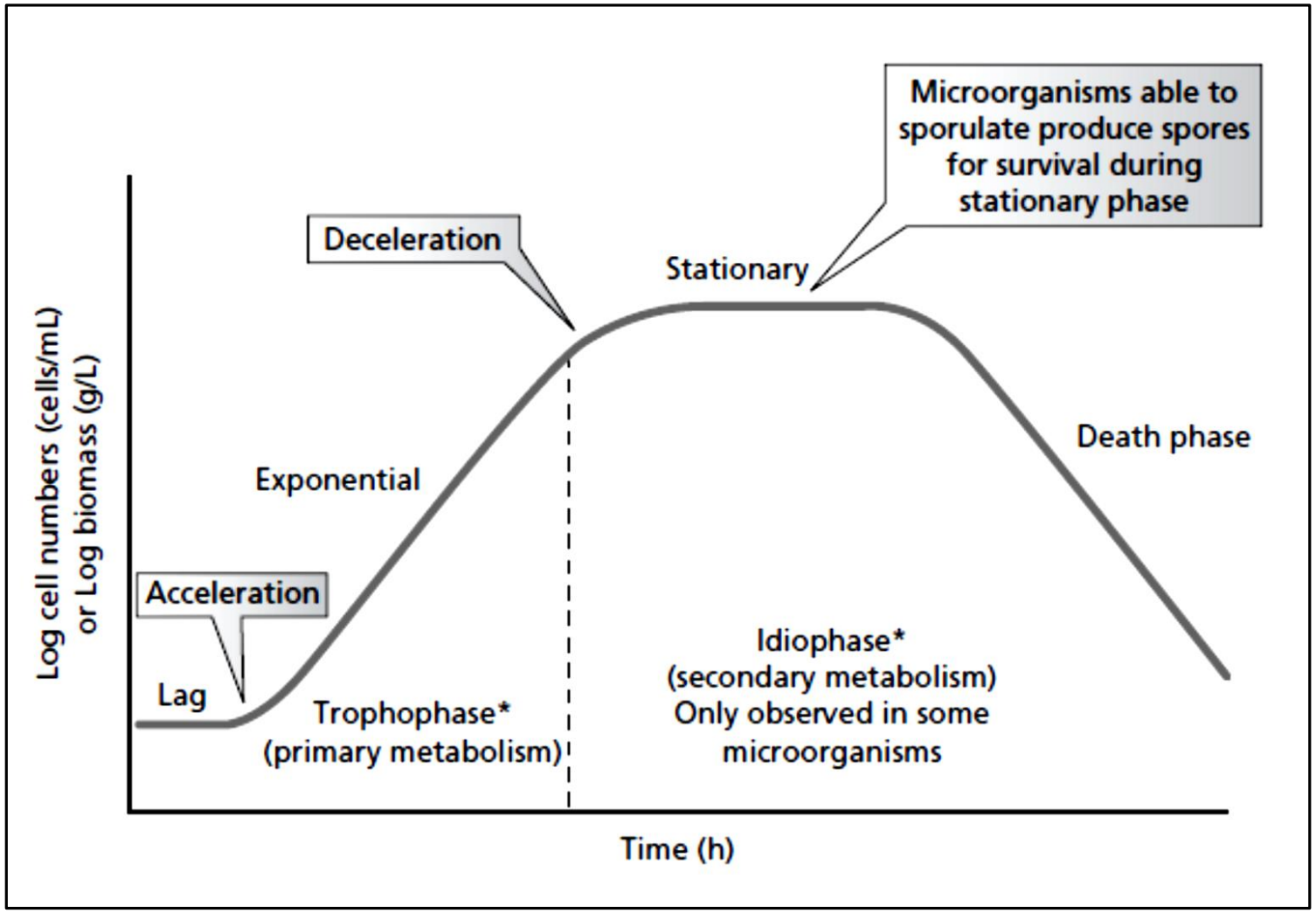
Typically, to understand and define the growth of a particular microbial isolate, cells are placed in a liquid medium in which the nutrients and environmental conditions are **controlled**.

The concept

If the medium supplies all nutrients required for growth and environmental parameters are optimal, the increase in numbers or bacterial mass can be measured as a function of time to obtain a growth curve. Several distinct growth phases can be observed within a growth curve:

- **lag phase**,
- **the exponential or log phase**
- **the stationary phase**
- **the death phase**

Each of these phases represents a **distinct period of growth** that is associated with typical **physiological changes** in the cell culture.



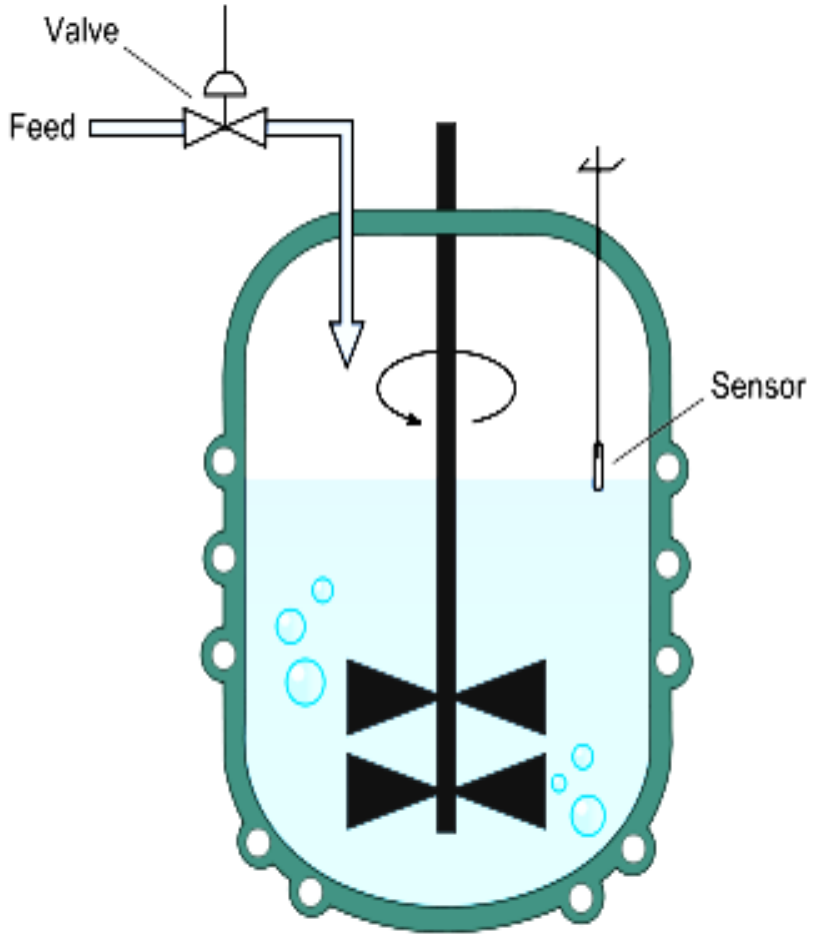


Figure . Growth curve

1. The Lag Phase

growth rate is essentially zero.
The lag phase is defined to transition to the exponential phase after the initial population has doubled.
The lag phase is thought to be due to the **physiological adaptation** of the cell to the culture conditions. This may involve a time requirement for induction of specific messenger **RNA (mRNA) and protein synthesis** to meet new culture requirements.
The lag phase may also be due to **low initial densities** of organisms that result in dilution of exoenzymes (enzymes released from the cell) and of nutrients that leak from growing cells. Normally, such materials are shared by cells in **close proximity**. But when cell density is low, these materials are diluted and not as easily taken up. As a result, initiation of cell growth and division and the transition to exponential phase may be slowed.

The lag phase usually lasts from minutes to several hours. The length of the lag phase can be controlled to some extent because it is dependent **on the type of medium as well as on the initial inoculum size**.

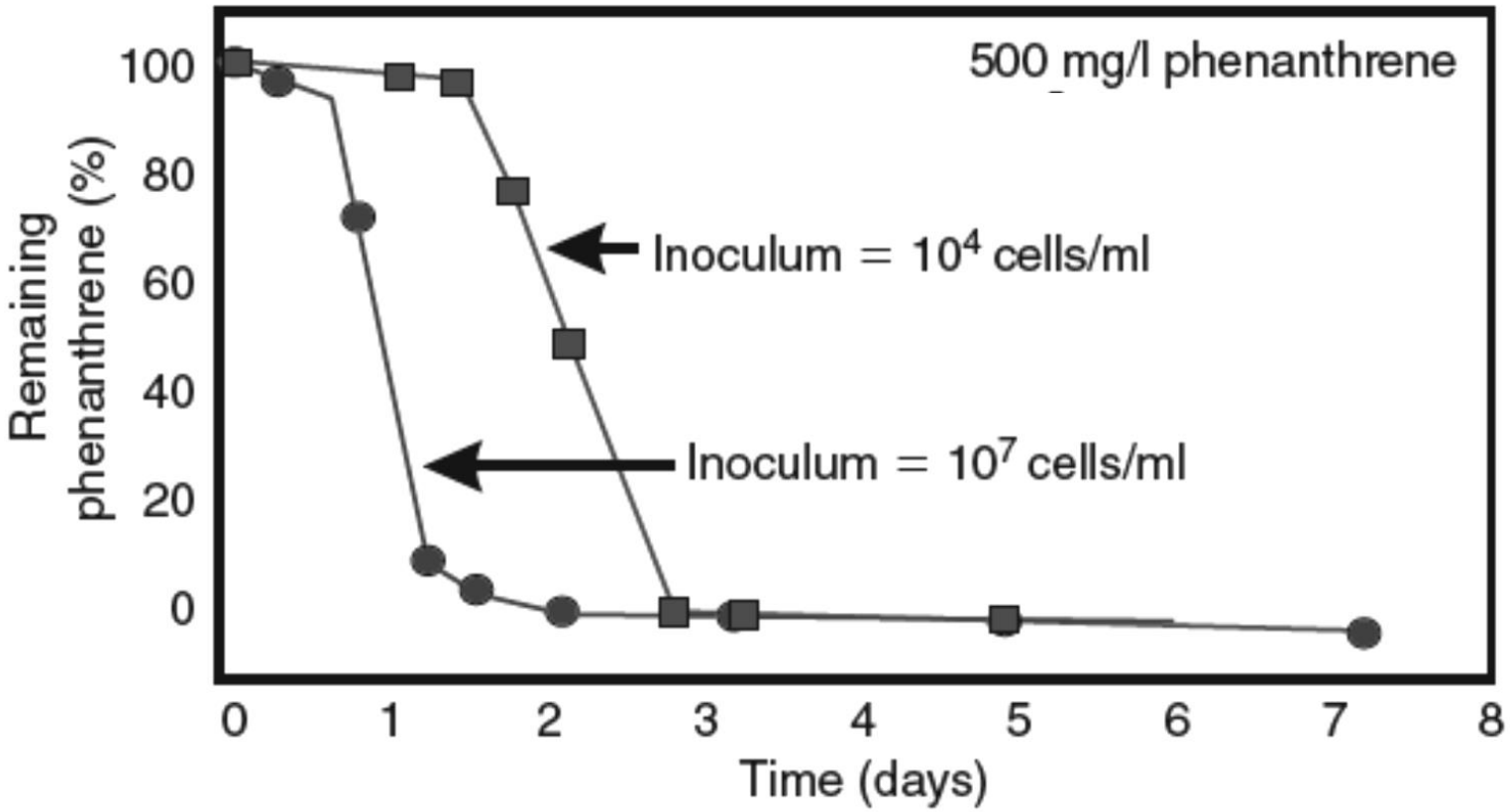


Figure . Effect of inoculum size on the lag phase during degradation of a polyaromatic hydrocarbon, phenanthrene.

2. The Exponential Phase

- Once the cells have adapted to their new environment they enter the acceleration phase. Cell division occurs with increasing frequency until the maximum growth rate (μ_{max}) for the specific conditions of the batch fermentation is reached.
- The exponential phase is characterized by a period of the exponential growth, the most rapid growth possible under the conditions present in the batch system. **During exponential growth the rate of increase of cells in the culture is proportional to the number of cells present at any particular time**.
- During exponential growth the number of cells increases in the **geometric progression 2^0, 2^1, 2^2, 2^3 until, after *n* divisions, the number of cells is 2^n**.

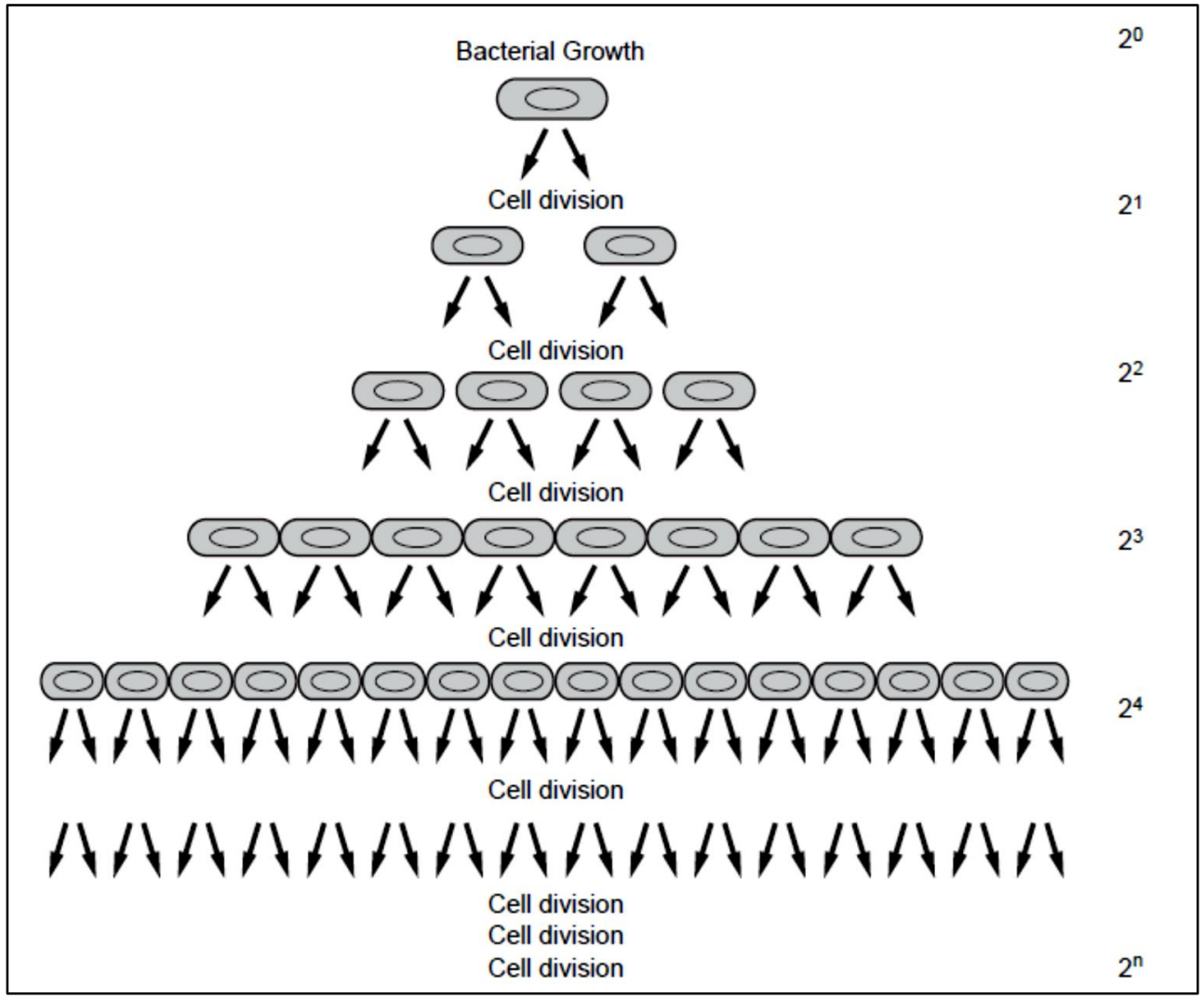


Figure . Geometric progression of cells divisions

3. The Stationary Phase

- **The batch culture may reach stationary phase when** the carbon and energy source or an essential nutrient becomes completely used up. When a carbon source is used up it does not necessarily **mean that all growth stops**.

- This is because dying cells can lyse and provide a source of nutrients. In addition, the stationary phase may be observed is that waste products build up to a point where they begin to inhibit cell growth or are toxic to cells. This generally occurs only in cultures with high cell density.

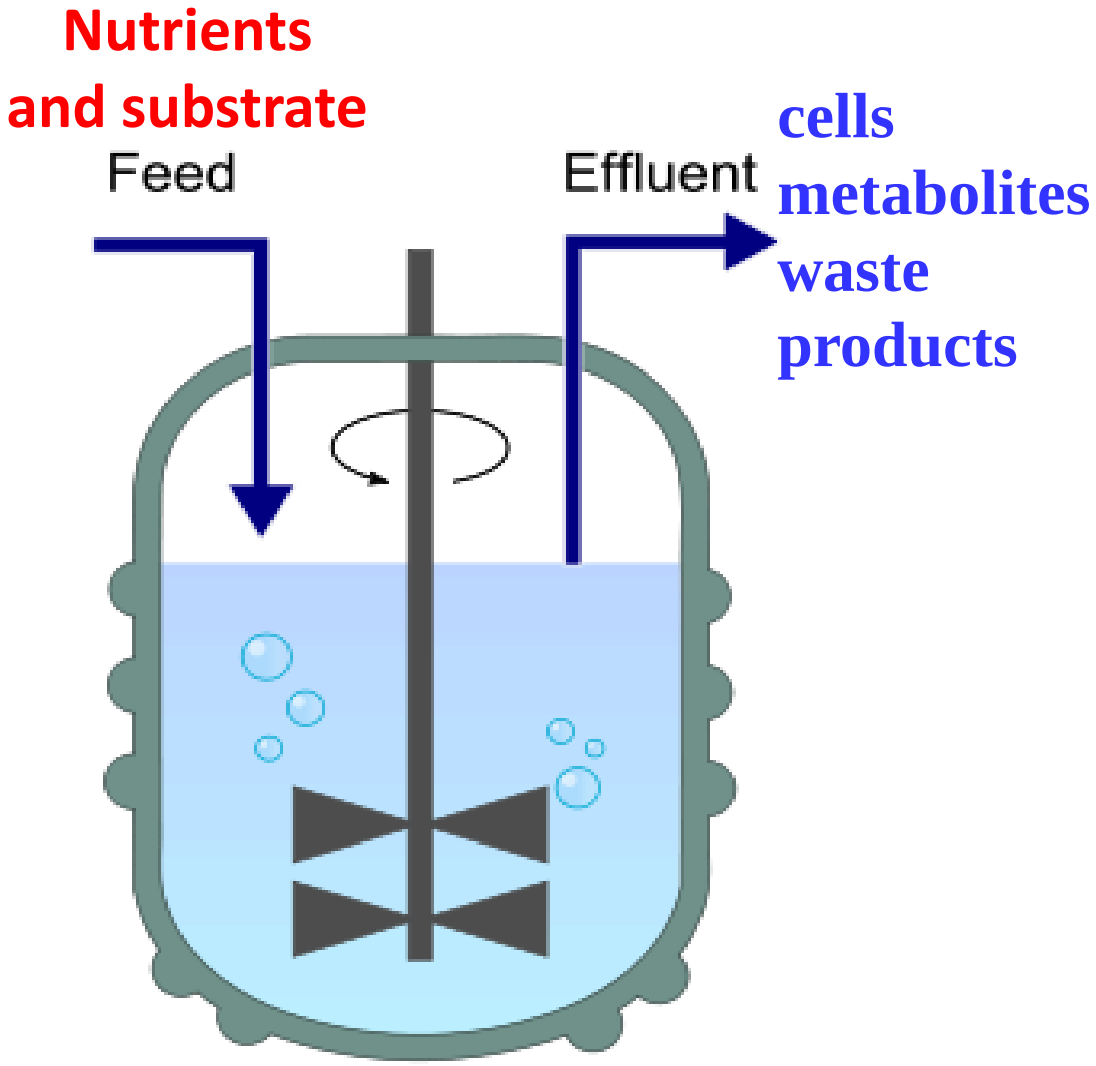
4. The Death Phase

- The final phase of the growth curve is the death phase, which is characterized by a net loss of culturable cells. Even in the death phase there may be individual cells that are metabolizing and dividing, but more viable cells are lost than are gained so there is a net loss of viable cells.

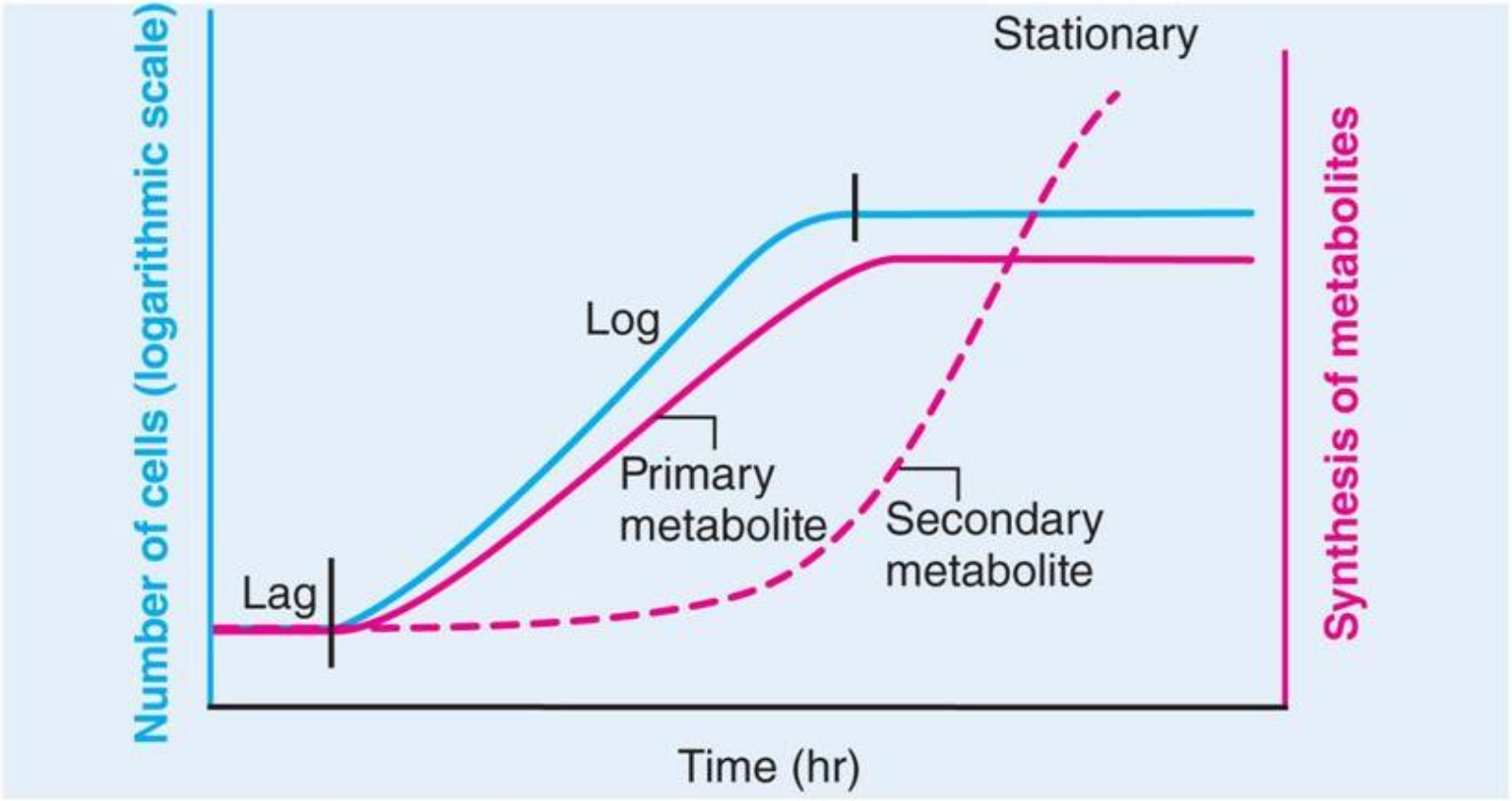
- The death phase is often exponential, although the rate of cell death is usually slower than the rate of growth during the exponential phase.

(B) Continuous Culture

In batch cultures, nutrients are not renewed and so growth remains exponential for only a few generations. Continuous culture is a system that is designed for **long-term operation**. This is known as **steady state or balanced growth**. Continuous culture can be operated over the long term because it is an open system with a **continuous feed** of influent solution that contains nutrients and substrate, as well as a **continuous drain** of effluent solution that contains cells, metabolites, waste products, and any unused nutrients and substrate.



exponential growth will continue by addition of new fresh medium



_____ Metabolites are required for growth

_____ Metabolites are required for survival

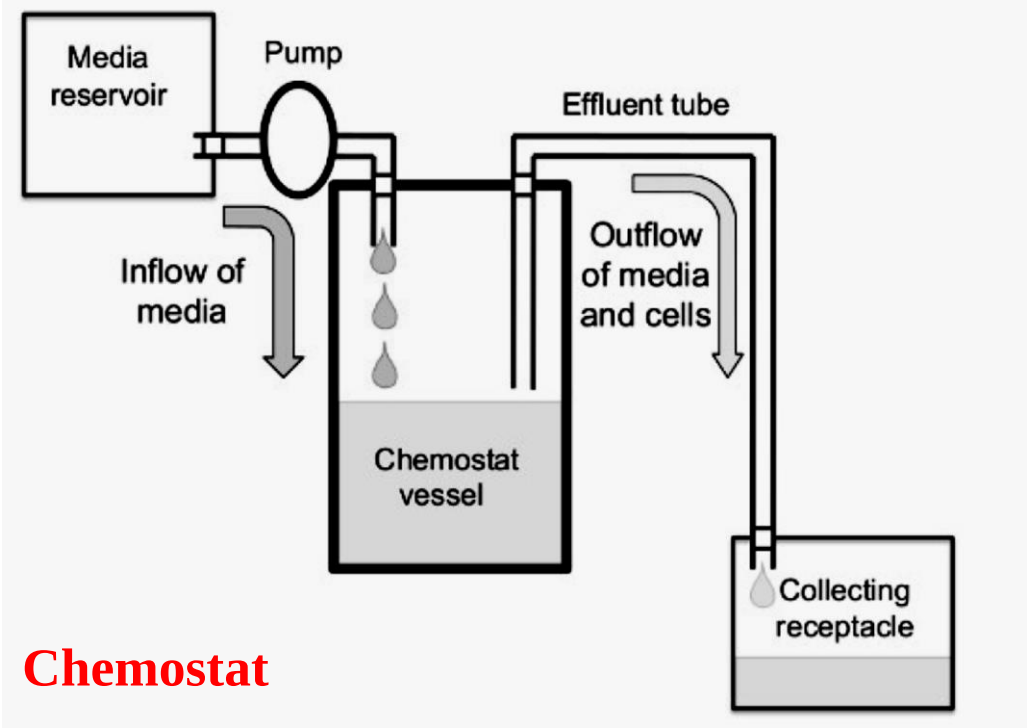
Figure . Secondary metabolites *vs* Primary metabolites.

Continuous culture is obtained by using two different types of devices:

A) Chemostat

- into which fresh medium is continuously introduced at a **constant rate**, and
- the culture **volume** is kept **constant** by continuous removal of culture at the same rate, and
- in which the supply of **a single nutrient controls growth rate**.

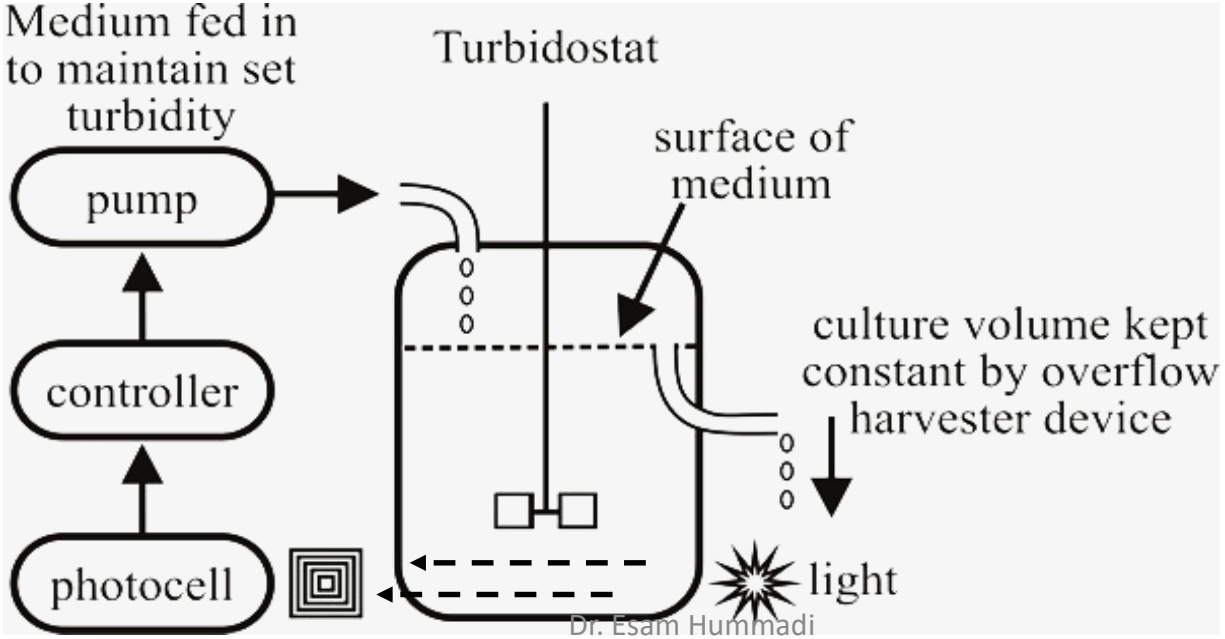
By controlling the rate at which nutrients are added to the chemostat, the rate of growth of cells is also controlled. A gradual decrease in bacterial concentration and gradual increase in substrate concentration occur when **dilution rate** begins to increase i.e. more bacteria are **washed out** of culture vessel than are produced by cell division. The problem associated with the chemostat is the increase or decrease in dilution rate and hence decreases or increases in microbial population, respectively.

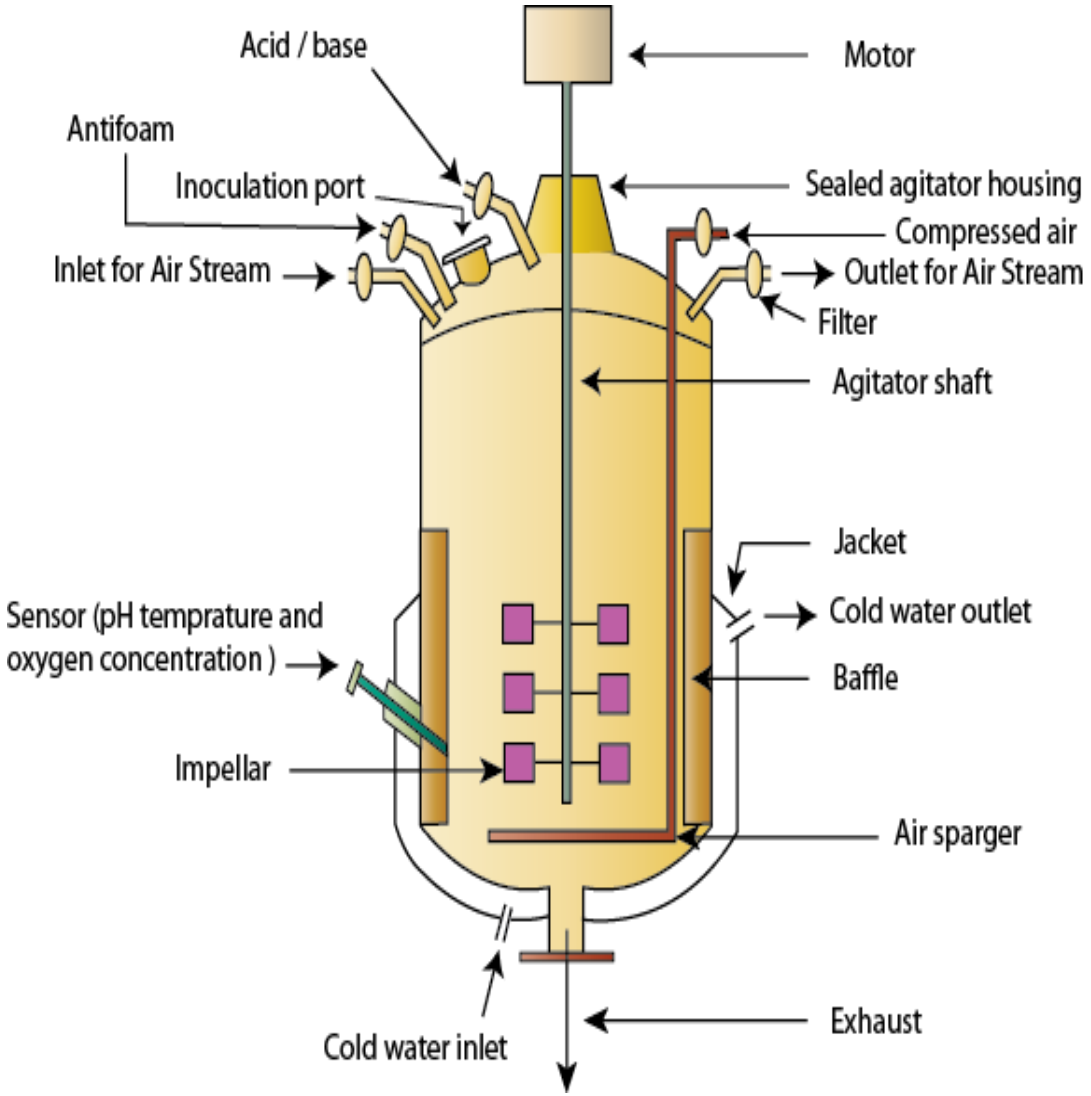


Chemostat

B) Turbidostat

The cell density in turbidostat is measured by **photo electric device**, which sends signal to the turbidostat to increase or decrease the flow rate of the medium to the fermenter vessel. The pump attached to the fermenter for controlling the flow rate will turn on or off depending on the increase or decrease in the level of biomass beyond set point.




Acid / base
Motor
Antifoam
Inoculation port
Sealed agitator housing
Compressed air
Inlet for Air Stream
Outlet for Air Stream
Filter
Agitator shaft
Jacket
Sensor (pH temprature and oxygen concentration)
Cold water outlet
Baffle
Impellar
Air sparger
Exhaust
Cold water inlet


Phosphate Solubilizing Bacteria
Bioreactor 500 Liter

Lecture 3

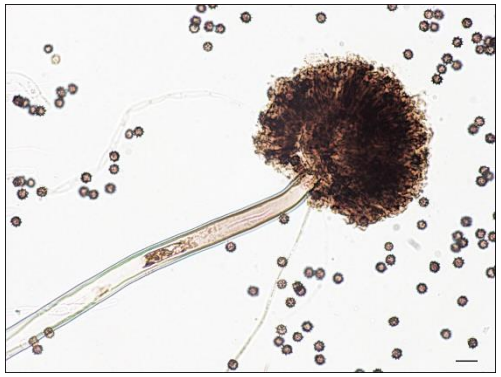
Fermentation Technologies:

Solid Substrate Fermentation (SSF)

Definition

Cultivation of microorganisms on moist solid materials such grains without or near-absence of free water.

Solid state fermentation is suitable for cultivation of **filamentous fungi** such as *Aspergillus*. The process was carried out under aeration conditions. The source of nutrients are usually comes from decomposition of solid-complex materials such as agro waste.

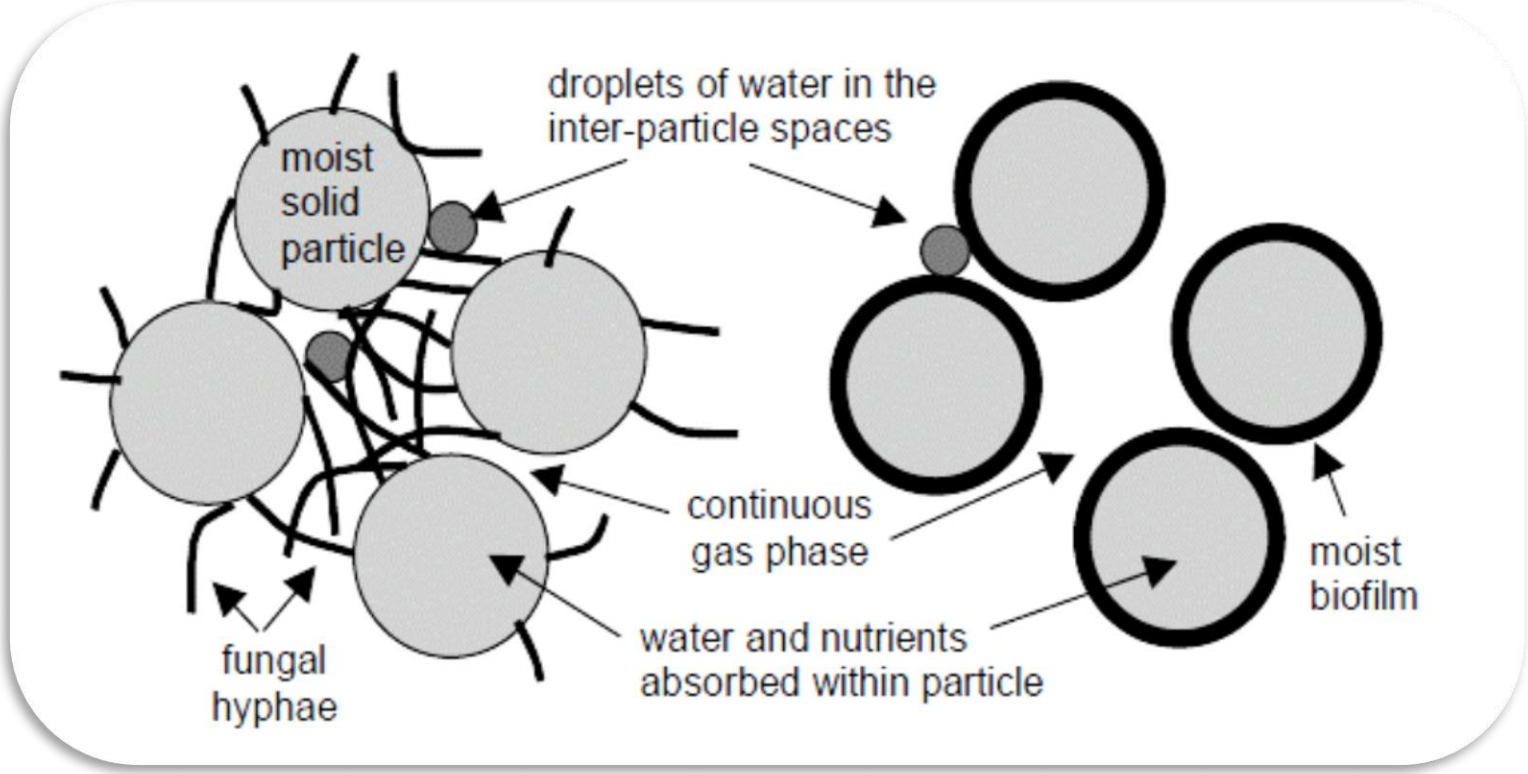


Aspergillus

Solid Substrate Fermentation

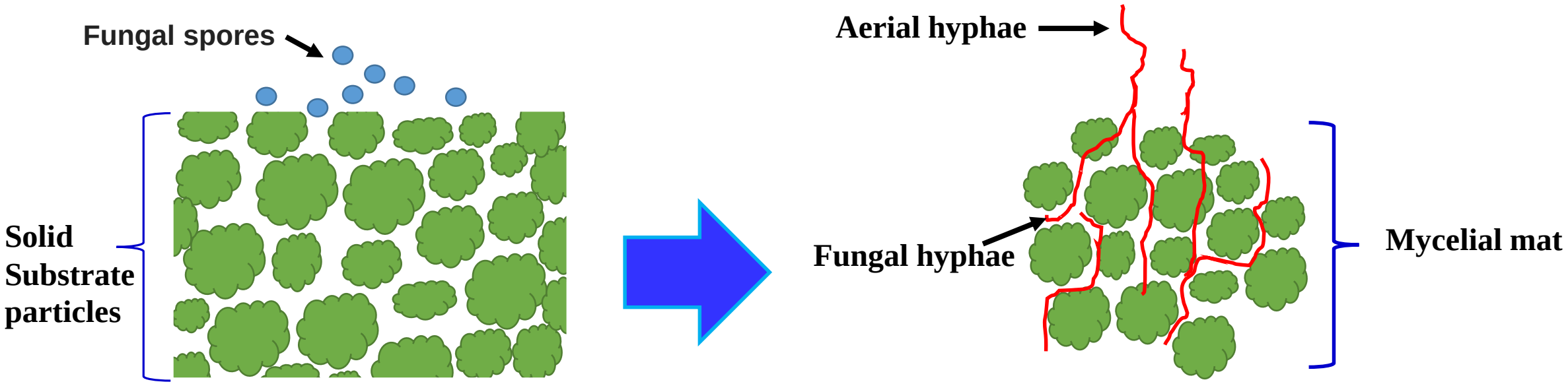
Gas phase in the spaces between the particles

Aqueous phase as film of water surrounding the particles surfaces



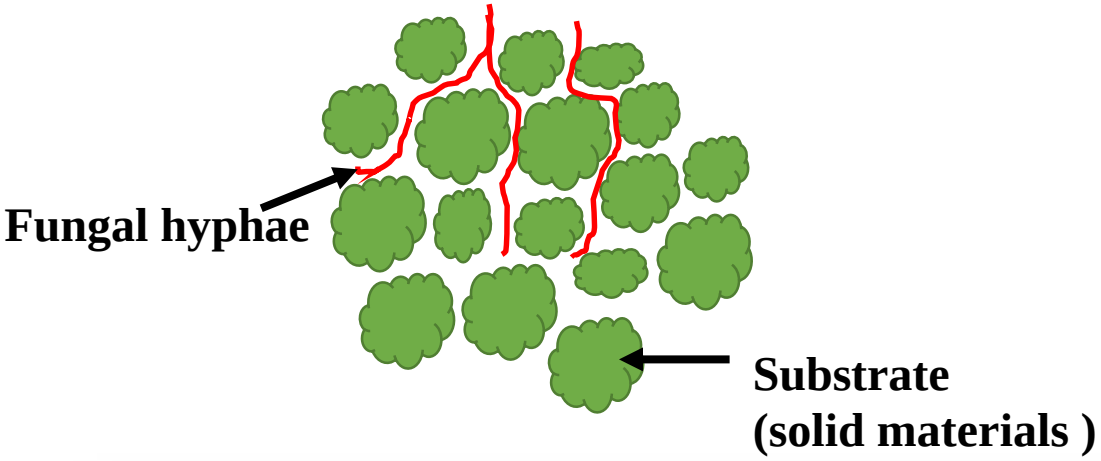
Sporulation

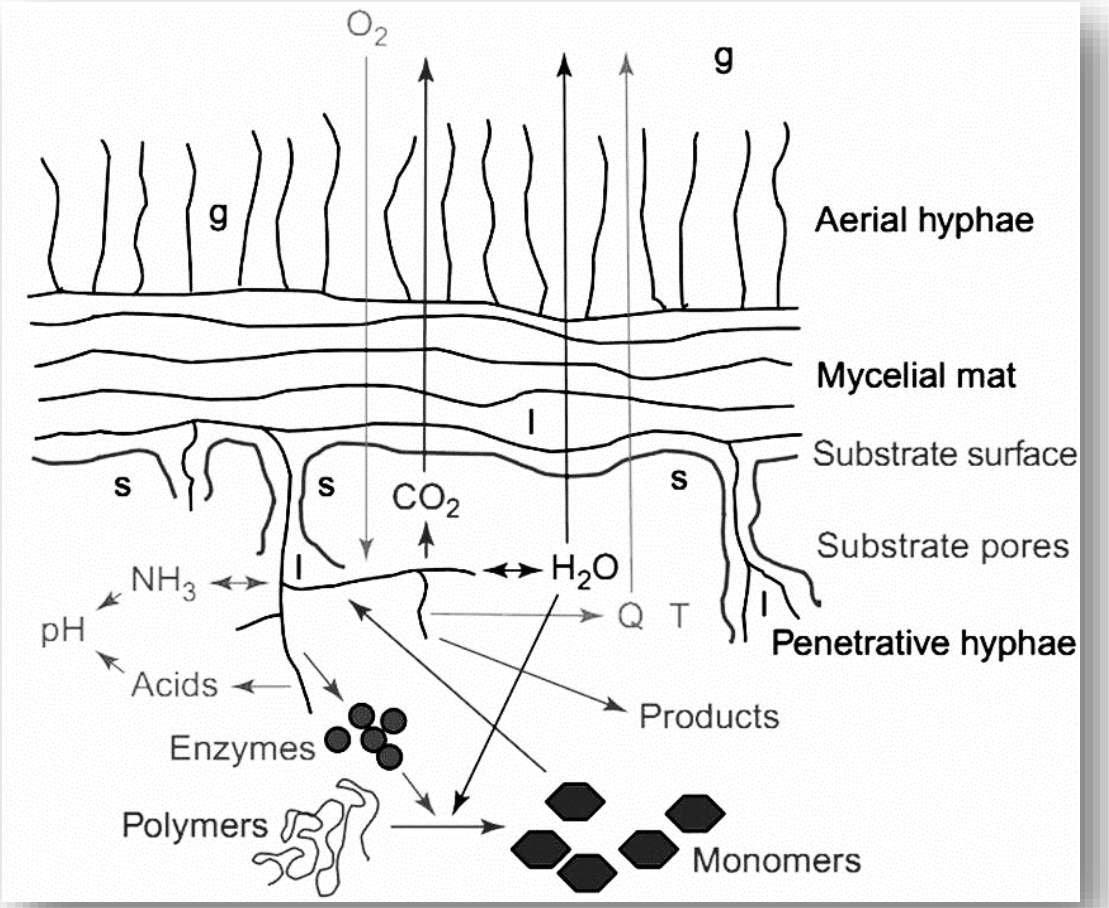
Fungal hyphae development



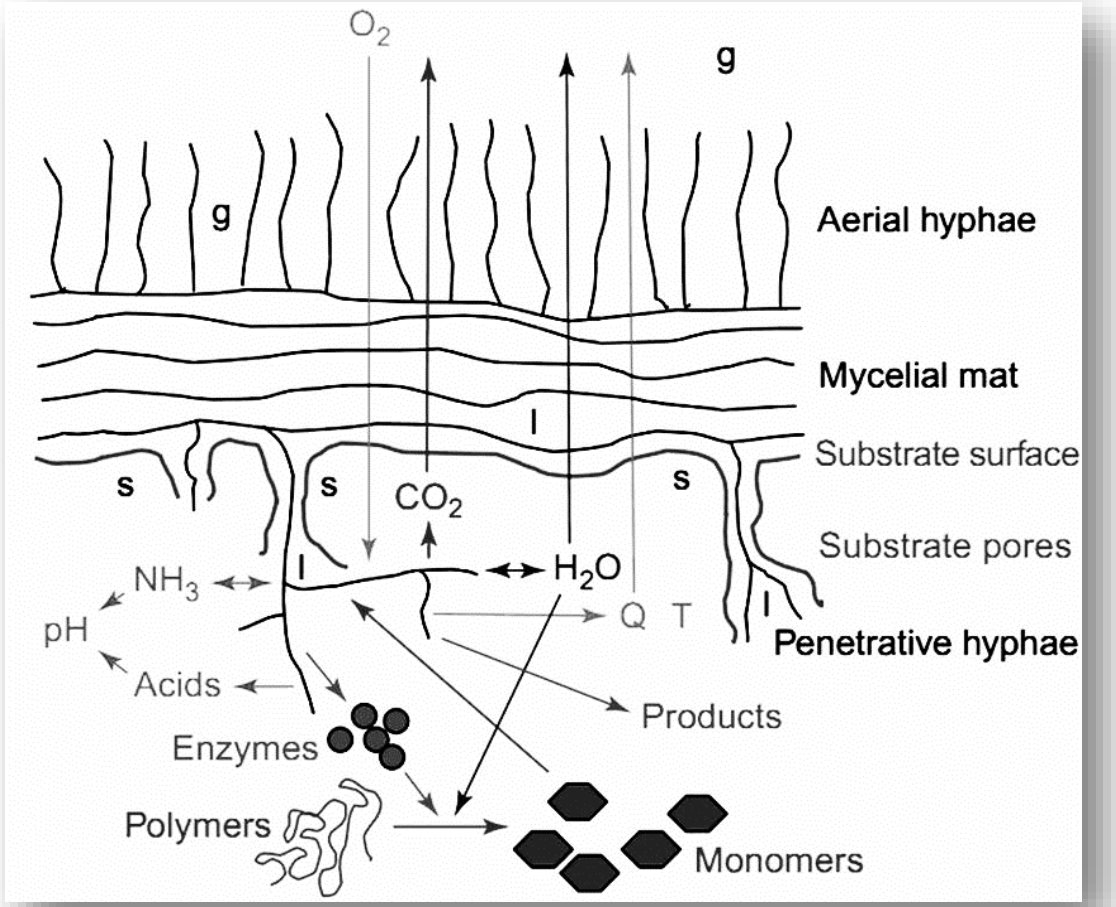
The biochemical processes of SSF

- After sporulation, fungal hyphae develop into a mycelial mat, which spreads over the surface of the particles that comprise the solid substrate. From the mycelial mat, aerial hyphae protrude into the gaseous space, whereas others penetrate the substrate by growing into liquid-filled pores.
- At normal moisture levels, the void spaces between the aerial hyphae are most likely to be filled with gas, whereas the void spaces within the mycelial mat and within the substrate are filled with liquid.
- The metabolic activities shown mainly occur **near the substrate surface and within the pores**; however, exposed regions of the mycelium (for instance the aerial hyphae) also show metabolism and there can be a transport of substances from the penetrative to the aerial hyphae.

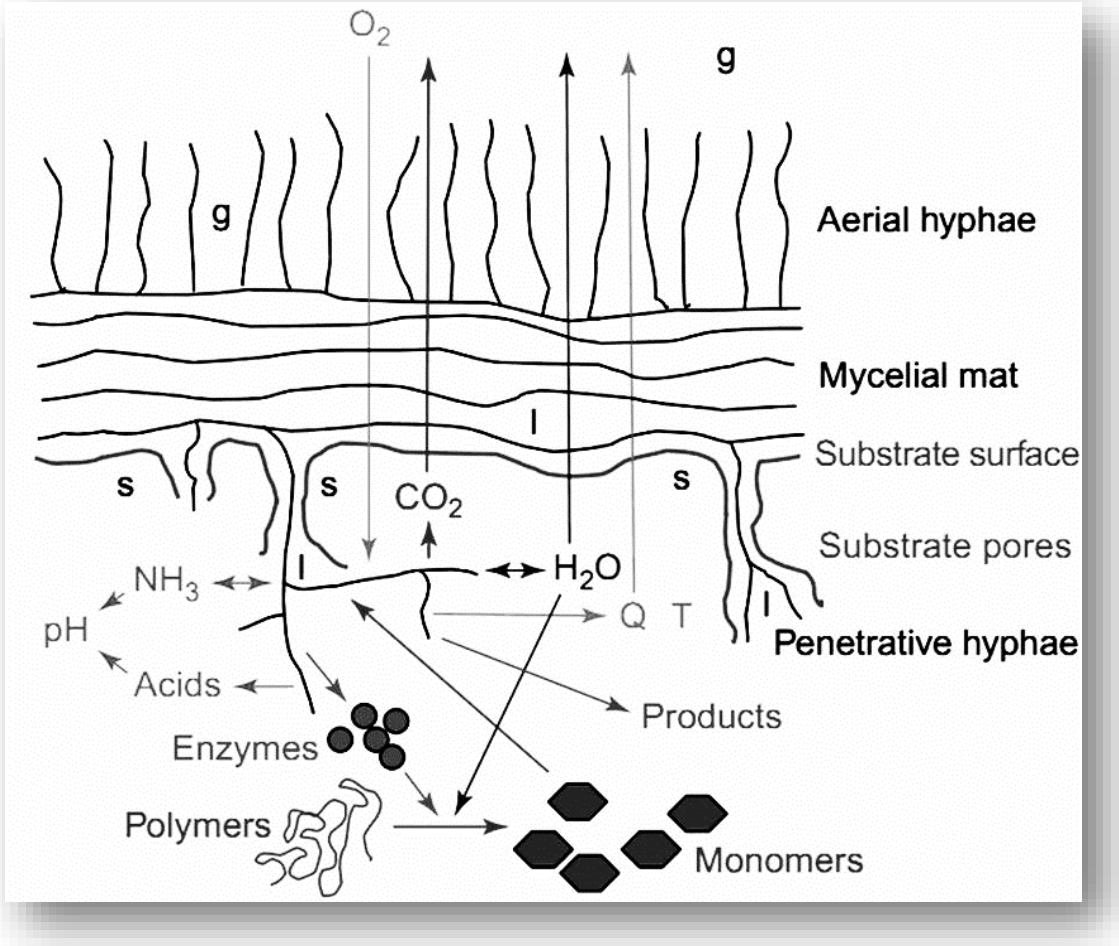




- **Hydrolytic enzymes**, which are produced by the mycelium, diffuse to the solid matrix and catalyse the degradation of macromolecules into smaller units. The latter are taken up by the fungus to serve as **nutrients**.
- O_2 is consumed and CO_2, H_2O, heat and interesting biochemical products are produced during fermentation. Hence, gradients develop within the biofilm that, for instance, force O_2 to diffuse from the gaseous phase into deeper regions of the biofilm and CO_2 to diffuse from these regions to the gaseous phase.
- Heat development leads to a **fast increase in temperature**, which is a **serious problem** during SSF. Heat is therefore removed from the substrate not only *via* conduction but also by evaporation, which is part of the complex balance of water in the system.



- Beside evaporation, water balance includes water uptake by the mycelium in the course of growth, water consumption during hydrolysis reactions and water production through respiration.
- As another important factor, local pH, might be **changed** owing to the release of **carbon acids** and the exchange of ammonia.
- The biochemical products of interest that are released into the solid matrix and the liquid-filled spaces during fermentation might **absorb to the solid and might have to be extracted for further use at the end of the process.**



Factors that influence SSF

Broadly, the factors that influence the performance of SSF can be divided into three major categories, namely:

1. **Biological factors** (the type of microorganism, inoculum, substrates).
2. **Physico-chemical factors** (moisture content, pH, temperature, gaseous environment, aeration, particle size).
3. **Mechanical factors** (agitation/mixing, particular design of bioreactors).

Classification of bioreactors for SSF

Good bioreactor performance is controlled by two factors:

- The interactions between the microorganism and its local environment.
- Influence of the design and operating strategies on conditions in the microorganism’s local environment.

SSF processes, resulting in **effective oxygen transfer**, **efficient heat removal**, **excellent water distribution** and **good substrate mixing** with minimal mycelia damage. The two basic strategies of aeration and mixing are as follows:

1. The air supply circulates around the fermented solid substrate particles.
2. The air goes through the inter-particles of fermented solid substrate particles. According to this strategy, three mixing types can be used on the fermented beds:

- **Static (unmixed)**
- **Agitated intermittently mixing or**
- **Agitated continuous mixing**

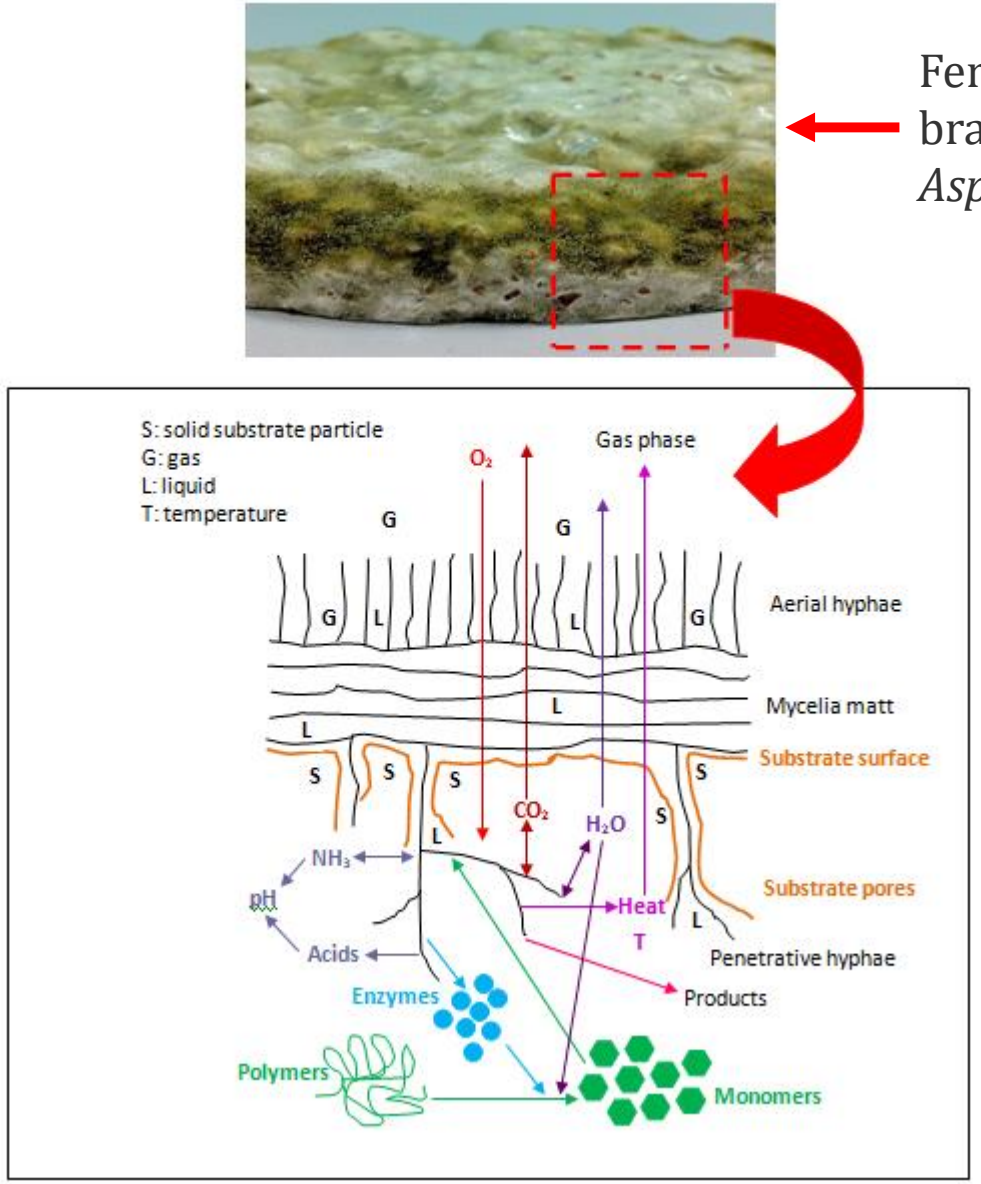
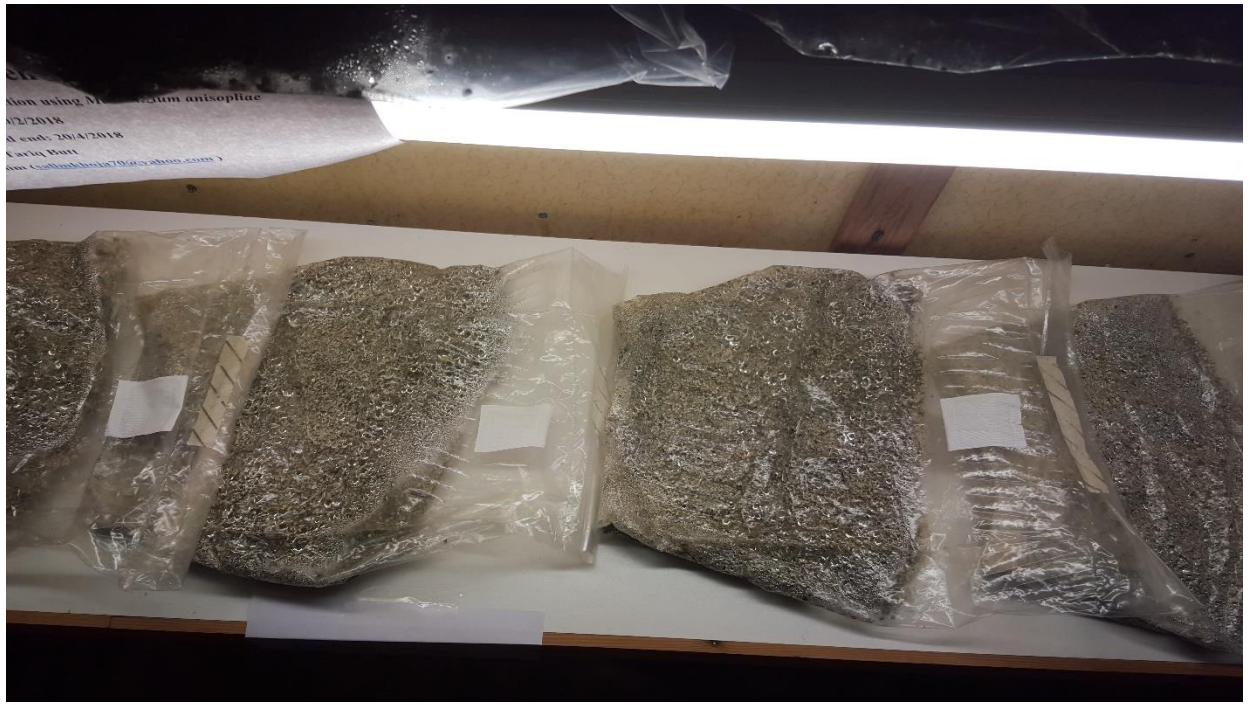


Figure . Schematic of the micro-scale processes that occur during SSF involving fungi. Above Fermented wheat bran with *Aspergillus oryzae* from the current study.



Bioreactors for SSF

SSF bioreactor design greatly depends on the solid substrate. There are four major roles of the bioreactor, which are:

1. To contain the substrate.
2. To contain the process microorganism.
3. To protect the process microorganism against contamination.
4. To control environmental conditions to optimize growth and product formation.

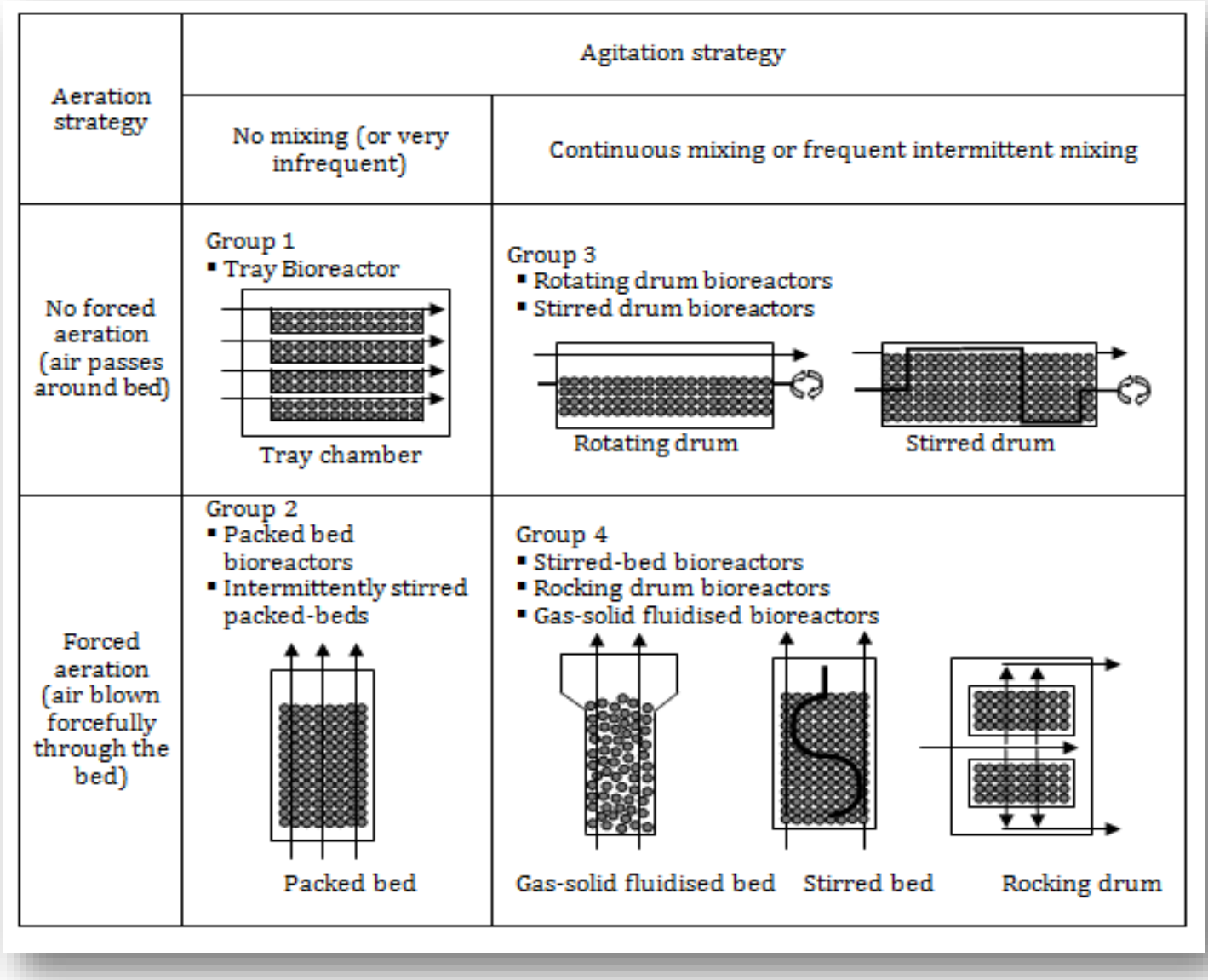


Figure . Types of SSF bioreactor

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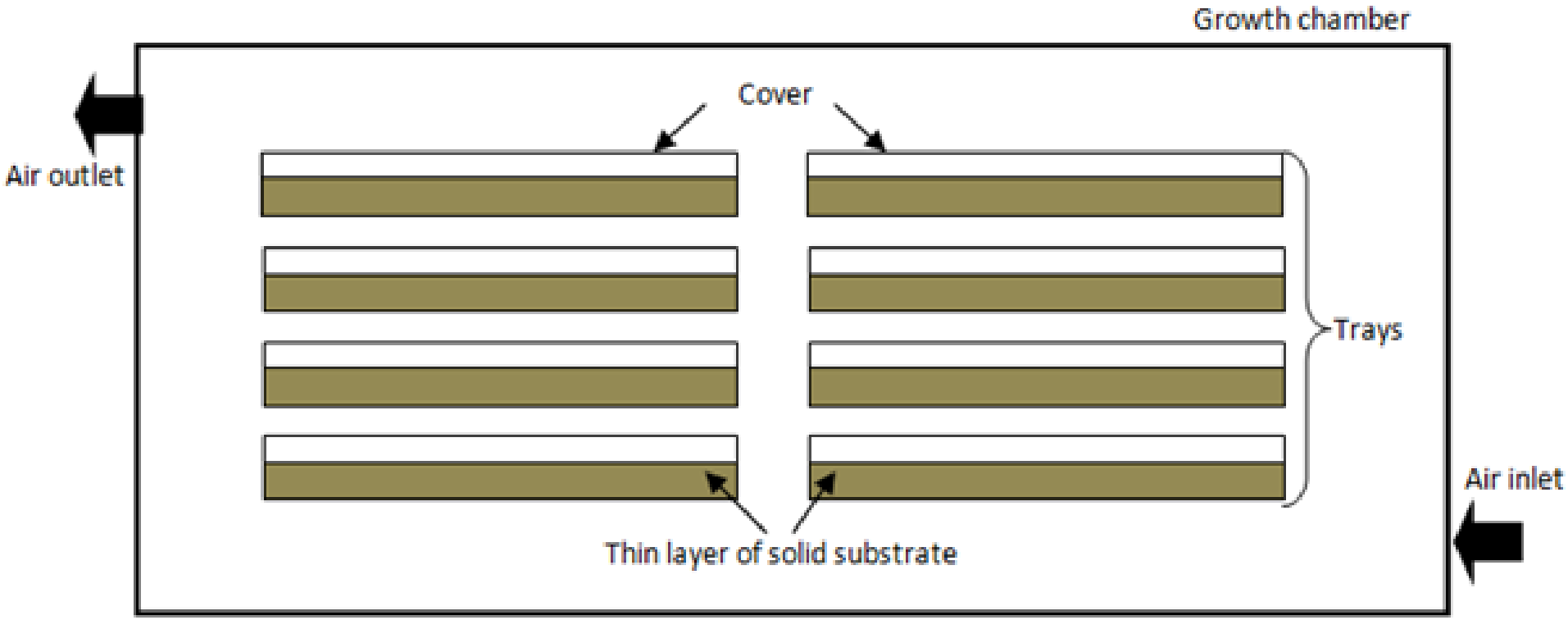

Figure . Tray bioreactor

Lecture 4

Downstream Processing

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Now that the organism has been grown (fermentation) and the product produced (upstream step), how do we get the product?

Biological synthesis of goods is not the only part of a production process. Before it can be offered on the market, invariably the product of interest has to be separated from the biomass and more or less purified **depending on its application**. Possibly the **most expensive part of the entire process** is extraction and purification of the product. For e.g. Recovery of antibiotics from fermentation broth to make it as a tablet form. The main aim is to purify the bioactive (e.g. pharmaceutical) ingredients to make it in a tablet form.

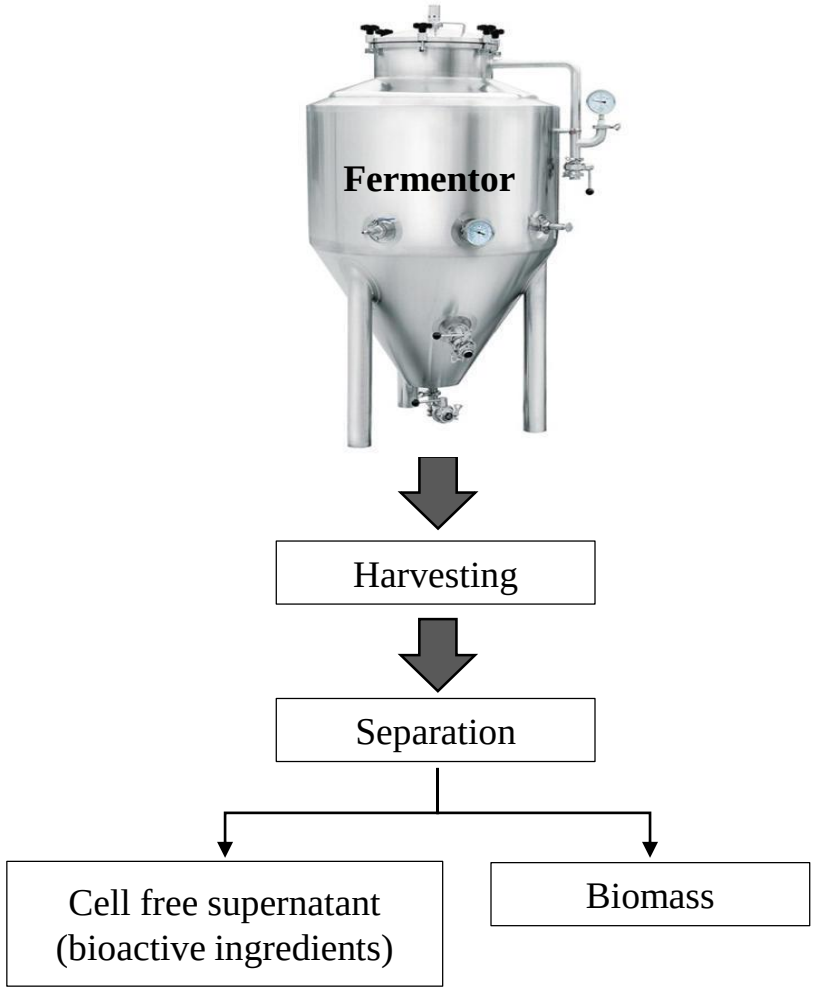

Figure 1. Downstream process.

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Choice of downstream process is based upon:

1) Location of the product in the organism; **intracellular** or **extracellular**.
2) Concentration of the product in the growth medium.
3) Physical and chemical properties of the product.
4) Intended use of the product.
5) Minimal standard of **purity**.
6) **Bio-hazard** level of medium, cells, or product.
7) Impurities present.
8) Market **price** of the product.

Unit operations in downstream processing are individual processes that operate separately from each other. Optimization of downstream processing costs can be achieved by as much integration of unit operations.

- Cell separation
  - Flocculation
  - Centrifugation
  - Filtration
- Cell disruption
  - Homogenizers
  - Hydrolytic enzymes
- Clarification
  - Centrifugation
  - Filtration
- Concentration
  - Precipitation
  - Chromatography
  - Ultrafiltration
  - Partitioning
  - Distillation
- High resolution techniques
  - Chromatography
  - Electrophoresis
  - Dialysis
- Finishing/packaging
  - Crystallization
  - Filtration
  - Gel chromatography
  - Drying

Overview of downstream process

The main objective of the first stage for the recovery of an extracellular product is the removal of large solid particles and microbial cells usually by centrifugation or filtration (Figure 2). In the next stage, the broth is fractionated or extracted into major fractions using ultrafiltration, reverse osmosis, adsorption/ion-exchange/gel filtration or affinity chromatography, liquid-liquid extraction, two phase aqueous extraction or precipitation. Afterwards, the product-containing fraction is purified by fractional precipitation, further more precise chromatographic techniques and crystallization to obtain a product which is highly concentrated and essentially free from impurities. Other products are isolated using modifications of this flow-stream.

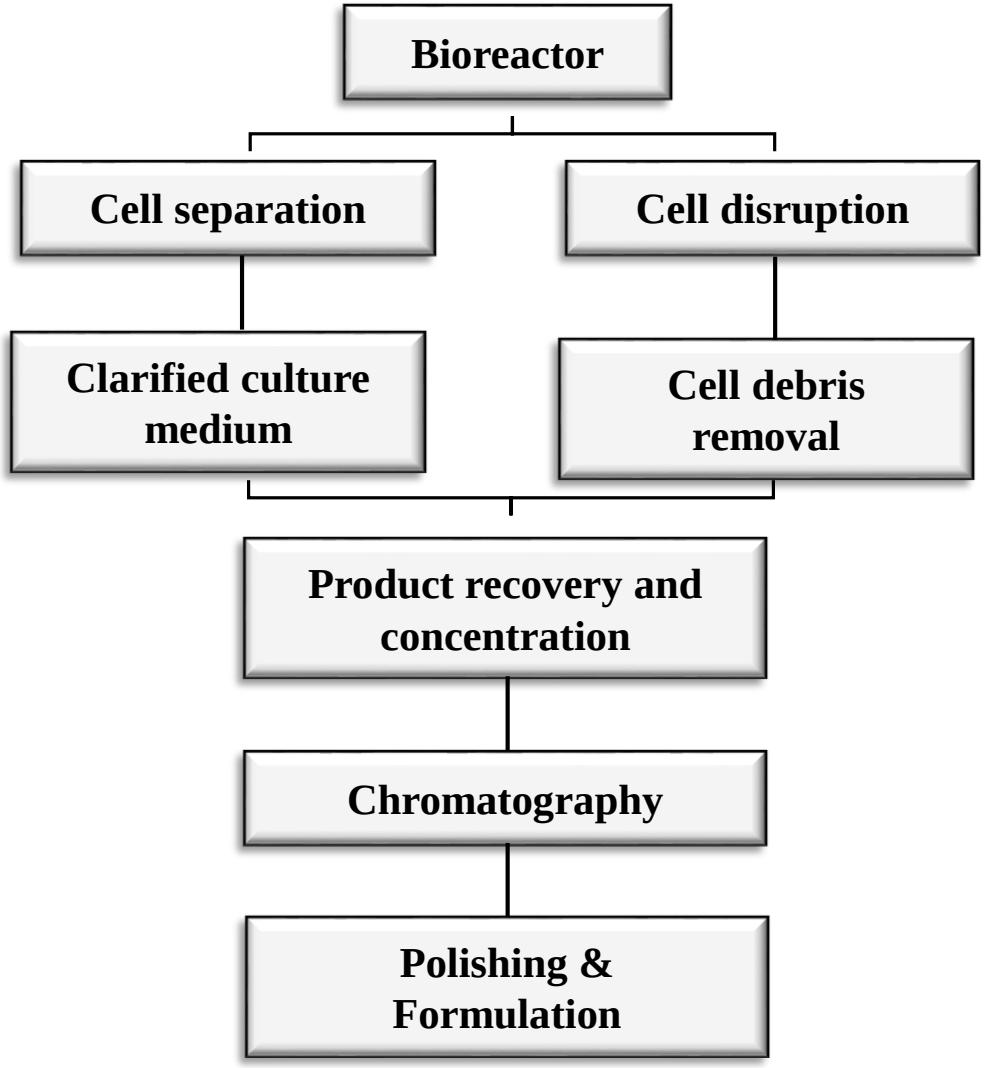
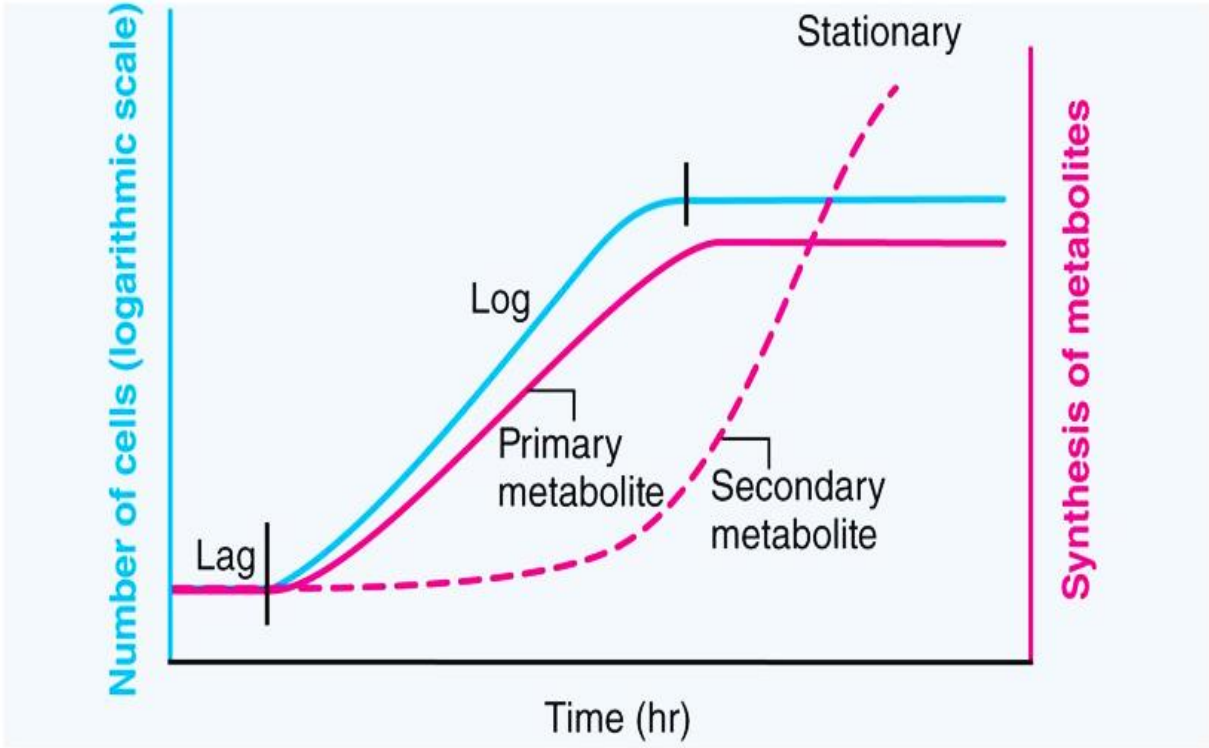


Figure 2. Stages in the recovery of product from a harvested fermentation broth.

It may be possible to modify the handling characteristics of the broth so that it can be handled simpler equipment making use of a number techniques:

1) Selection of organisms that do not produce undesirable pigments or metabolites.
2) Modify the fermentation conditions so that undesirables are not produced.
3) Precise timing of harvest.
4) pH control after harvesting.
5) Temperature control after harvest.
6) Addition of flocculating agents.
7) Addition of antifoams that do not cause purification problems.
8) Use of lytic enzymes to aid in cell wall disruption.



_____ Metabolites are required for growth

_____ Metabolites are required for survival

Lecture 5

Microbial Metabolism

Overview of microbial metabolism

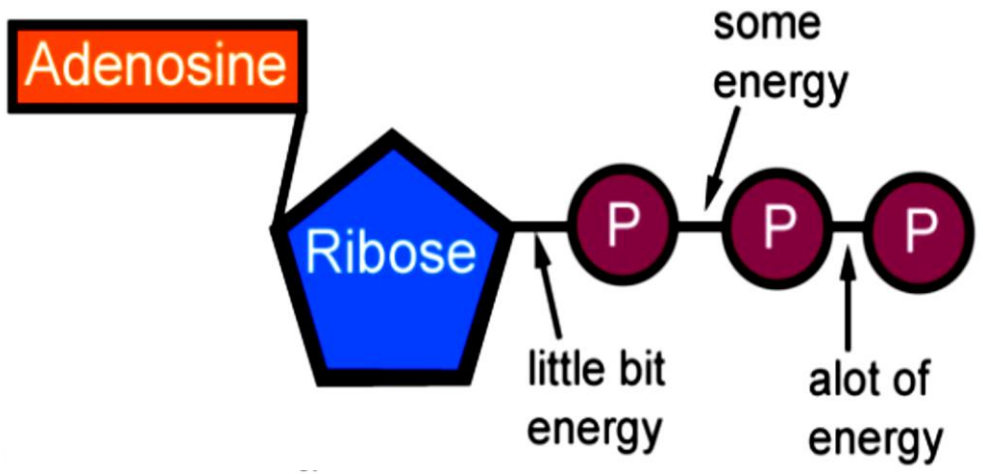
Dr. Esam Hummadi

Metabolism refers to the sum of all chemical reactions within a living organism. Chemical reactions either release or require energy. Metabolism can be viewed as an energy-balancing act.

Catabolism (reactions that release energy)
Complex organic compounds are broken down into simpler ones. These reactions are called catabolic or degradative reactions. They are generally hydrolytic reactions (reactions that use water and in which chemical bonds are broken), and they are exergonic (produce more energy than they consume). For example, cells break down **sugars** into CO_2 and H_2O.

Anabolism (energy requiring reactions)
The building of complex organic molecules from simpler ones. These reactions are called anabolic or biosynthetic and they are generally dehydration synthesis reactions (reactions that release water), and they are endergonic (consume more energy than they produce). For example, formation of **proteins** from amino acids, **nucleic acids** from nucleotides, **polysaccharides** from simple sugars)

These reactions generate the materials for growth. This coupling of energy requiring and energy-releasing reactions is made possible through the molecule **adenosine triphosphate (ATP)** (Figure 1). ATP **stores** energy derived from **catabolic reactions** and releases it later to drive anabolic reactions and perform other cellular work.



Catabolic reactions have been mostly studied with glucose. Four pathways of glucose breakdown to pyruvic acid (or glycolysis) are currently recognized:

1. The Embden-Meyerhof-Parnas Pathways
2. The pentose Phosphate Pathway
3. The Entner-Duodoroff Pathway
4. The Phosphoketolase Pathway

Catabolic reactions often furnish energy in the form of ATP and other high energy compounds, which are used for biosynthetic reactions. A second function of catabolic reactions is to **provide the carbon skeleton for biosynthesis**. Anabolic reactions lead to the formation of **larger molecules** some of which are constituents of the cell.

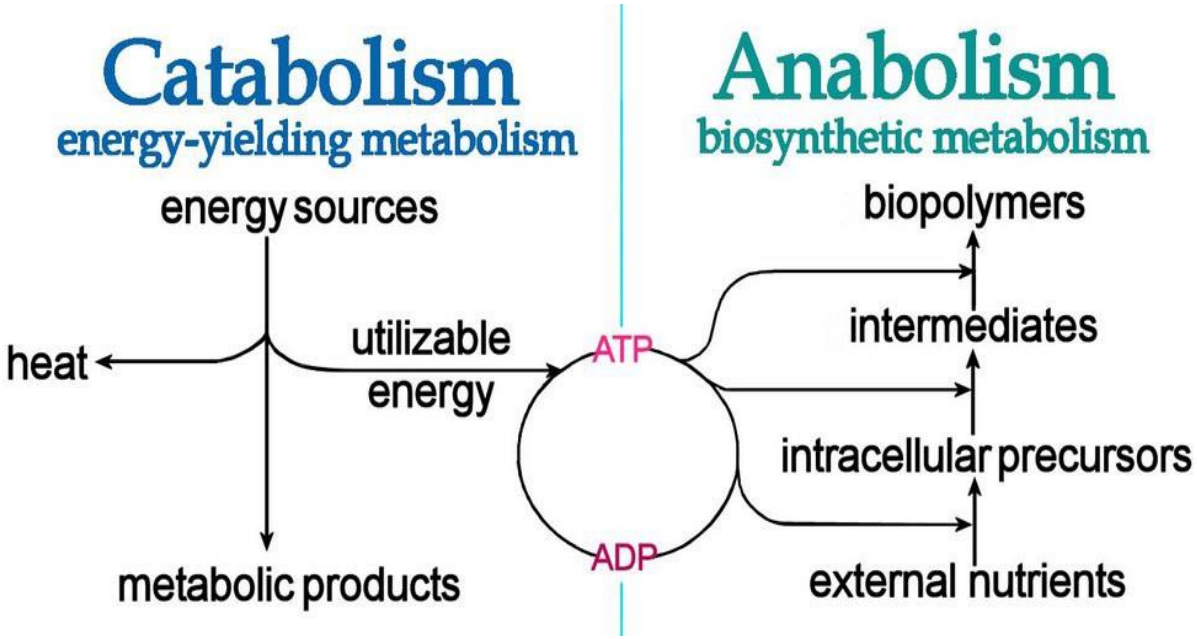


Figure 2. Metabolism: Relationship between Anabolism and Catabolism in a Cell.

1. Products of Primary Metabolism

Primary metabolism is the inter-related group of reactions within a microorganism which are associated with **growth** and the **maintenance of life**. Primary metabolism is essentially the same in all living things and is concerned with the release of energy, and the synthesis of important macromolecules such as **proteins**, **nucleic acids** and other **cell constituents**. When primary metabolism is stopped the **organism dies**. Products of primary metabolism are associated with growth and their maximum production occurs in the **logarithmic phase** of growth in a batch culture (see Lecture 2 Figure 6). Primary catabolic products include ethanol, lactic acid, and butanol while anabolic products include amino-acids, enzymes and nucleic acids. Single-cell proteins and yeasts would also be regarded as primary products (Table 1)

Table 1. Some industrial products resulting from primary metabolism.

Anabolic Products	Catabolic Products
1. Enzymes products, e.g. wines	1. Ethanol and ethanol-containing
2. Amino acids	2. Butanol
3. Vitamins	3. Acetone
4. Polysaccharides	4. Lactic acid
5. Yeast cells	5. Acetic acid (vinegar)
6. Single cell protein	
7. Nucleic acids	
8. Citric acid	

2. Products of Secondary Metabolism

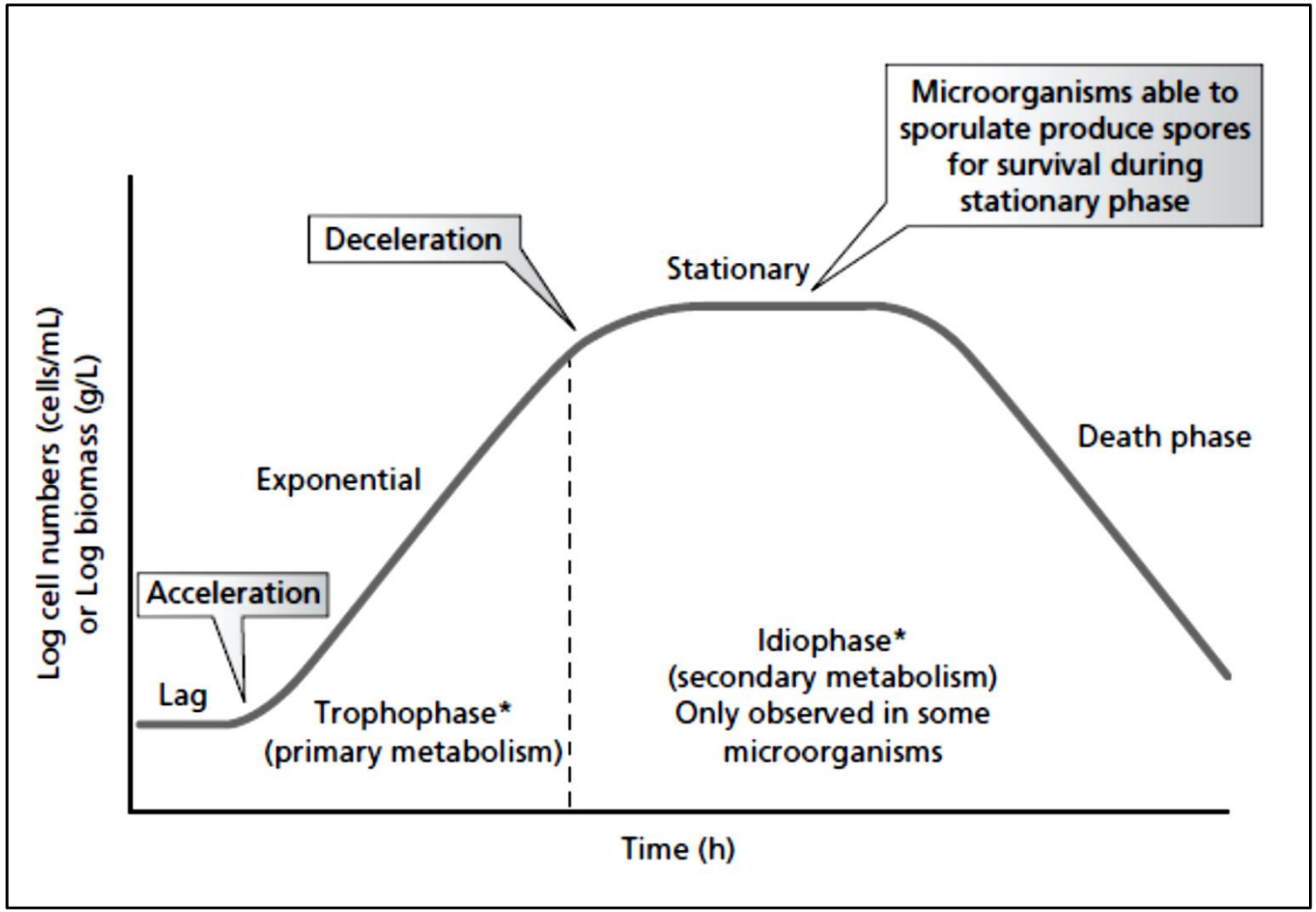
Secondary metabolism has no **apparent function** in the organism. The organism continues to exist if secondary metabolism is **blocked** by a suitable biochemical means. On the other hand it would die if primary metabolism were stopped. Secondary metabolites are produced in response to a **restriction** in nutrients. They are therefore produced after the growth phase, at the end of the logarithmic phase of growth and in the stationary phase (in a batch culture) (see Lecture 2 Figure 6). They can be more precisely controlled in a **continuous culture** (why?).

Table 2 Some industrial products of microbial secondary metabolism.

Product	Organism	Use/Importance
Antibiotics		
Penicillin	Penicillium chrysogenum	Clinical use
Streptomycin	Streptomyces griseus	Clinical use
Anti-tumor Agents		
Actinomyin	Streptomyces antibioticus	Clinical use
Bleomycin	Streptomyces verticulus	Clinical use
Toxins		
Aflatoxin	Aspergiulus flavous	Food toxin
Amanitine	Amanita sp	Food toxin
Alkaloids		
Ergot alkaloids	Claviceps purpurea	Pharmaceutical
Miscellaneous		
Gibberellic acid	Gibberalla fujikuroi	Plant growth hormone
Patulin	Penicillium urticae	Anti-microbial agent

Trophophase-idiophase relationships in the production of secondary products

From studies on *Penicillium urticae* the terms trophophase and idiophase were introduced to distinguish the two phases in the growth of organisms producing secondary metabolites (see Lecture 2 Figure 6). The trophophase (Greek, tropho = nutrient) is the **feeding phase** during which primary metabolism occurs. In a batch culture this would be in the logarithmic phase of the growth curve. Following the trophophase is the idio-phase (Greek, idio = peculiar) during which secondary metabolites peculiar to, or characteristic of, a given organism are synthesized. Secondary synthesis occurs in the **late logarithmic**, and in the stationary phase. It has been suggested that secondary metabolites be described as 'idiolites' to distinguish them from primary metabolites.


Microorganisms able to sporulate produce spores for survival during stationary phase
Deceleration
Stationary
Death phase
Exponential
Acceleration
Lag
Trophophase*
(primary metabolism)
Idiophase*
(secondary metabolism)
Only observed in some
microorganisms
Log cell numbers (cells/mL)
or Log biomass (g/L)
Time (h)

Lecture 6

Microorganisms in Bioprocesses

Dr. Esam Hummadi

Native Microbial Bioprocessors

Microbial bioprocessing in Nature

Microorganisms play a central role in the cycling of carbon in terrestrial environments. Cellulose degraders contribute to ca. 90% of global carbon cycling of plant-derived cellulose as complex microbial consortia composed of cellulolytic and saccharolytic microorganisms degrade and ferment cellulose anaerobically to CO2, H2, organic acids, alcohols, and fatty acids. In the absence of inorganic electron acceptors such as nitrate, Mn(IV), Fe(III) and sulfate, the products of cellulose fermentation serve as growth substrates for other microorganisms, such as methanogens and homoacetogens, which complete the C cycle by producing CO2 and CH4 .

Microbial bioprocessing in Industry:

Bioconversion to Ethanol

To convert biomass to ethanol, pretreated substrates must first be hydrolyzed to fermentable sugars. Cellulose is hydrolyzed by cellobiohydrolases (exoglucanases) that cleave cellobiose off the ends of cellulose chains, endoglucanases that hydrolyze internal bonds along the chain, and β-glucosidase that splits cellobiose into glucose units. Since hemicellulose is composed of a variety of hexose and pentose sugars, various other enzymes are required such as xylanases, mannases, and galactosidases depending on the biomass composition.

Cellulomonads

Cellulomonas spp. play a critical role in decomposition in many terrestrial ecosystems as they are the only bacteria known to decompose plant litter aerobically and anaerobically. Their versatile metabolism is due to their unique ability to produce free non-complexed enzymes, which are the major enzymatic strategy for aerobic decomposition, as well as enzyme complexes or cellulosomes, analogous to those produced by anaerobic decomposers.

The main habitat of this genus is soil, but they are often found in other cellulose enriched environments such as rumen, activated sluge, and sugar fields. Cellulomonads were of original interest to industry as efficient producers of single cell protein for use as a food additive. *Cellulomonas* spp. were used to digest agricultiral residues like sugar cane bagasse and rice straw to produce single cell protein.

Microbial bioprocessing in industry:

Wastewater treatment

Anaerobic digestion is a method of using a complex mixture of anaerobic microorganisms to degrade organic matter in industrial wastewater and solid wastes to reduce contaminants and release energy via biomethanation. The pulp and paper mill industry for example, began using anaerobic wastewater treatment in the 1970s. Various other industries have also employed similar methods including textile industries for the degradation of color effluents, fisheries as well as distilleries and the food industry.

Clostridia

The *Clostridium* genus encompasses a broad range of gram-positive, rod-shaped, endospore forming bacteria making up the 2nd largest bacterial genus. The group of organisms (organized into 19 clusters) is often associated with disease-causing bacteria and toxins, but these organisms also are filled with potential for biotechnological applications.

Lecture 7

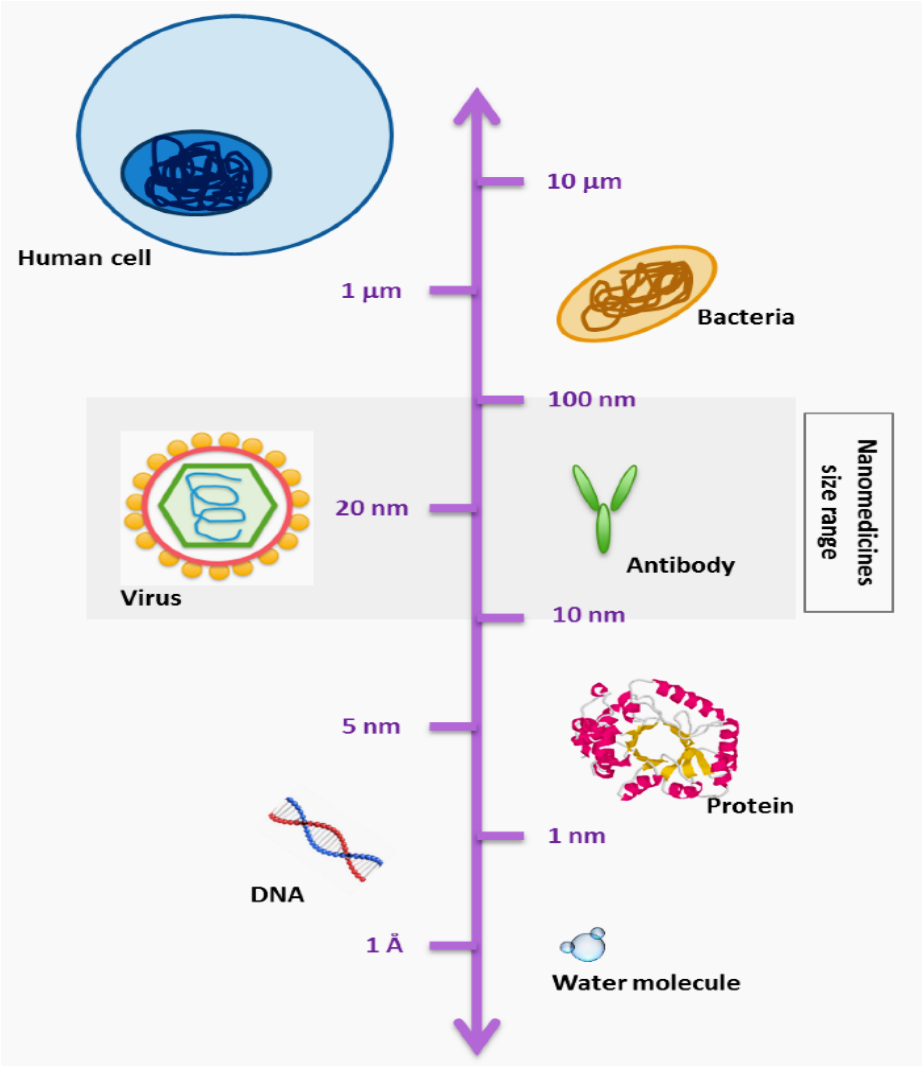
Nanobiotechnology

Dr. Esam Hummadi

Scientists always seek inspiration from nature for their research. The majority of biological systems found on Earth are at the nanoscale level. Incorporating all the biological principles, a scientist can get in-lab results using chemistry, physics, nanotechnology and engineering principles for the production, characterisation and formulation of nanoscale biological molecules and devices using biological systems.

Using these facts, a new technology came into existence, known as nanobiotechnology. **Nanobiotechnology is the fusion of biotechnology and nanotechnology**. It has a combination of molecular biological approaches with classical microtechnology. Biotechnology makes use of the knowledge and techniques of biology to manoeuvre genetic, molecular and cellular processes to build products and services, and is utilised in different fields from **agriculture to medicine**.

Any object possessing any one dimension **between 1-100 nm can be defined as "nanomaterial".** When we deal with nanostructures, the ratio between surface (or interface) and inner atoms becomes significant. This means that the quantistic effect and surface atoms with partial coordination influences strongly the physical and chemical behavior of the nanomaterials with that of the bulk solids.



Nanobiotechnology is a young and rapidly evolving field of research in nanoscience and it is an interdisciplinary area which complies advances in Science and Engineering. **Nanobiotechnology is a field that concerns the utilization of biological system optimized through evolution, such as cells, cellular components, nucleic acid, and proteins to facilitate functional nanostructured and mesoscopic architecture comprised of organic and inorganic materials.**

Biofunctionlization of nanoparticles is an important contribution of present day nanobiotechnology. On the other hand, bionanotechnology generally refers to the study of how the goals of nanotechnology can be guided by studying how biological "machines" work and adapting these biological motifs into improving existing nanotechnologies or creating new ones.

Overview of Emerging Nanobiotechnology Field

- Imagine cancer drugs that travel through the body attacking only cancer cells.
- Imagine bandages that release special particles to stop bleeding and infection.
- Imagine safer cheaper vaccines and faster, cheaper, more sensitive blood tests for common diseases.
- Imagine hospital bed sheets that prevent bedsores.

In their 2003 book The Next Big Thing is Really Small, Deb Newberry and Jack Uldrich define nanotechnology as:
“the willful manipulation of matter at the atomic level to create better and entirely new materials, devices, and systems.”

The National Nanotechnology Initiative (United States National Nanotechnology Initiative, n.d.) defines nanotechnology as:
“the understanding and control of matter at dimensions between approximately 1 and 100 nanometers (nm), where unique phenomena enable novel applications not feasible when working with bulk materials or even with single atoms or molecules.”

Nanometers (nm)= 1×10^{-9} meter

Three areas in medicine already being transformed by nanobiotechnology are:

(1) drugdelivery systems
(2) diagnostic tests
(3) biocompatible coatings for implants, such as replacement joints.

Nanobiotechnology at a glance

Biotechnology and nanotechnology are two of the 21st century's most promising technologies. Nanotechnology (sometimes referred to as nanotech) is defined as the design, development and application of materials & devices whose least functional make up is on a nanometer scale.

Generally, nanotechnology deals with developing materials, devices, or other structures possessing at least one dimension sized from 1 to 100 nanometers. Meanwhile, Biotechnology deals with metabolic and other physiological processes of biological subjects including microorganisms. Association of these two technologies, i.e. nanobiotechnology can play a vital role in developing and implementing many useful tools in the study of life.

Advantages of nanobiotechnology

The pathophysiological conditions and anatomical changes of diseased or inflamed tissues can potentially trigger a great deal of scopes for the development of various targeted nanotechnological products. This development is like to be advantageous in the following ways:

1. Drug targeting can be achieved by taking advantage of the distinct pathophysiological features of diseased tissues.
2. Various nanoproducts can be accumulated at higher concentrations than normal drugs.
3. Increased vascular permeability coupled with an impaired lymphatic drainage in tumors improve the effect of the nanosystems in the tumors or inflamed tissues through better transmission and retention.
4. Nanosystems have capacity of selective localization in inflammed tissues.
5. Nanoparticles can be effectively used to deliver/transport relevant drugs to the brain overcoming the presence of blood–brain barrier (meninges).
6. Drug loading onto nanoparticles modifies cell and tissue distribution and leads to a more selective delivery of biologically active compounds to enhance drug efficacy and reduces drug toxicity.

Applications of nanobiotechnology in medical and clinical fields

A number of clinical applications of nanobiotechnology, such as disease diagnosis, target-specific drug delivery, and molecular imaging are being laboriously investigated at present. Some new promising products are also undergoing clinical trials [12,13]. Such advanced applications of this approach to biological systems will undoubtedly transform the foundations of diagnosis, treatment, and prevention of disease in future. Some of these applications are discussed below.

Diagnostic applications

Current diagnostic methods for most diseases depend on the manifestation of visible symptoms before medical professionals can recognize that the patient suffers from a specific illness. But by the time those symptoms have appeared, treatment may have a decreased chance of being effective.
Therefore the earlier a disease can be detected, the better the chance for a cure is. Optimally, diseases should be diagnosed and cured before symptoms even manifest themselves. Nucleic acid diagnostics will play a crucial role in that process, as they allow the detection of pathogens and diseases/diseased cells at such an early symptomless stage of disease progression that effective treatment is more feasible. Current technology, such as- polymerase chain reaction (PCR) leads toward such tests and devices, but nanotechnology is expanding the options currently available, which will result in greater sensitivity and far better efficiency and economy.

Detection

Many currently used/conventional clinical tests reveal the presence of a molecule or a disease causing organism by detecting the binding of a specific antibody to the disease-related target.

Traditionally, such tests are performed by conjugating the antibodies with inorganic/ organic dyes and visualizing the signals within the samples through fluorescence microscopy or electronic microscopy. However, dyes often limit the specificity and practicality of the detection methods. Nanobiotechnology offers a solution by using semiconductor nanocrystals (also referred to as "quantum dots"). These minuscule probes can withstand significantly more cycles of excitations and light emissions than typical organic molecules, which more readily decompose.

Therapeutic applications

Nanotechnology can provide new formulations of drugs with less side effects and routes for drug delivery.

Drug Delivery

Nanoparticles as therapeutics can be delivered to targeted sites, including locations that cannot be easily reached by standard drugs. For instance, if a therapeutic can be chemically attached to a nanoparticle, it can then be guided to the site of the disease or infection by radio or magnetic signals. These nanodrugs can also be designed to "release" only at times when specific molecules are
present or when external triggers (such as infrared heat) are provided. At the same time, harmful side effects from potent medications can be avoided by reducing the effective dosage needed to treat the patient. By encapsulating drugs in nanosized materials (such as organic dendrimers, hollow polymer capsules, and nanoshells), release can be controlled much more precisely than ever before. Drugs are designed to carry a therapeutic payload (radiation, chemotherapy or gene therapy) as well as for imaging applications.