SUBJECT «BIOCHEMICAL ENGINEERING»
FINAL LECTURE ON MODULE 1

LECTURE 5 – PRESS-CONFERENCE
INTRODUCTION: BIOPROCESS PRODUCTS

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What is bioprocess?

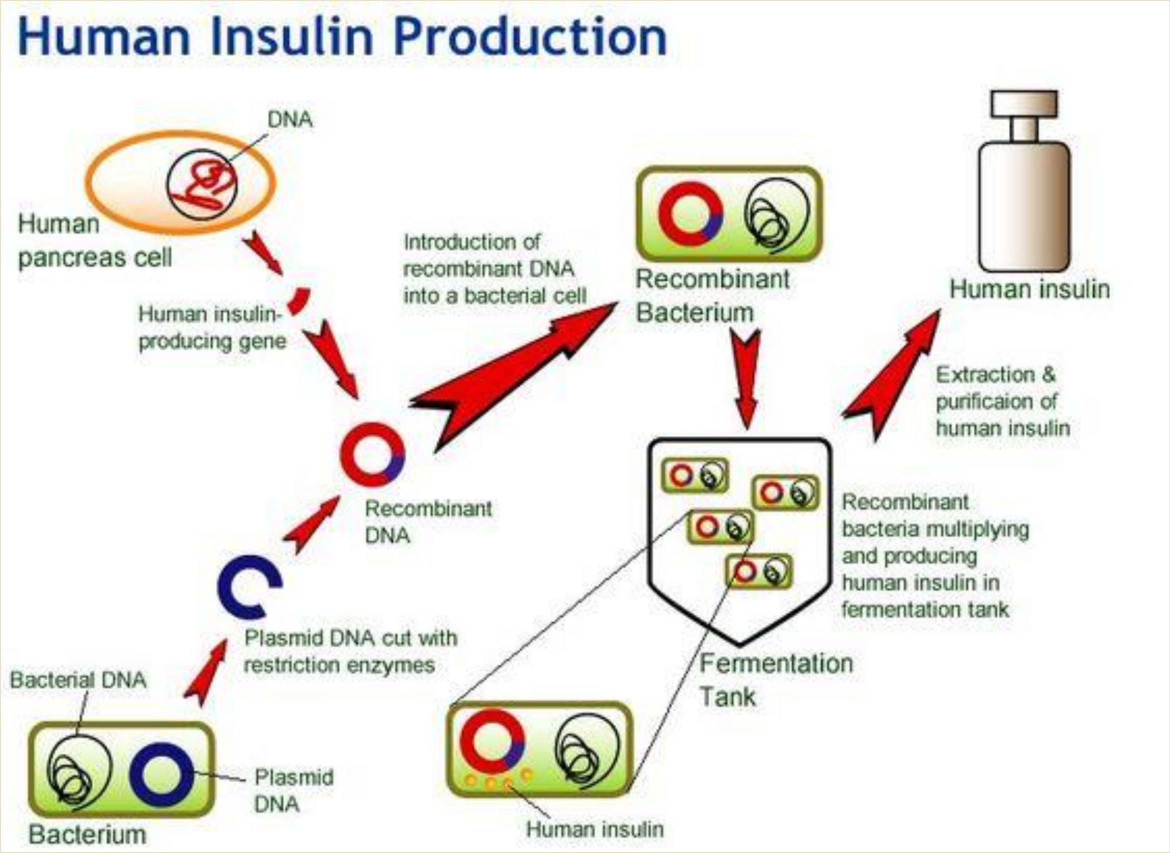
- A **bioprocess** is a specific process that uses complete living cells or their components (e.g., bacteria, enzymes, chloroplasts) to obtain desired products.
- Transport of energy and mass is fundamental to many biological and environmental processes. Areas, from
  - food processing (including brewing beer)
  - thermal design of buildings
  - biomedical devices
  - pollution control and global warming
- require knowledge of how energy and mass can be transported through materials (momentum, heat transfer, etc.).

Bioprocess engineering

- **Bioprocess engineering**, also biochemical engineering, is a specialization of chemical engineering or Biological engineering, It deals with the design and development of equipment and processes for the manufacturing of products such as ***agriculture, food, feed, pharmaceuticals, nutraceuticals, chemicals***, and ***polymers*** and ***paper from biological materials & treatment of waste water.***
- Bioprocess engineering is a conglomerate of ***mathematics***, ***biology*** and ***industrial design***, and consists of various spectrums like designing of bioreactors, study of fermenters (mode of operations etc.). It also deals with studying various biotechnological processes used in industries for large scale production of biological product for optimization of yield in the end product and the quality of end product.
- Bioprocess engineering may include the work of mechanical, electrical, and industrial engineers to apply principles of their disciplines to processes based on using living cells or sub component of such cells.

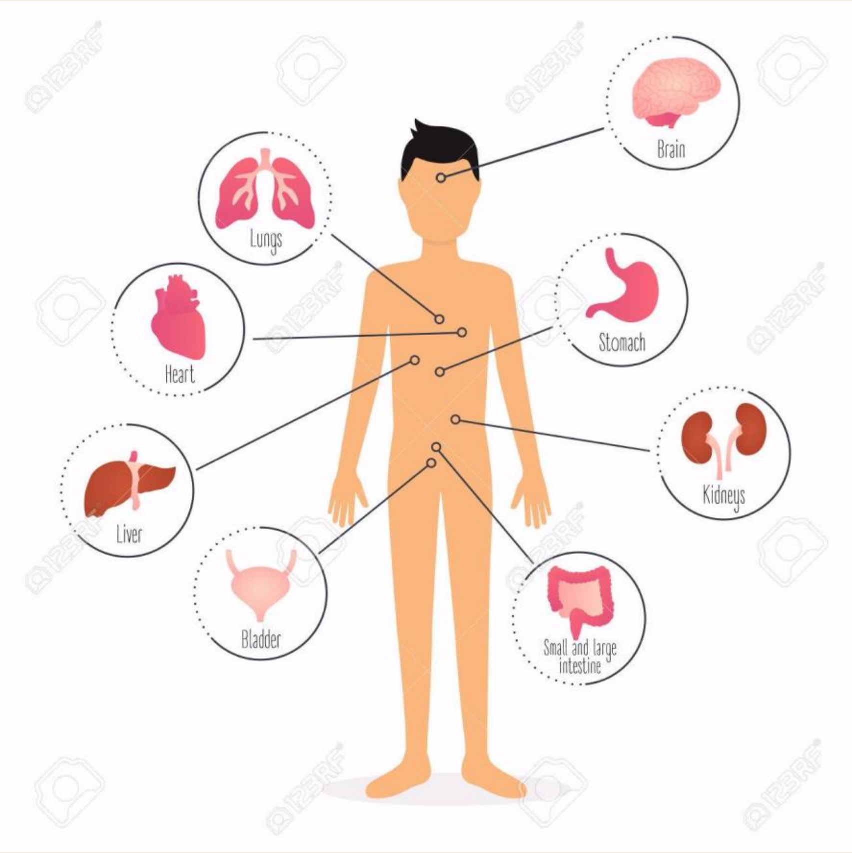
Historical development

- Engineering processes have been required for human survival and for satisfying to different requirements throughout human culture. Current bio-process technology is an extension of ancient techniques for developing significant products by taking uses of **natural biological activities**. Early biotechnological processes that use microbes to produce a fixed product have been used for many thousand years. When our early ancestors made alcoholic liquid beverages, they used a bioprocess method the combination of live yeast cells and nutrients (like cereal grains) **create a fermentation system** in which the organisms used the nutrients for their own growth and produced several by-products (as alcohol and carbon dioxide gas) that helped to produce the liquid beverage.



- Although today's modern bioprocess technology is based on the **same principle or method**: combining living matter (whole organisms or enzymes) with nutrient supplements under the controlled conditions require to make the specific end product. Modern biotechnology started when general knowledge about biological system, their components, and interaction between components grew. In the first half of the **20th century**, the first biggest scale fermentation processes, namely ***penicillin and citric acid***, were realized. The process of recombinant DNA technology or genetic engineering then led to a substantial increase in the number of bioprocesses and their manufacturing volume starting with ***insulin***, the first product produced with recombinant DNA technology.

- The most highly visible significance has been in the area of **human health care**, with product such as interferon, tissue plasminogen activator, human insulin, erythropoietin, and monoclonal antibody-based bio products. In addition, usage new products for agriculture, food industry, good chemicals and the environments are also under intense development. Thus the field of bioprocessing has become a very significant part of biotechnology.



hhc

human health care

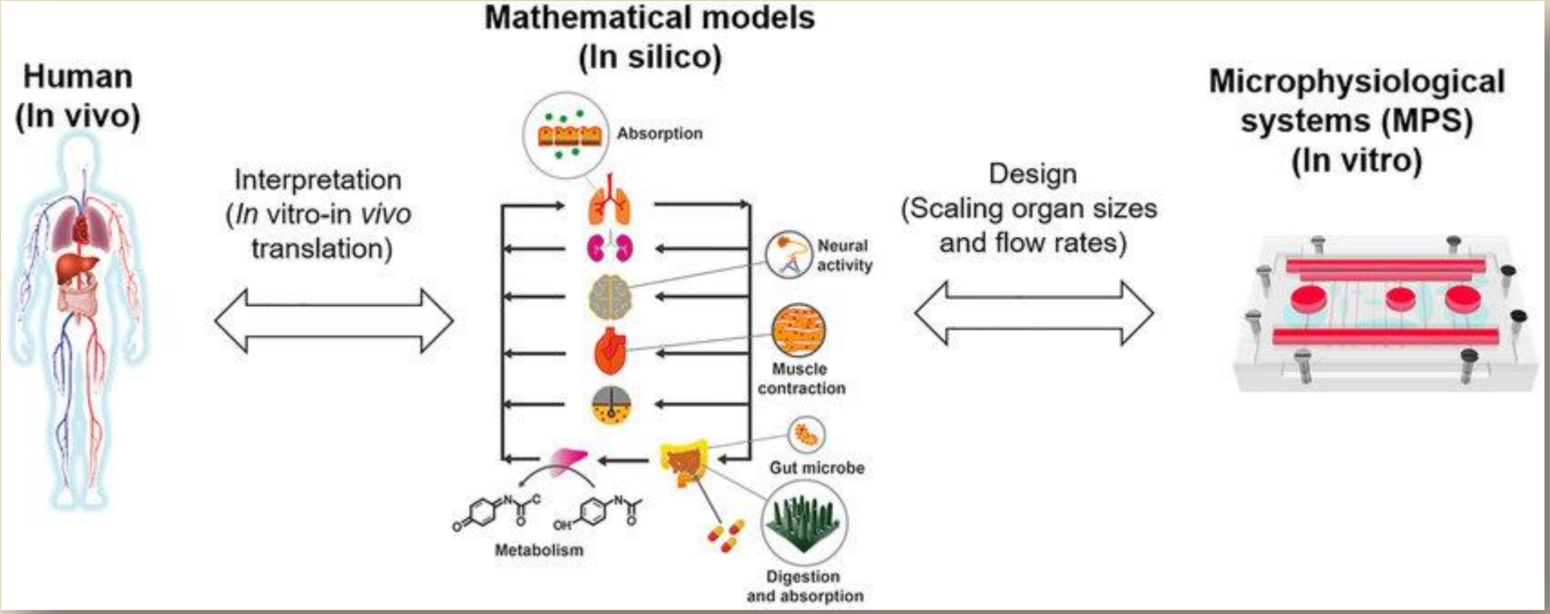
Milestones of bioprocess development

Chronology	Milestones
6000 BC	Brewing (Sumeria, Babylonia)
2400 BC	**The first bioprocess complete description (ancient Egyptian)**
1680	**Yeast under the microscope (van Leeuwenhoek)**
1835	Alcoholic fermentation associated with yeast
1857	Fermentation correlated with metabolism (Pasteur)
1877	Term “enzyme” (in yeast) introduced (Kühne)
1923	Industrial production of Citric acid
1930s	Industrial production of Amino acids
1940s	**Industrial production of Antibiotics**
1979	**Monoclonal antibody production by hybridoma cell**
1982	**Industrial Production of rHuman Insulin in *E. coli* (Eli Lily)**
1984	**First commercial production of therapeutic MAb (Anti CD3)**
1994	First commercial vaccine from recombinant yeast (Hepatitis B vaccine)
1996	**Completion of the yeast genome project for *S. cerevisiae*.**
2000	**10 m^3 STR for Mammalian cell culture**
2002	Disposable bioreactors were used in industrial scale
2007	**The Bioprocess products market exceeded 700 Billion US$. Only the Biopharmaceuticals market reached 70 Billion US$. Mammalian cell culture products reached 25 Billions US$**

Applications and research performance

- Bioprocess engineering uses the abilities of microbes to produce a great variety of bio products for medical, industrial and agricultural significance. In current time computer based bioreactors are used to create large or best quantities or quality of important biological materials such as ***recombinant proteins, antibiotics or viral vaccines, amino acids.***
- Many of the potential new drugs are generated in eukaryotic living cells which express poor productivity vs to classical fermentation processes. To take the full potential of biotech based production process, highly developed cell culture technologies and sophisticated product recovery steps have to be established.
- Furthermore, current progress in high-throughput analysis of DNA and protein expression, protein function or signal transduction will help to elucidate methods of cellular behavior and offer tremendous opportunities for optimizing bioprocesses or makes a new opportunity in pharmacy and medicine field.

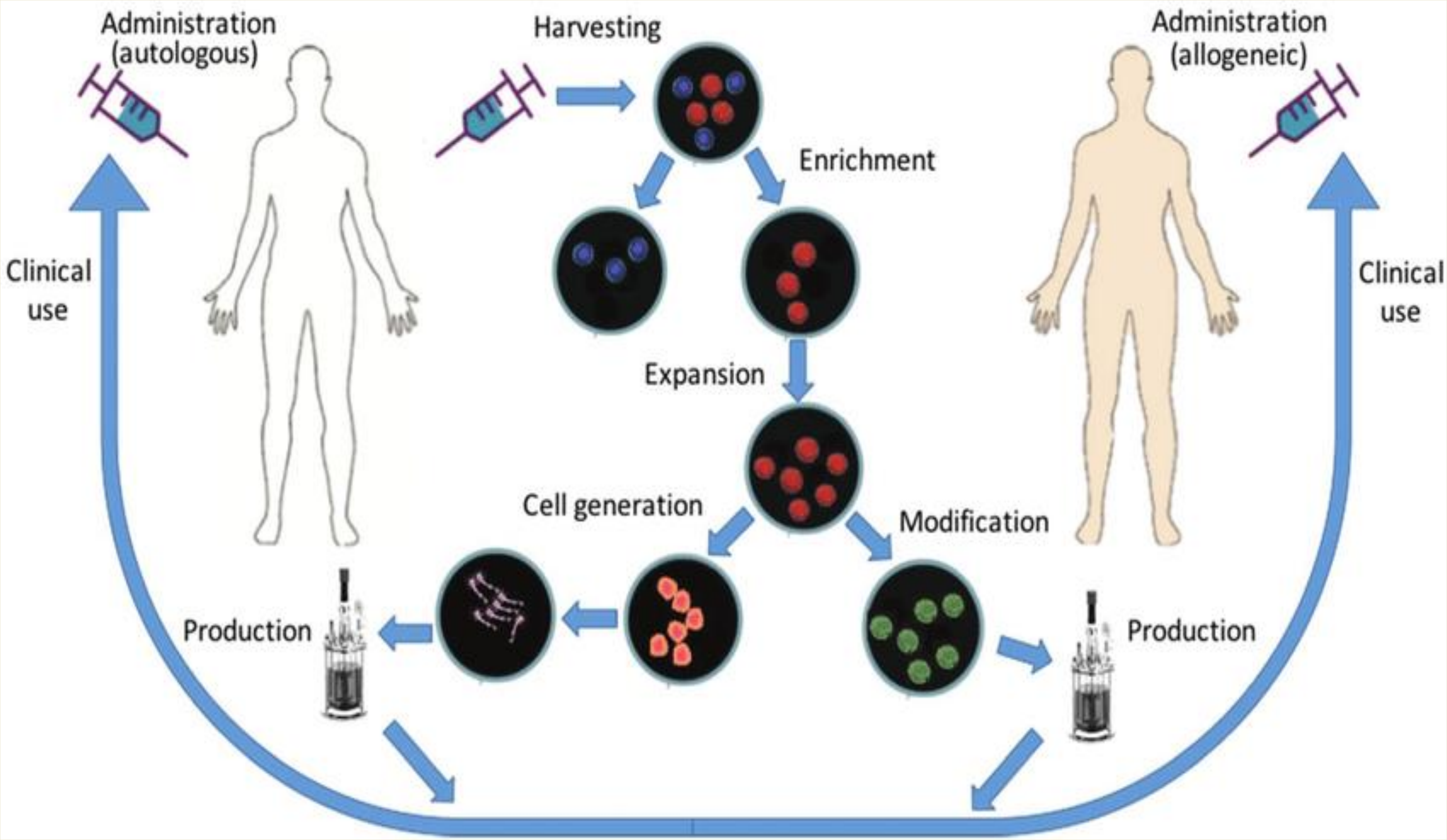
- **Math based modeling** plays a crucial role in testing and viewing in vitro cell culture technology. Without such models it is manually impossible to quantitatively understand metabolic pathways and regulatory networks of living cells, compact interactions between microbes and their environment in a bioreactor or to allow for a rational design of downstream processing steps to larger yield and purity of the last product or byproduct.
- Bioprocess engineering encompass many different aspects of biotechnology and genetic engineering, including fundamental work in bimolecular interactions and many different significances are being researched, including food preservation and food processing, the manufacturing of pharmaceuticals, air and wastewater treatment; bio-based structural motifs for supra-molecular architectures; microfluidics as used to bioreactors.



Cell bioprocessing

- **Cell therapy bioprocessing** is a discipline that bridges the fields of cell therapy and bioprocessing (i.e., biopharmaceutical manufacturing), and is a sub-field of bioprocess engineering. The goals of cell therapy bioprocessing are to establish reproducible and robust manufacturing processes for the production of therapeutic cells. Commercially relevant bioprocesses will:

1. Produce products that maintain all of the quality standards of biopharmaceutical drugs
2. Supply both clinical and commercial quantities of therapeutic cells throughout the various stages of development. The processes and production technologies must be scalable
3. Control the cost of goods (CoGs) of the final drug product. This aspect is critical to building the foundation for a commercially viable industry.



Administration (autologous)
Harvesting
Administration (allogeneic)
Enrichment
Clinical use
Clinical use
Expansion
Cell generation
Modification
Production
Production

Fermentation

- **Fermentation** is a metabolic process that produces chemical changes in organic substrates through the action of enzymes. In biochemistry, it is narrowly defined as the extraction of energy from carbohydrates in the absence of oxygen. In food production, it may more broadly refer to any process in which the activity of microorganisms brings about a desirable change to a foodstuff or beverage. The science of fermentation is known as **zymology.**
- In microorganisms, fermentation is the primary means of producing adenosine triphosphate (ATP) by the degradation of organic nutrients anaerobically.

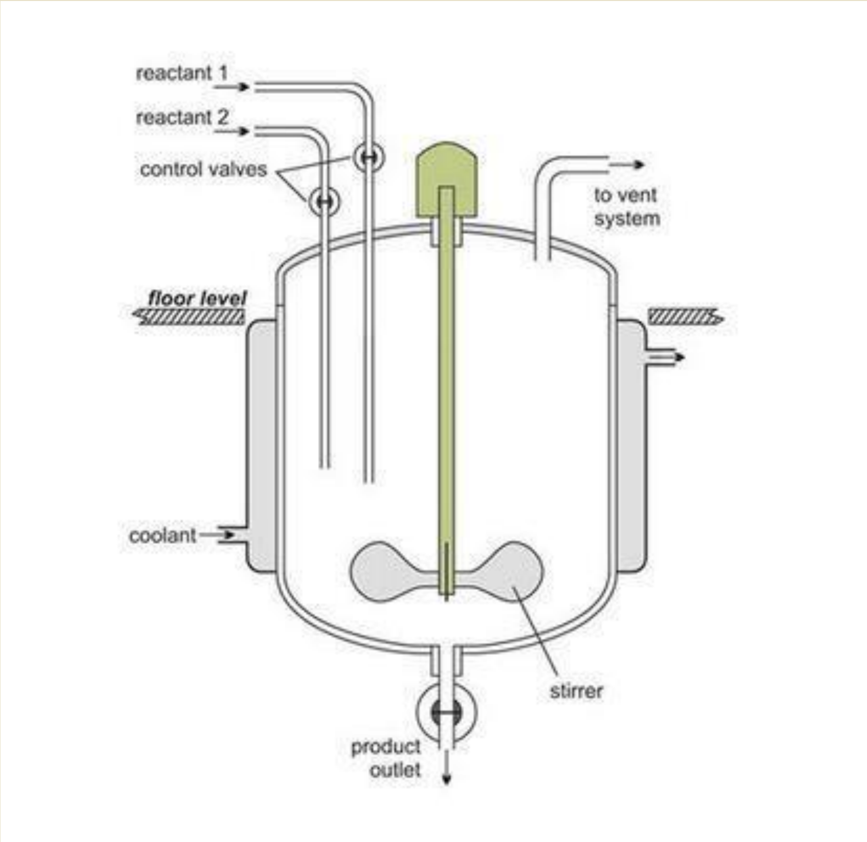
fermentation

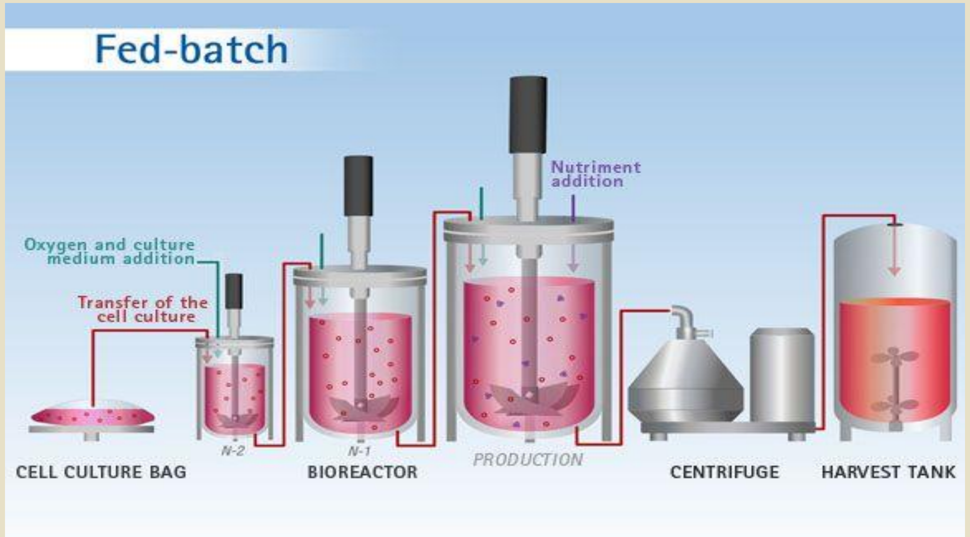
microbes + time = food & drink

Types of Fermentation process

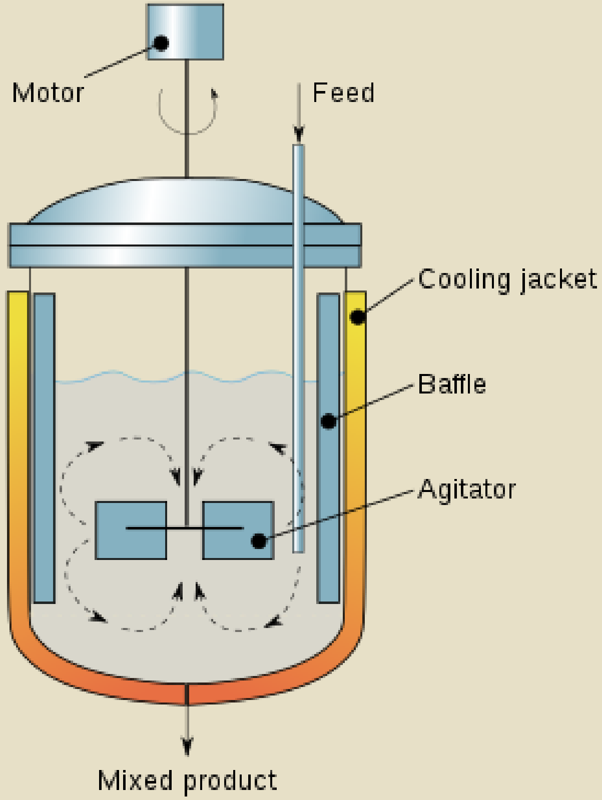
1. **Batch Fermentation**
2. **Continuous Fermentation**
3. **Fed batch**

- **Batch reactors**, simplest type. Reactor is filled with medium and the fermentation is allowed. Fermentation has finished, contents are emptied for downstream processing. The reactor is then cleaned, re-filled, re-inoculated and the fermentation process starts again.



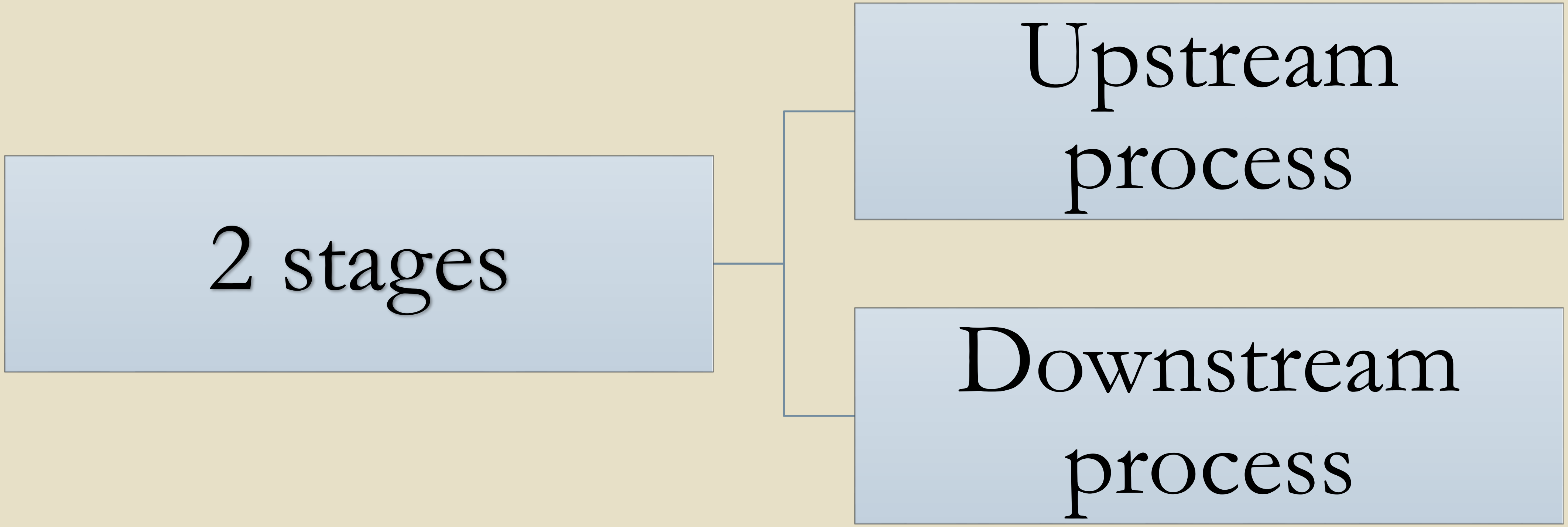


- **Fed batch reactor,** most common type of reactor used in industry. Fresh media is continuous or sometimes periodically added.



- **Continuous reactors,** where fresh media is continuously added and bioreactor fluid is continuously removed. As a result, cells continuously receive fresh medium and products and waste products and cells are continuously removed for processing. The reactor can thus be operated for long periods of time without having to be shut down. Many times more productive than batch reactors. Does not have to be shut down as regularly the growth rate of the bacteria in the reactor can be more easily controlled and optimized cells can also be immobilized in continuous reactors, to prevent their removal.

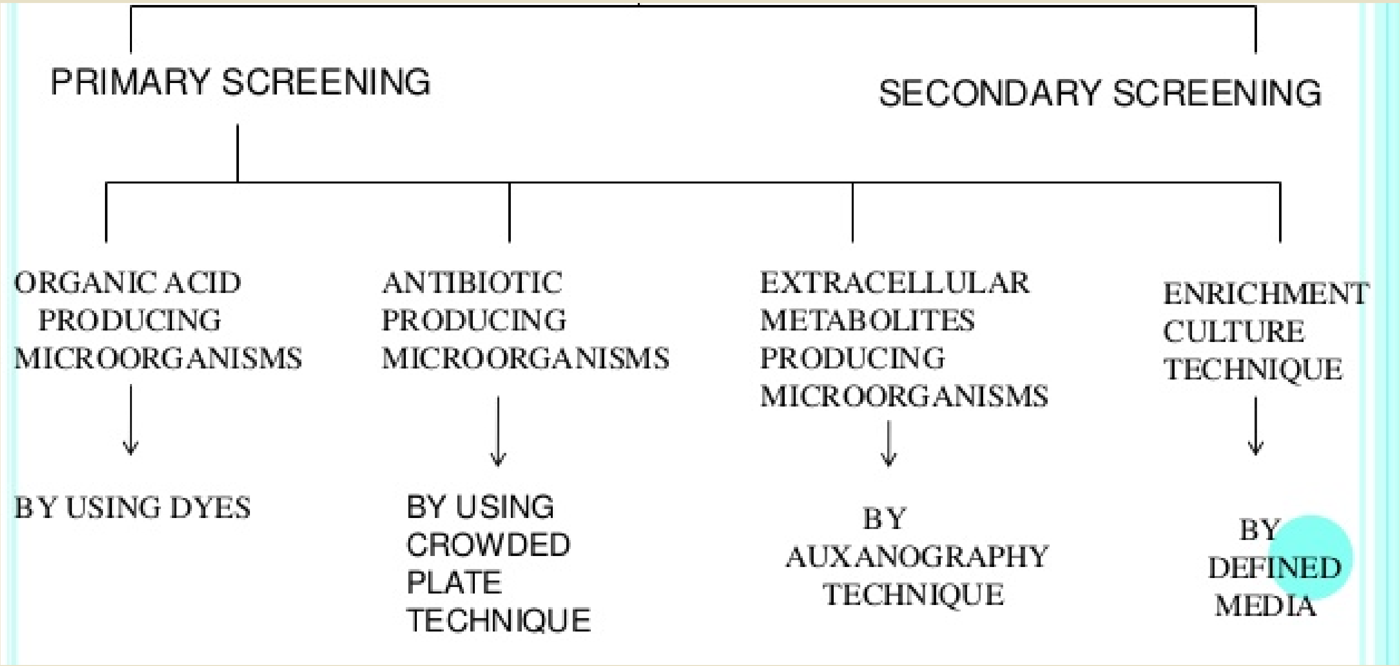
The stages of Fermentation



Upstream processing

- The **upstream process** is defined as the entire process from early cell isolation and cultivation, to cell banking and culture expansion of the cells until final harvest (termination of the culture and collection of the live cell batch).
- The upstream part of a bioprocess refers to the first step in which microbes/cells are grown, e.g. bacterial or mammalian cell lines, in bioreactors. Upstream processing involves all the steps related to inoculum development, media development, improvement of inoculum by genetic engineering process, optimization of growth kinetics so that product development can improve tremendously.

- The pre-fermentation stage:
  - Isolation
  - Improvement
  - Producing of microorganisms
- **Screening method** – process that extracts, isolates and identifies a compound or group of components in a sample with the minimum number of steps and the least manipulation of the sample. There are two types:



- Microbes isolated from natural sources thus is improved to get ***productive strains*** by using
  - Recombination
  - Mutation
  - Cell fusion
  - Gene cloning
- **Media formulation**: growth medium must have essential nutrients for microbial growth for successful fermentation process. There are two kinds of media.

1. ***Inoculum media:*** enrich the culture
2. ***Production media*****:** contain carbon & nitrogen

- **Raw materials:** corn molasses, cellulose, corn, steep liquor soybean, sugar, beet molasses, malt extract etc.

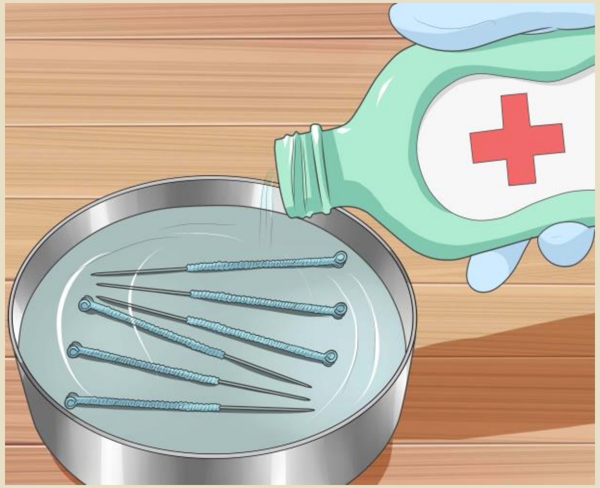
- Upstream processing includes formulation of the fermentation medium, sterilization of air, fermentation medium and the Fermenter, inoculum preparation and inoculation of the medium.
- The **fermentation medium** should contain an energy source, a carbon source, a nitrogen source and micronutrients required for the growth of the microorganism along with water and oxygen, if necessary.
- A medium which is used for a **large scale fermentation**, in order to ensure the sustainability of the operation, should have the following characteristics:

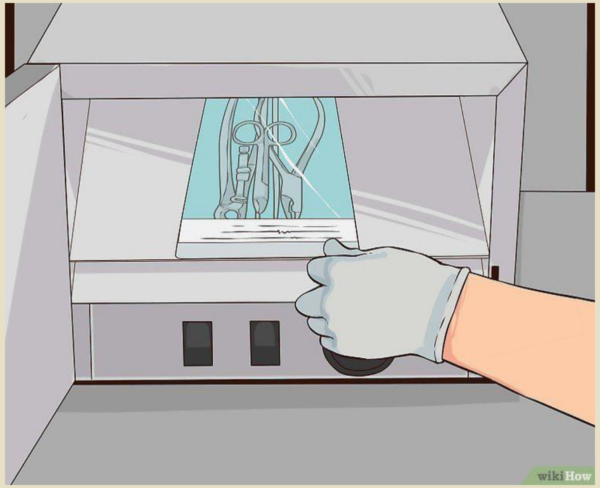
1. It should be cheap and easily available

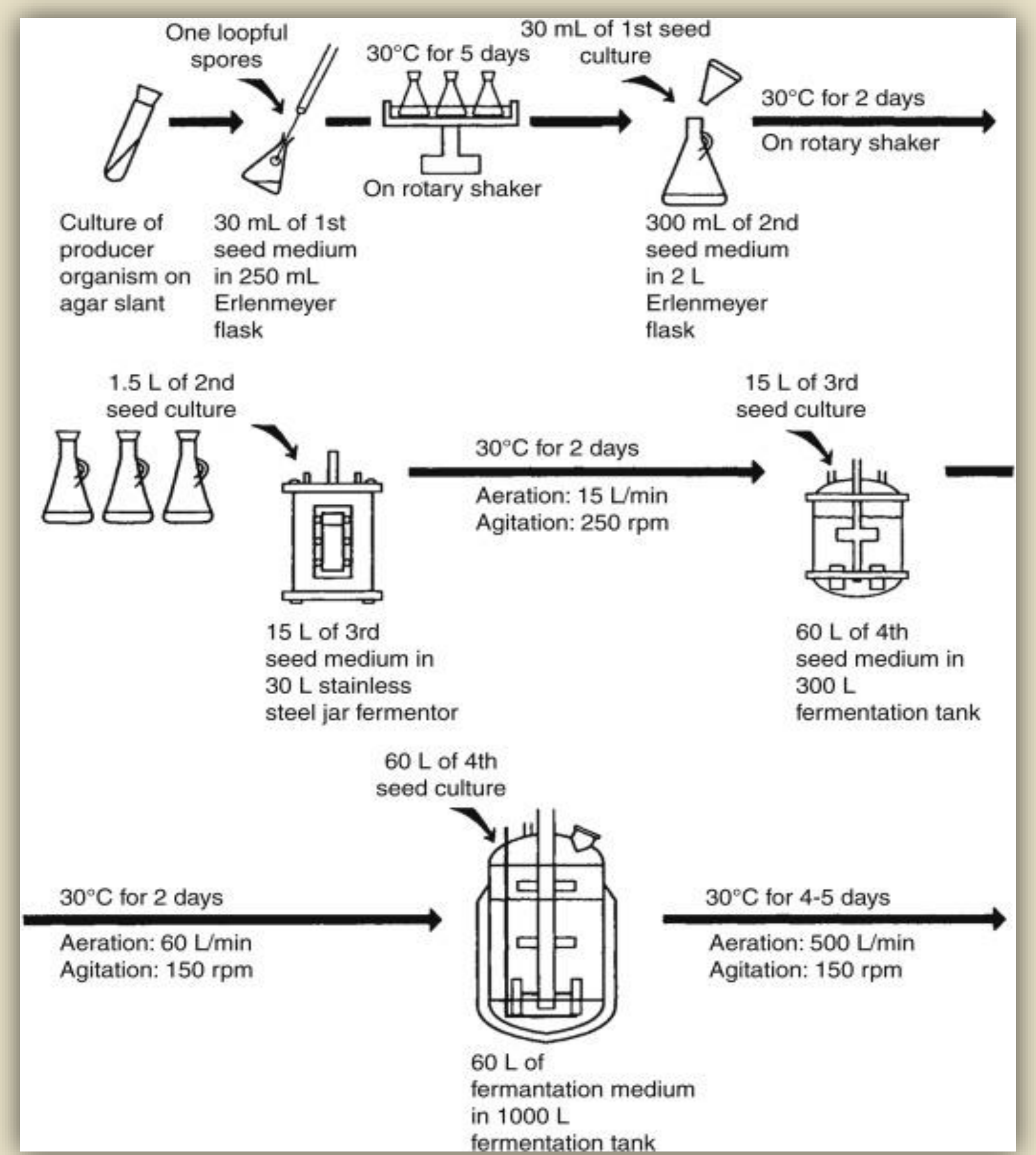
2. It should maximize the growth of the microorganism, productivity and the rate of formation of the desired product

3. It should minimize the formation of undesired products

- Usually, waste products from other industrial processes, such as molasses, lignocelluloses wastes, cheese whey and corn steep liquor, after modifying with the incorporation of additional nutrients, are used as the substrate for many industrial fermentations.
- **Sterilization** is essential for preventing the contamination with any undesired microorganisms.
- **Air** is sterilized by ***membrane filtration*** while the **medium** is usually ***heat sterilized***.
- Any nutrient component which is heat labile is ***filter-sterilized*** and later added to the sterilized medium.
- The fermenter may be sterilized together with the medium or separately.





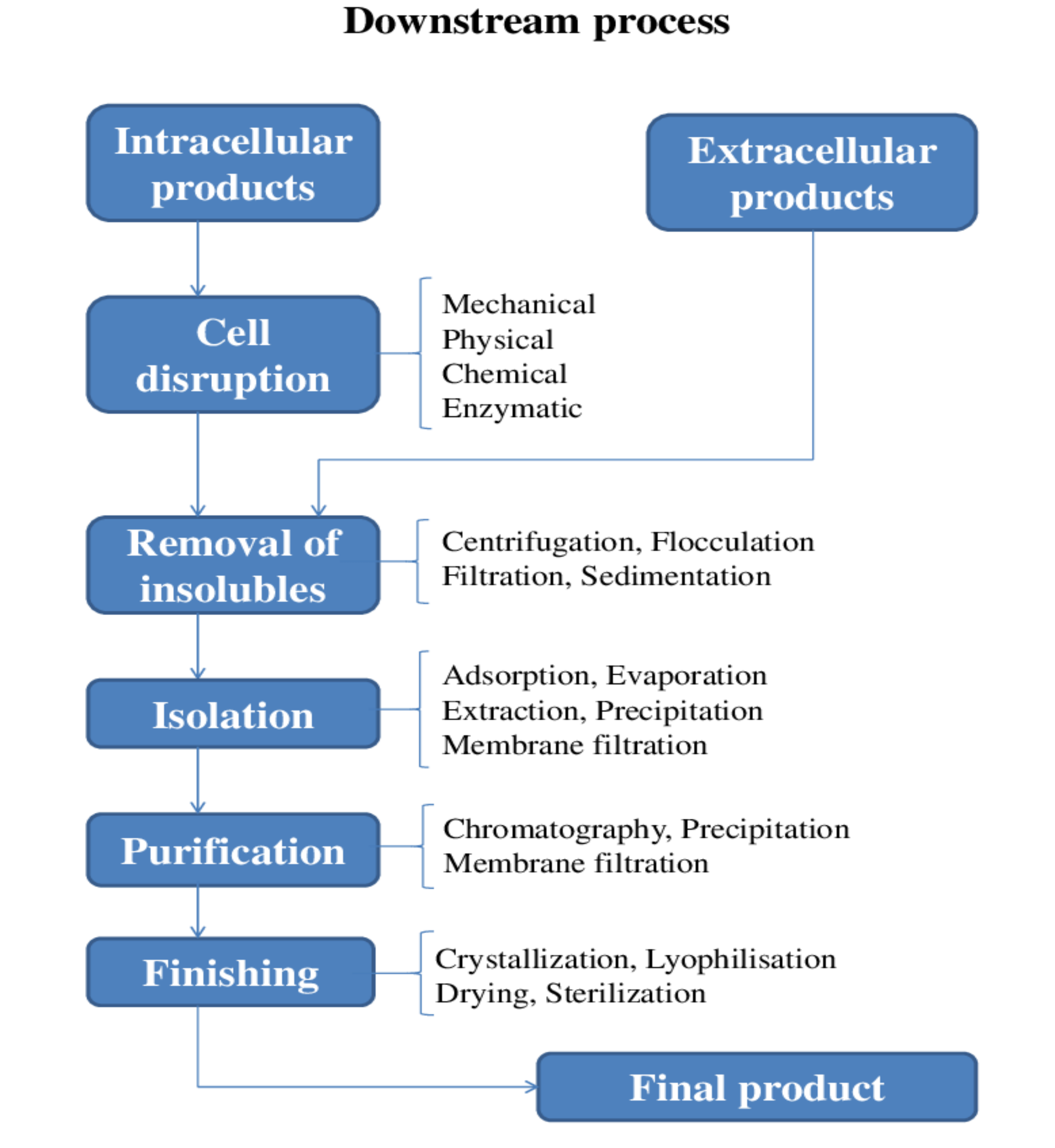


- **Inoculum build up** is the preparation of the seed culture in amounts sufficient to be used in the large Fermenter vessel. This involves growing the microorganisms obtained from the pure stock culture in several consecutive fermenter.
- This process cuts down the time required for the growth of microorganisms in the fermenter, thereby ***increasing the rate of productivity***. Then the seed culture obtained through this process is used to inoculate the fermentation medium.

Downstream processing

- **Downstream processing**, the various stages that follow the fermentation process, involves suitable techniques and methods for ***recovery, purification, and characterization*** of the desired fermentation product. A vast array of methods for downstream processing, such as ***centrifugation, filtration, and chromatography,*** may be applied. These methods vary according to the chemical and physical nature, as well as the desired grade, of the final product.
- Downstream processing encompasses all processes following the fermentation. It has the **primary aim** of efficiently, reproducibly and safely recovering the target product to the required specifications (biological activity, purity) while maximizing recovery yield and minimizing costs.
- Each stage in the overall recovery procedure is strongly dependent on the **protocol of the preceding fermentation**. Fermentation factors affecting downstream processing include the properties of microorganisms, particularly morphology, flocculation characteristics, size and cell wall rigidity. These factors have major influences on the ***filterability, sedimentation and homogenization efficiency***. The presence of fermentation by-products, media impurities and fermentations additives such as antifoams may interfere with downstream processing steps and accompanying product analysis.

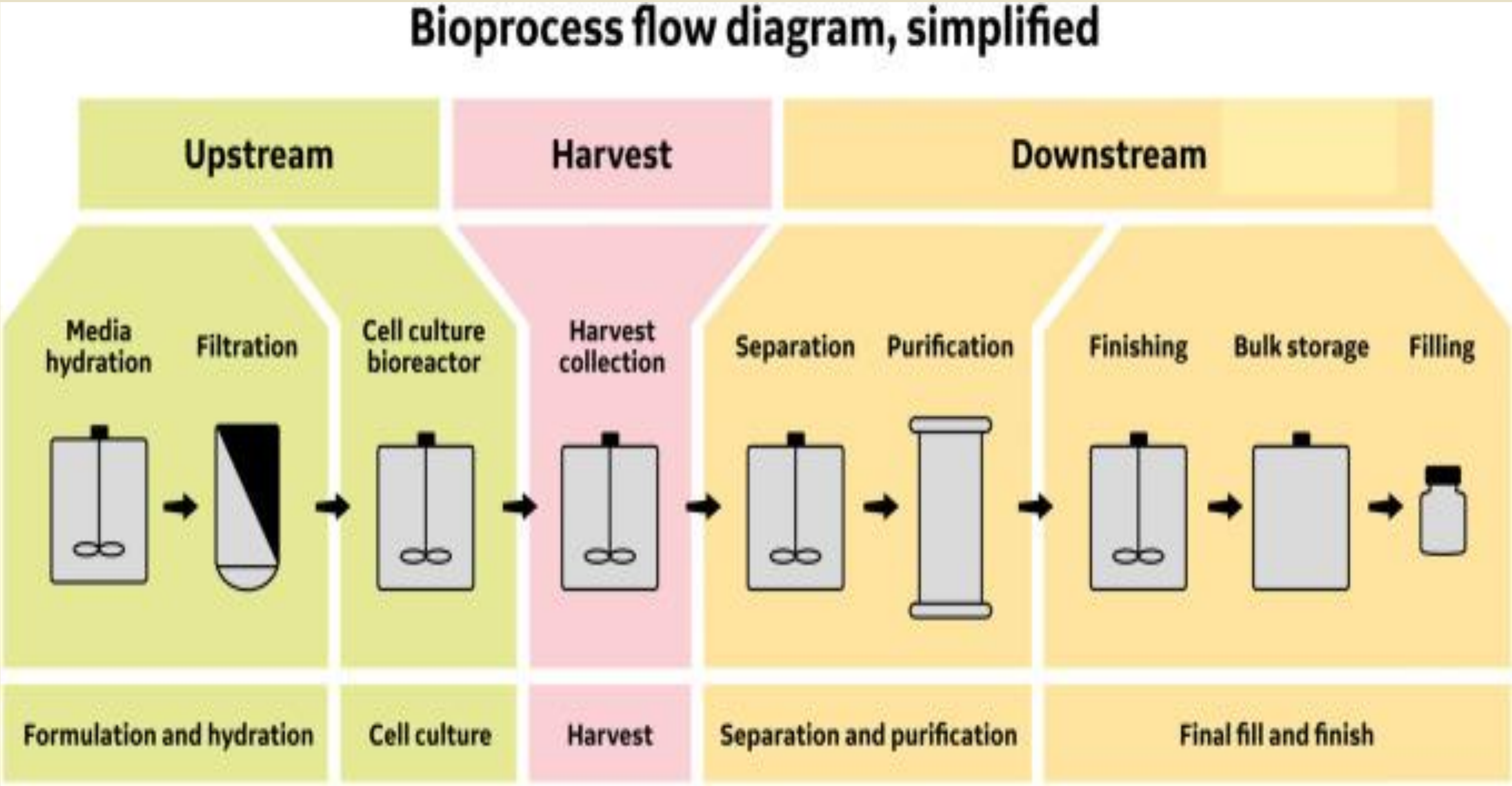
- The products of fermentation are usually found in **complex mixtures** of dilute solutions and must be ***concentrated*** and ***purified***. The typical downstream operations involved in the processing of fermentation broth are shown in this diagram.



The steps of downstream processing

1. **Separation of biomass:** Separating the biomass (microbial cells) generally carried out by centrifugation or ultra-centrifugation. If the product is biomass, then it is recovered for processing and spent medium is discarded. If the product is extra cellular the biomass will be discarded. Ultra filtration is an alternative to the centrifugation.
2. **Cell disruption:** If the desired product is intra cellular the cell biomass can be disrupted so that the product should be released. The solid-liquid is separated by centrifugation or filtration and cell debris are discarded.
3. **Concentration of broth:** The spent medium is concentrated if the product is extracellular.
4. **Initial purification of metabolites:** According to the physico-chemical nature of the product molecule several methods for recovery of product from the clarified fermented broth were used (precipitation, solvent extraction, ultra-centrifugation, ion-exchange chromatography, adsorption and solvent extraction).

5. **Metabolite specific purification:** specific purification methods are used when the desired metabolite is purified to a very high degree.
6. **De-watering:** If low amount of product is found in very large volume of spent medium, the volume is reduced by removing water to concentrate the product. It is done by vacuum drying or reverse osmosis.
7. **Polishing of metabolites:** This is the final step of making the product to 98 to 100% pure. The purified product is mixed with several inert ingredients called excipients. The formulated product is packed and sent to the market for the consumers.


Bioprocess flow diagram, simplified
Upstream
Harvest
Downstream
Media hydration
Filtration
Cell culture bioreactor
Harvest collection
Separation
Purification
Finishing
Bulk storage
Filling
Formulation and hydration
Cell culture
Harvest
Separation and purification
Final fill and finish

Various downstream processes

1. **Filtration**

Filtration is one of the most widely used and efficient processes for solid-liquid separation with different types of filtration systems. The process uses a porous medium that allows the flow of gas or liquids but not the solid material.

A simple filtration apparatus consists of a filtration cloth supported by a porous material. When the broth passes through the filter cloth, the solid material is deposited on it and a cake is formed. Due to the gradual deposition of solid on the cloth, the cake increases in thickness and a resistance in the flow gradually builds up. To make the rate of flow constant, an increasing pressure has to be applied on the cloth with the increase in thickness of the cake and resistance of flow. Sometimes the pores of the filtering cloth may be closed due to the clogging or the flow may be stopped due to the compression of the particles. In that case pressure can not be applied for filtration especially when the particles are compressible.

- Following factors influence the choice of the most suitable equipment :

✓ Viscosity and density of the filtrate

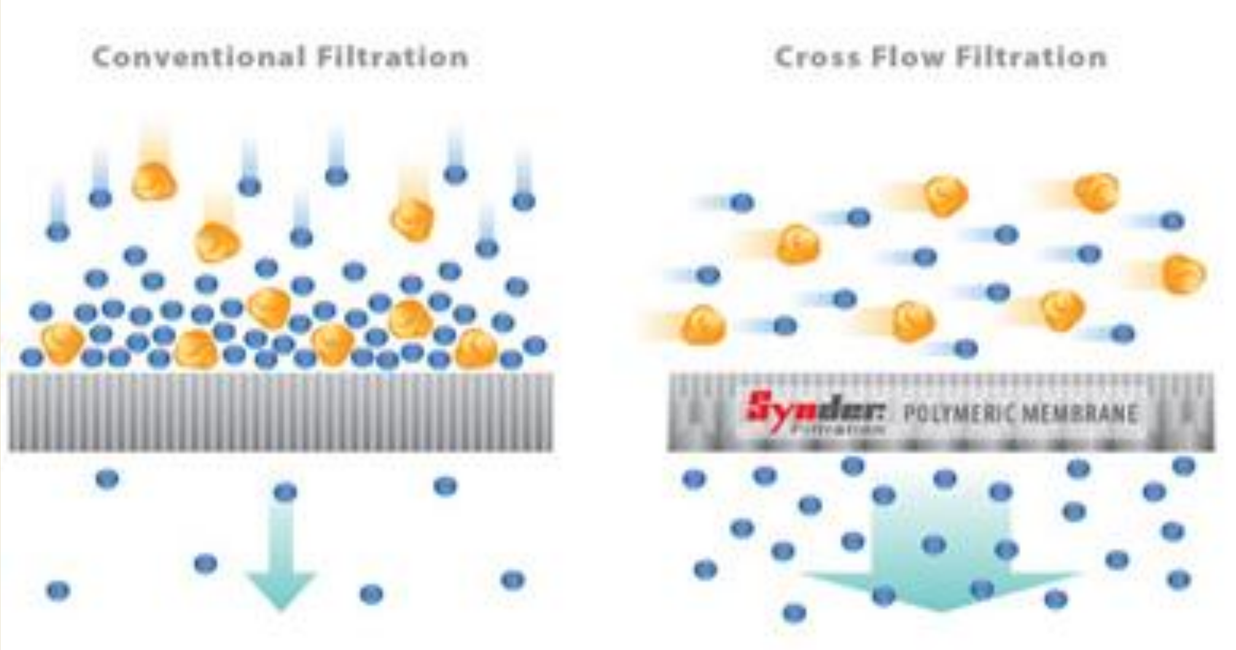
✓ Nature of the solid particles: shape, size, distribution and packing characteristics

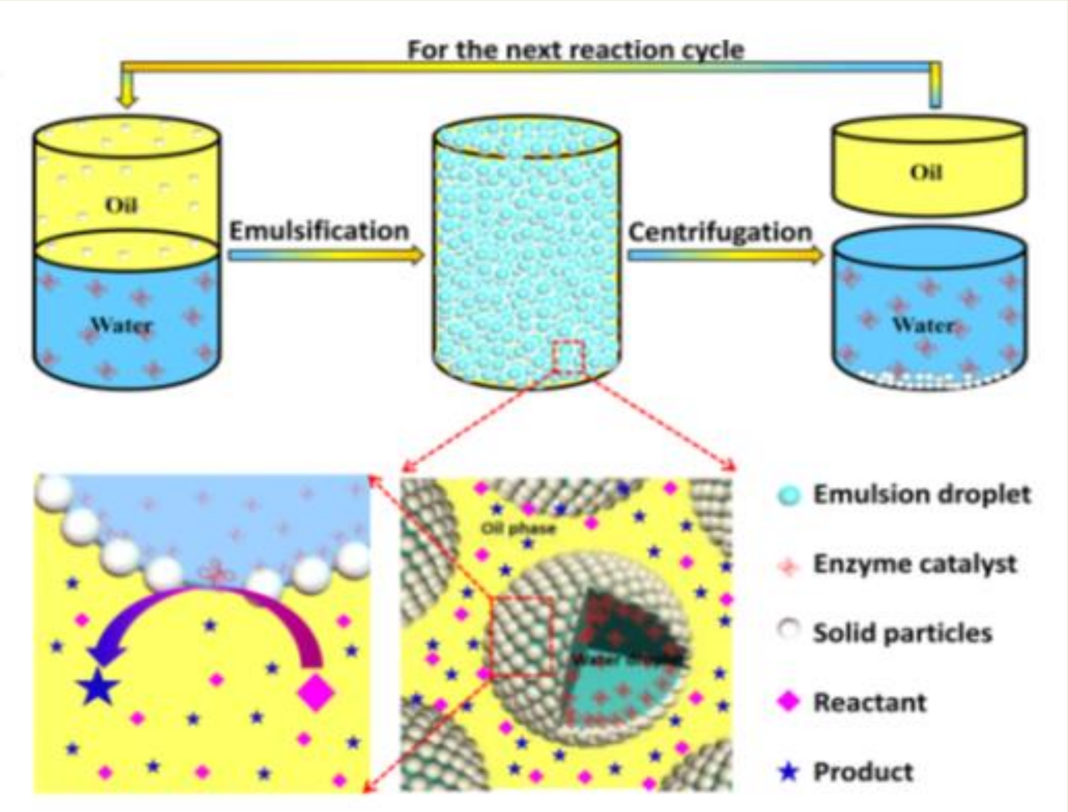
✓ Solid: liquid ratio

✓ Material required to be recovered i.e. solid or liquid or both

✓ Mode of operation: continuous or batch

✓ Need for additional attachment for vacuum suction or need for low temperature





2. Centrifugation

When solid separation is not satisfactory by filtration or a very high degree of separation is required, centrifugation is the method of separation. The main principle of centrifugation process is the sedimentation under centrifugation force.

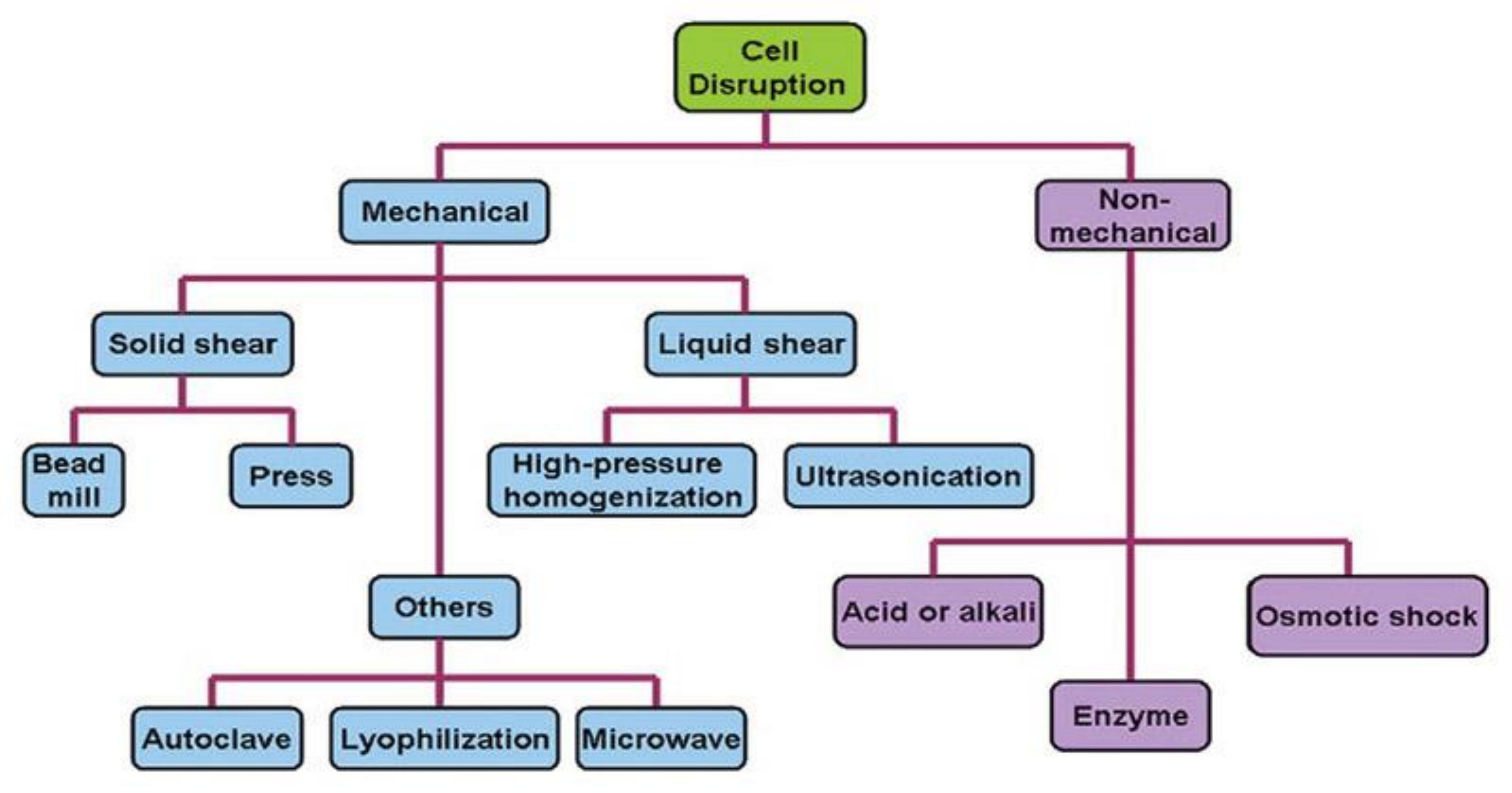
Application

Centrifugation is one of the preferred methods of clarification of wine, removal of solids from the fermented broths etc. In this, high speed rotation impels suspended material out of the wine. Speed of clarification is the primary advantages of centrifugation. It is especially useful when early bottling is desired. Centrifugation is also particularly valuable with very turbid wines. They frequently produce off-odors during spontaneous clarification and typically clog filter units. Centrifugation also increases the efficient use of polishing filtration by removing the most remaining suspended material.

3. Cell Disruption

Microbes are protected from the outside environment by rigid cell wall. The cell wall may be extremely hard and the recovery of the intracellular products requires the breakage of the cell wall. A number of cell disintegration methods are available but the choice of the method depends on its suitability for the particular substance. Though in some cases by the application of a particular method, a specific product can be recovered from inside the cells with greater yield and purity, most of the times these types of applications are not feasible.

On the other hand, gross disruption of the cell releases a huge number of products and its down stream processing becomes difficult. Sometimes application of a particular process is only feasible in the laboratory scale and therefore, choice of suitable methods for the industrial application is a matter of investigation. In recent times, enzymes are the most important intercellular products of interest. But the disruption methods applied for the release of enzymes must keep them active and properly folded, at the same time release yield must be high.


Cell Disruption
Mechanical
Non-mechanical
Solid shear
Liquid shear
Bead mill
Press
High-pressure homogenization
Ultrasonication
Others
Autoclave
Lyophilization
Microwave
Acid or alkali
Enzyme
Osmotic shock

Current bioprocess technology & products

- Products and services that depend on bioprocessing can be grouped broadly into:

➢ **Biopharmaceuticals:** Mainly includes therapeutic proteins, antibiotics, antibodies, polysaccharides, hormones, vaccines, and diagnostic agents. The success of biotechnology is seen in the impact of new products and processes.

➢ **Industrial chemicals and Energy:** Mainly includes alcohols, organic acids, fuels, several chemicals and fiber from renewable resources. It is an essential component for rapid transition of bio-products from the laboratory to a manufacturing scale able to provide the benefits of biotechnology on a large scale at a reasonable cost.

- **Food Industries:** Mainly includes alcoholic beverages and dairy products, several novel foods (soya sauce) and mushrooms (starch and vitamins) and food additives (anti-oxidants) and value added products. Bioprocess engineering has been used in food industries for long time ago.
- **Agriculture:** Mainly includes single cell protein, veterinary vaccine and antibiotics, use of nitrogen fixing bacteria, to produce transgenic plants and genetic modification of foods.
- **Environmental-management:** Bioprocessing products and services used to control or remediate toxic wastes. Mainly includes municipal wastewater treatment plants to provide clean and safe drinking water, bio composting methods for waste management. The recent attention to the environment has focused some bioprocessing technology on the transformation of hazardous wastes and the use of biological processes that produce desired products but little or no waste byproduct.

Opportunities in bioprocessing

- Bioprocess engineering is the established and applied field of biology and has a lot of opportunities or applications are present. Some of them are given below:
- ❑ Bioprocess engineering enables transition of biotechnology into products that benefit society
- ❑ Applications of bioprocessing to higher value products obtained through recombinant-DNA and cell culture technology
- ❑ It also gives opportunity to production of biofuels and other types of biological energy source
- ❑ It can also contribute in design and operation of fermentations systems
- ❑ Also used in the development of food processing systems
- ❑ It can contribute in application and testing of product separation technologies
- ❑ It can be used for designing of instrumentation to monitor and control biological processes
- The demand for bioprocess engineers continues to grow. They provide a bridge between the research lab and the economic large scale implementations of biotechnologies and food production systems.

Future perspectives

- The knowledge of molecular breeding, stem cell technology and pharmacogenomics might way to high personalized therapies and therapeutics. It can expect that biocatalyst such as insect and plant cell and transgenic plants and animal's sooner or later will reach much broader significantly, although this might not happen in next decade. Apart from the genetic engineering, the naturally occurring organism also provides the huge reservoir of new products.
- Bioprocesses will be applicable in the industries where they are not used current or where only lab scale processes are developed. It is expected that the combination of biotech, nanotech and IT will lead to substantial rate of progress and expansion. The use of IT has already way to improvement in the screening and development of new drugs and in the understanding of biological system.

- It might also lead to bio-chips for computers that replace silicon based chips. The bio-industries are still immature and productions are relatively low. Therefore, not only do the strain and fermentation have to be optimized and production scales increased, but also a substantial progress in downstream technologies is necessary.
- Modeling, simulation and accompanying sustainability assessment will play a crucial role in achieving a full exploitation of the potential of bioprocessing. However, in some areas in the expected positive development will reach its full potential only if the public acceptance of biotechnology can be improved considerably.
- The expending development of biofuels is a good example. Here, an open and constructive dialogue based on the sound sustainability is crucial, and scientist can make a valuable contribution to this discussion. well trained bio-experts are essential for the existing potential of biotechnology to be realized.