

FERMENTATION TECHNOLOGY

Abstract

Food production and processing by using fermentation process is about 6000 years old practices when human being had no specific idea about the existence and uses of microorganisms. Now a days, fermentation is a biochemical process by which we can synthesize various food and other products like: wine, cheese, bread, antibiotics, curds, biofuels, enzymes, pigments and many others in a large quantity with the help of several microorganisms. We can subdivide the process of large-scale fermentation in several steps: Upstream processing, microbial fermentation of molecules and downstream process. In upstream process selection of microorganism, strain improvement and selection, media preparation are the fundamental steps by which we can start the biotransformation process of fermentation in a controlled way to get desired products. After the performing of upstream process, we need to do the downstream process by which we can get the products in its pure form. The downstream process includes several steps like: cell harvesting, broth conditioning, sedimentation, centrifugation, filtration, cell disruption and most importantly product recovery. Interference of different microorganisms and maintenance of chemical as well as physical factors are very important in the process of fermentation. In recent days, fermentation technology is a part of food production and processing industries explores ways to overcome food scarcity and secure the future's food economy.

Keywords: Fermentation Technology, Food Production, Biotransformation, Physical Factors, Microorganisms.

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Fermentation is an age-old food processing technique. The term "fermentation" is derived from the Latin verb 'fervere,' which means 'to boil,' although the fermentation process doesn't always require high temperatures or boiling. It's a diverse process that signifies the growth or propagation of microorganisms under various conditions or environments, such as anaerobic or aerobic settings. The fermentation process begins with the introduction of desired microorganisms into a substrate, which is then maintained under appropriate environmental conditions. These inoculated microorganisms produce the desired product, which can be used directly or further processed to isolate specific molecules. When fermentation is employed on a large scale in fields like chemical, pharmaceutical, and food industries to produce substantial quantities of products using various microorganisms, it's referred to as fermentation technology.

The fundamental principles of the fermentation process encompass three steps: a) upstream processing, b) microbial fermentation of molecules, and c) downstream processing. Upstream processing involves preparing and purifying the medium and selecting the appropriate microorganism to achieve the desired products. The second phase involves the biotransformation process, where the selected medium yields the desired product, and the third step involves collecting and purifying these products. All three of these processes require specific equipment known as fermenters, where biotransformation occurs under optimal physical and chemical conditions, including factors like pH, temperature, and oxygen levels.

I. UPSTREAM PROCESSING

1. Selection and Isolation of Suitable Microorganisms: The initial stage in the upstream fermentation process involves the selection of the most suitable microorganism. To accomplish this, the screening and identification of the optimal microorganism strain are pivotal tasks among the available microorganisms. The ideal strain is the one capable of efficiently producing the desired product using the selected medium. Diverse industrially significant microorganisms, encompassing bacteria, fungi, actinomycetes, and algae, possess the potential to generate significant quantities of the crude product. For the isolation of suitable industrial microorganisms, two primary strategies, referred to as the shotgun and objective approaches, are employed.

In the shotgun approach, samples of free-living microorganisms, biofilms, or other microbial communities are gathered from a wide range of sources, including soil, sewage, water bodies, anthropogenic or natural environments, as well as floral and faunal origins. These collected microorganisms undergo diverse screening procedures for isolation. Conversely, the objective approach is a more focused method, involving the collection of samples from particular locations where the desired microorganisms are anticipated to be present within the natural microflora.

Several criteria are taken into account when choosing the appropriate microorganism strain. It should demonstrate the following characteristics:

- Effective production of the desired or target products.
- Genetic stability and reliability.
- Minimal or no requirement for vitamins and additional growth factors.

- Capability to utilize a wide range of carbon sources.
- Non-pathogenic nature - should not produce any toxic products or metabolites.
- Ease of harvesting from the fermentation process.
Collected microorganism are then grown in suitable culture media.

2. Strain Improvement: Strain enhancement is a pivotal factor in the initial stages of fermentation technology. Genetic recombination is a fundamental technique employed in this regard. With the recombination method, two separate genomes are fused to form a singular entity with novel genotypic attributes. This procedure entails the utilization of specific restriction endonucleases.

Table 1: Some examples of recombinant DNA technology mediated changes in the production of different metabolites are given below:

Approach	Features	Yields
1. Product modification	New enzymes modify the product of existing biosynthetic pathway.	Conversion of encephalosprin C into &-aminocephalosproric acid by D amino biosynthetic pathway.
2. New substrate utilization	Inaccessible substrate converted into accessible substrate	Beer fermentation by yeast: Cyclodextrins converted into glucose which is utilized by yeast
3. Completely new metabolites	All the genes of a new pathway transferred	<i>E. coli</i> ; transfer of two genes for polyhydroxybutyrate synthesis from <i>Alcaligenes eutrophus</i>
4. Stimulated metabolic production	Amplification of genes or enzyme whose activity is rate limiting	Gene <i>cefG</i> of <i>C.acremonium</i> catalyzing the conversion of Penicillin N: increased cyclosporin yields.
5. Enhanced growth	Enhanced substrate utilization	<i>E. coli</i> glutamate dehydrogenase into <i>M. methylotrophus</i> ; carbon conversion increased from 4% to 7%.

Mutagenesis is a traditional method for strain enhancement. Normally, the natural mutation rate is quite low in bacterial cells, approximately one mutation per 10^7 to 10^{11} cells. However, the application of mutagens substantially increases the mutation rate. Various physical mutagens, such as UV radiation, X radiation, and gamma radiation, as well as chemical mutagens like Ethen methane sulphonate, nitroso methene guanidine, nitrous acid, and acridine mustards, are used for this purpose. These agents provoke changes in the DNA's base sequences.

Protoplast fusion offers an alternative method for strain enhancement. This procedure entails the use of PEG (polyethylene glycol) treatment, which results in the amalgamation of desirable characteristics from two parental cultures. For instance, the fusion of protoplasts between non-producing strains of *Streptomyces griseus* and *Streptomyces tenjimariensis* has yielded a strain with the ability to produce indolizomycin, a novel indolizine antibiotic.

3. Strain Stability: The foremost consideration for the recently created strain revolves around its stability, which can be preserved through either storage in liquid nitrogen or

lyophilization. In the case of strains transformed by plasmids, it is crucial to keep them under constant selection to guarantee the maintenance of plasmid stability. Recombinant plasmids can experience instability primarily due to two factors: structural instability, stemming from deletions or recombination events, and segregationally instability, which results from the complete loss of the plasmid.

- 4. Media Selection:** The fermentation media plays a pivotal role in the overall process, with both liquid (broth) and solid media being employed in large-scale fermentation processes. The primary objective of any medium is to fulfill the nutritional requirements of microorganisms, while any additional components should enhance the rate of product synthesis. Large-scale fermentation necessitates the development of suitable media and a well-planned inoculation step. During the initial phases of media preparation, it is vital to provide appropriate carbon and nitrogen sources, minerals, and oxygen, which are subsequently converted into cell biomass, metabolites, carbon dioxide, water, and heat. Typically, microbial cells have a chemical composition of approximately $C_4H_7O_2N$, with their dry weight consisting of 48% carbon, 7% hydrogen, 32% oxygen, and 14% nitrogen. In various industrial processes, suitable nutrient sources can be integrated into the media to fulfill these requirements. It's important to highlight that specific nutritional and environmental conditions can influence the physiological, biochemical, and even morphological aspects of microorganisms. Each individual requirement plays a pivotal role in achieving optimal yields of fermentation products. For example, carbon sources are essential for cell reproduction, product synthesis, and overall cell maintenance. Additionally, most carbon sources serve as energy sources, facilitating ATP generation. In industrial fermentation processes, carbohydrates have traditionally served as primary carbon and energy sources. However, alternative carbon sources include alkanes, alcohols, various organic acids, among others. Moreover, many microorganisms possess the capability to utilize both inorganic and organic nitrogen sources. Inorganic nitrogen sources comprise ammonium salts like ammonium sulfate, diammonium hydrogen phosphate, or ammonia, while organic nitrogen sources in various industrial fermentation processes encompass amino acids, proteins, and urea.

In the context of broth or suspended fermentation, water plays a pivotal role in the media by supplying vital trace minerals like calcium carbonate, iron, and chloride. Nonetheless, it is essential to subject the water to treatment before its direct utilization from its sources. This water treatment procedure involves the removal of suspended solid particles, unwanted salts, and particularly other microorganisms.

Other Requirements for Media Formulation: To ensure the adequate growth and production of metabolites or fermented products in the desired quantity and quality, various other factors and components must be present in suitable proportions for each chosen fermentation process. These supplementary prerequisites include minerals, vitamins, growth factors, precursor molecules, inducers, inhibitors, oxygen, cell permeability modifiers, and antifoams, among others.

For example, minerals like copper, cobalt, iron, manganese, zinc, and molybdenum are essential necessities. Corn steep liquor, known for its abundant mineral content, is frequently employed in commercial product manufacturing. In the synthesis of compounds such as Penicillin, precursor molecules like phenylacetic acid or

phenylacetamide hold significant importance, while D-threonine acts as a precursor in the production of L-isoleucine.

In plant cell culture, the existence of elicitors, which are derived from plant pathogens, initiates the synthesis of secondary metabolites such as terpenoids and flavonoids. Genetically modified microorganisms use inducers to activate cloned genes, guiding the formation of intermediate molecules. In processes like yeast-based glycerol production, inhibitors play a role in redirecting metabolism, as exemplified by sodium bisulfite.

Oxygen is another crucial factor to consider, and its necessity varies depending on whether the fermentation process is aerobic or anaerobic. Cell permeability modifiers are employed to improve cell permeability by modifying cell membranes and walls. For instance, in amino acid fermentations like L-glutamate fermentation, substances such as penicillin and surfactants are introduced into the fermentation media.

The occurrence of foam formation can present challenges in numerous fermentation processes, often attributed to the presence of proteins in the media, which can impede product synthesis. To address this issue, antifoaming agents are introduced to diminish foam formation. These substances work by reducing the surface tension that binds foam together, and they should possess qualities such as being non-toxic, thermally stable, and effective even at low concentrations. Natural antifoaming agents include soya oil, sunflower oil, rapeseed oil, deionized fish oil, mineral oils, and tallow, while synthetic antifoaming agents comprise silicon oils, poly alcohols, and alkylated glycols, among others.

II. TYPES OF FERMENTATION PROCESSES

There are several types of fermentation processes: Submerged fermentation and Solid-state fermentation.

- 1. Solid State Fermentation:** In the process of solid-state fermentation, solid materials are used as the substrate for the growth of microorganisms. These substrates can include a wide range of materials such as sawdust, wheat bran, cereal grains, wood shavings, as well as various animal and plant substrates. Different species of fungi and actinomycetes are commonly utilized in this type of fermentation. Fungi, especially, are grown in environments with reduced water content, allowing their hyphae to effectively penetrate the substrate and produce exoenzymes responsible for the degradation of polysaccharides.

Solid-state fermentation is widely utilized in the manufacturing of various products, including traditional foods such as cheese, natto, koji, sake, soy sauce, and ragi. It is also applied in the production of alcoholic beverages like ethanol and enzymes such as alpha amylase, cellulase, rennin, protease, pectinase, and beta galactosidase. Additionally, solid-state fermentation plays a crucial role in antibiotic production, resulting in antibiotics like penicillin, tetracycline, cephalosporins, Iturin, surfactin, and more.

In solid-state fermentation, two essential conditions are necessary: sufficient air supply and precise temperature control, both of which are managed through forced

aeration. Forced aeration plays a critical role in promoting the exchange of oxygen, carbon dioxide, and temperature within the fermentation bed. In specific instances, enclosed bioreactors are employed in solid-state fermentation to exert meticulous control over temperature and other critical parameters.

Maintaining sterile conditions is a critical aspect of solid-state fermentation. Before inoculation, thorough sterilization of fermenter trays is carried out, and once the fermentation process is completed, the trays are taken out to gather the fully fermented mash for subsequent processing and product recovery. Tray fermenters are a widely used choice for solid-state fermentation and are available in various materials, including plastic, wood, or metal, often equipped with perforated bottoms to ensure adequate aeration.

Table 2: Some Examples of Solid-State Fermentation Products with Required Microorganisms and Substrates:

Products	Microorganisms	Substrate
Enzymes:		
a. α -amylase	<i>Aspergillus oryzae</i> , <i>Rhizopus</i> sp, <i>Bacillus licheniformis</i> .	Wheat bran, cassava
b. Cellulase	<i>Aspergillus niger</i> , <i>Trichoderma reesei</i>	Wheat bran, wheat straw
c. Glucoamylase	<i>Aspergillus niger</i> , <i>Rhizopus</i> sp	Corn, cassava, wheat bran
d. β -galactosidase	<i>Kluyveromyces laccis</i>	Whey, corn or wheat bran
e. Pectinase	<i>Talaromyces flavus</i> , <i>Aspergillus niger</i> , <i>Aspergillus carbanerius</i>	Wheat bran, fruit pomace, wheat bran, coffee pulp
f. Rennin	<i>Mucor pusillus</i> , <i>Mucor miehei</i>	Wheat bran
g. Protease	<i>Penicillium caseicolum</i> , <i>martierella renispora</i>	Dried skim milk, wheat bran
Metabolites:		
a. Citric acid	<i>Aspergillus niger</i>	Sugarcane, fruit pomace, wheat bran.
b. Ethanol	<i>Saccharomyces cerevisiae</i>	Fruit pomace, beet, carob pods, sweet sorghum.
c. Gibberellic acid	<i>Gibberella fujikuroi</i>	Wheat bran
d. Lactic acid	<i>Lactobacillus</i> sp, <i>Rhizopus oryzae</i>	Sugarcane, sweet sorghum
Food products:		
a. Cheese	<i>Penicillium roqueforti</i>	Milk curd
b. Bread dough	<i>Saccharomyces cerevisiae</i> , <i>Lactobacillus sanfrancisco</i>	Wheat powder
c. Soy sauce	<i>Aspergillus sojae</i>	Soy bean. Wheat
d. Miso	<i>Aspergillus oryzae</i>	Soy bean, rice
e. Natto	<i>Bacillus natto</i>	Soy bean
f. Tempeh	<i>Rhizopus oligosporus</i>	Soy bean
g. Tape	<i>Amylomyces rouxii</i> , <i>Rhizobium chinensis</i>	Rice, Cassava, maize

There are some Advantages in the process of solid-state fermentation:

- The process is much easier,
- Low-cost rate to carry down the whole process,
- It is performed in low moisture condition which help for the lower contamination rate of bacterium and fungus,
- Different waste(e.g., agricultural) utilization as substrate,
- Product recovery much easier,
- Product yields good.

There are also some Disadvantages of solid-state fermentation:

- Slower microbial growth
- Bacterial contamination can hamper the process
- Difficulties often encountered on scale-up
- Substrate moisture level difficult to control.

2. **Submerged Fermentation:** This enclosed fermentation system entails the cultivation and development of microorganisms in the presence of free water and a range of nutrients, usually in suspended or dissolved states. This culture medium is often known as broth media. Submerged fermentation forms the foundation for the manufacturing of numerous enzymes, antibiotics, and other valuable substances.

The submerged fermentation process can be categorized into three primary types: Batch fermentation, Fed-batch fermentation, and Continuous fermentation.

- **Batch Fermentation:** This fermentation process takes place within a closed system, typically in a sealed fermenter vessel or bioreactor. Under aseptic conditions, a specific microorganism is inoculated into the closed bioreactor, along with a suitable medium and nutrients. It's crucial to maintain a constant volume of added media or broth, as even a slight change in volume can impact the pH level. In batch culture, microorganisms progress through distinct growth phases, including the lag phase, log phase, exponential phase, and death phase. To achieve optimal results in batch culture, it's essential to minimize nonreproductive stages, maximize the rate of product synthesis, optimize productivity, and maximize the yield of end products. Extending the growth phase is particularly beneficial for efficiently producing biomass (such as baker's yeast or feed biomass) and primary metabolites (like ethanol, acetic acid, citric acid, or lactic acid). Conversely, for the production of secondary metabolites, the exponential phase is shortened (typically by limiting one nutrient, usually the nitrogen source), and the stationary phase is prolonged to achieve the highest possible product yield.

Applications:

- This method enables microbiologists to monitor the metabolic activity of bacteria and aids in the study of microbial cell physiology.
- It proves effective in the synthesis of secondary metabolites, including antibiotics.

There are some Advantages of batch fermentation process:

- Simple and easy to operate; only one reactor is used.
- Contamination rate low because of short growth periods and closed system.

- Capital investment low as compared with others fermentation process.
- High raw materials conversion level resulting from a controlled growth period.

Disadvantages of batch fermentation process:

- Time consuming as compared with others processes.
- Batch viability.
- Organisms are not found in their specific growth phase.
- Any kind of technical error changes the media composition which may affect the microbial exponential growth pattern
- After each cycle of product recovery, system has to be cleaned and filled with fresh feed.

● **Fed Batch Fermentation:**

- This is a partially open system in which one or more nutrients are introduced into the bioreactor under sterile conditions, and the product remains within the bioreactor over time. This approach is commonly employed in mammalian and insect cell cultures. Recent advancements have broadened the capabilities of high-density fed-batch cultures by incorporating advanced media formulations and feeding strategies that extend beyond providing carbon and energy substrates. In a fed-batch culture, fresh nutrient medium is continuously supplied to the culture without removing the growing microbial culture. This allows for the addition of nutrients that become depleted or are required for later stages of the culture. Four primary types of fed-batch fermentation processes include Fixed Volume Fed-Batch Culture, Variable Volume Fed-Batch Culture, Repeated or Cyclic Fed-Batch Culture, and Single-Feed Batch Process.
- In the fixed-volume fed-batch process, a limited substrate is introduced into the reactor in a highly concentrated form to prevent a significant increase in volume. Typically, concentrated growth-limiting gas or liquid substrates are added, and these substrates must undergo sterilization through methods like dialysis or radiation sterilization. This constant-volume fed-batch system has been applied in the cultivation of hyperthermophilic Archaea under aerobic conditions.
- In a variable fed-batch fermentation process, the addition of substrate feed leads to changes in the working volume as the fermentation advances. These volume adjustments are dictated by process requirements, the duration of fermentation, and the primary objectives of the fermentation process. Variable feeding follows particular guidelines, such as the use of an appropriate medium composition as needed and the gradual introduction of concentrated forms to prevent dilution of the fermentation broth.
- Cyclic or repeated fed-batch culture is a method in which a defined volume of spent broth is taken out, and fresh nutrient medium is added during the stationary phase. This repetitive cycle of medium withdrawal and addition results in an augmentation of the specific growth rate. For instance, in the context of penicillin G biosynthesis, a repeated fed-batch cultivation strategy was utilized to optimize conditions through a phosphorus feeding approach.
- In a single-feed batch culture process, the spent broth remains within the reactor without being removed, and a substantial portion of the fermentation broth

remains unused until the entire process is finished. This can result in the reactor volume becoming a significant limiting factor throughout the fermentation period.

Applications:

- Fed-batch culture is employed in the production of antibiotics like penicillin, where precise nutrient feeding is essential.
- Incorrect substrate feeding, either insufficient or excessive, can lead to nutrient depletion or product dilution, respectively. Therefore, fed-batch processes ensure a regulated nutrient supply to optimize the production of desired products or to achieve high cell density.
- In the production of baker's yeast, fed-batch culture is utilized to prevent the Crabtree effect, which inhibits respiration when the substrate concentration exceeds a certain threshold.

Advantages of Fed Batch Fermentation Includes:

- Product recovery rate high because of higher cell density utilization which is proportional to the concentration of biomass.
- Fed batch process limit the production of inhibitory product or desired inoculum.
- Use for those which are toxic for inoculum.

Disadvantages of the fed batch include:

- Lower productivity levels due to time for filling, heating, sterilization, cooling, emptying and cleaning the reactor.
 - Not easy to handle as like batch fermentation.
 - High investment for multiple tasks.
 - Productivity level low as compared with batch fed fermentation.
- **Continuous Fermentation:** Continuous fermentation represents a fully open system, where nutrients and microorganisms are consistently introduced into specific bioreactors under aseptic conditions. In this configuration, the culture volume remains stable, and nutrient concentrations remain constant and unchanged. This process is chiefly controlled by microbial growth activity or the production of by-products. There are two primary categories of continuous fermentation processes: Turbidostat and chemostat methods.
 - In the turbidostat technique, a constant biomass concentration is maintained by modulating the dilution rate, and in this method, the flow rate of fresh media varies. The regulation of cell density primarily revolves around the supervision of the turbidity value, which indicates the number of cells per unit volume within the population.
 - In the chemostat technique, a constant cell growth rate, matching the dilution rate, is maintained, and this rate is controlled by the presence of the limiting nutrient. In this situation, the growth rate is adjusted by controlling the levels of substrates like carbon, nitrogen, and phosphorus, among others.

Applications: It has some industrial applications. Primary metabolites are the main products of this process. Single-Cell Proteins (SCPs), for example.

Advantages of Continuous Fermentation Includes:

- Overcome the batch viability
- Can produce primary metabolites in their large quantity
- Can maintain the culture in fresh and healthy state by addition of nutrients.

Disadvantages of continuous fermentation include:

- Risk of contamination is high due to in and out of medium.
- In long term process, genetic mutation of strains may occur.
- The process is expensive as compared with batch and fed batch process and need expert staffs to maintain.

Distinguish between Batch and Fed Batch Fermentation Process:

Batch Fermentation	Fed Batch Fermentation
Closed type process	Semi open type process
It is fill and forgot type	Not fill and forgot type
Easy to operate	Not easy as batch process
Fermenter is filled up to 3/4 th portion with medium	Fermenter is partially filled
Organisms maintain in their log phase for short period of time	Organisms maintain in their log phase for more time period.
Contamination rate very low	Contamination rate higher than batch feed.

Distinguish between Fed Batch and Continuous Fermentation Process:

Feb Batch Fermentation	Continuous Fermentation
Discontinuously addition of nutrients	Continuous addition of nutrients
No simultaneous product recovery	Product recovery simultaneous
Product toxicity cannot be avoided	Product toxicity can be avoided
Batch variability is found	Batch variability not found
It may produce lower quantity of primary metabolites	Produce large quantities of primary metabolites.
Content of vessel may vary with time	Content of vessel is fixed

III. DOWNSTREAM PROCESSING

The effective operation of a bioreactor relies on the correct execution of upstream processing and the subsequent recovery of valuable products through downstream processing after fermentation. The product can be found either within the cells, in the medium, or in both. To ensure proper product recovery and purification, a series of steps in downstream processes must be followed. The entire downstream processing can be segmented into individual unit processes, interconnected to ultimately achieve a final and purified product. Therefore, downstream processing represents a multi-stage product recovery and purification process.

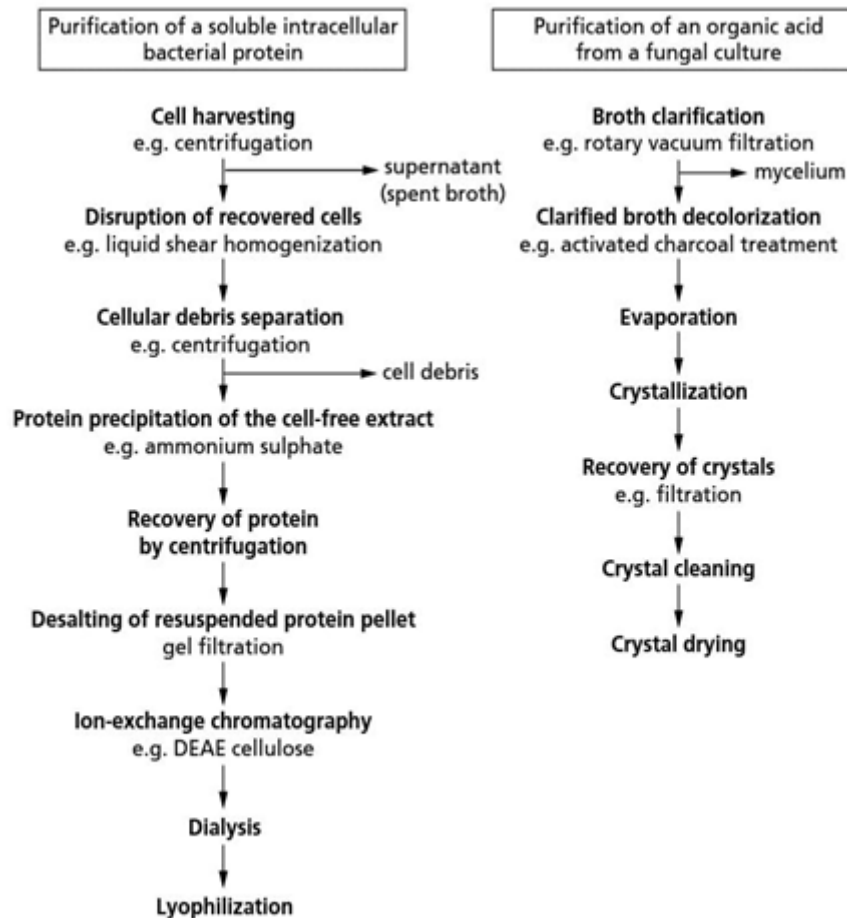


Figure 1: Examples of unit downstream processing (Ref: Industrial microbiology: An introduction by Michael J. Waites, Neil L. Morgan, John S. Rockey, Gary Higton).

- 1. Cell Harvesting:** The first phase of downstream processing encompasses cell harvesting, a pivotal step centered on the separation of cells from the medium. The choice of a solid-liquid separation technique depends on several crucial considerations, including the microorganisms' size, their specific gravity, and the viscosity and rheological characteristics of the spent fermentation medium.
- 2. Broth Conditioning:** The process of isolating cells from liquid media encompasses methods associated with sedimentation and centrifugation, often referred to as broth conditioning. Broth conditioning is also applied to promote flotation, which naturally occurs in traditional ale and baker's yeast fermentations. Furthermore, distinct techniques involving floc precipitation are employed towards the end of various traditional beer and wine fermentation procedures. These approaches may entail the utilization of finings such as egg albumen or isinglass to induce the precipitation of yeast cells. These methods are preferred for their cost-effectiveness and their ability to efficiently separate microbial cells from significant volumes of medium.
- 3. Sedimentation:** This is a cost-effective and somewhat gradual technique suitable for larger flocs, typically exceeding 100µm in diameter. Examples include the primary yeast separation in alcoholic beverage production and wastewater treatments. The speed of

sedimentation is contingent on both particle size and the medium's density. In simple terms, larger particles with higher densities lead to faster sedimentation rates. These rates adhere to Stokes' Law.:

$$V_g = \frac{d_p^2(p_s - p_l)g}{18\eta}$$

In summary, the rate of particle sedimentation (V_g) is determined by various factors, including the particle's diameter (d_p), the density contrast between the particle and the surrounding medium ($p_s - p_l$), gravitational acceleration (g), and the viscosity of the medium (η) measured in Pascal seconds (Pa s). To achieve swift sedimentation, it's essential for the density contrast between the particle and the medium to be substantial, and the medium's viscosity should be minimal.

- 4. Centrifugation:** Centrifugation is a widely recognized and extensively utilized method in downstream processing. It relies on the principle of exploiting density disparities between the particles intended for separation and the surrounding medium, instead of relying solely on gravitational force. In this method, a centrifugal field is employed to efficiently separate solid particles from the liquid phase, making it suitable for applications in both laboratory settings and large-scale industrial processes.

$$V_c = \frac{d_p^2(p_s - p_l)\omega^2 r}{18\eta}$$

Here, V_c represents the centrifugal sedimentation rate or particle velocity (m/s), ω stands for the angular velocity of the centrifuge (rad/s), and r denotes the distance of the particle from the center of rotation (m), which affects parameters such as η , $p_s - p_l$, and d_p . Consequently, a higher operating speed (ω) and a greater distance from the center of rotation lead to a swifter sedimentation rate (V_c). Centrifuges can be compared using the relative centrifugal force (RCF) or g number (which is the ratio of the velocity in a centrifuge to the velocity under gravity, expressed as $\omega^2 * r/g$).

Currently, various centrifugation methods are employed to segregate solid particles from the liquid phase in industrial processes. Four primary types of centrifuges are commonly utilized in these processes: the tubular centrifuge, multi-chamber bowl centrifuge, disc stack centrifuge, and screw-decanter centrifuge.

- **Tubular Centrifuge:** This is a compact and straightforward type of centrifuge capable of generating a significant centrifugal force ranging from 13,000 to 17,000g. It finds frequent use in pilot plants. The device comprises a hollow tubular rotor bowl that offers an extended path for the suspension. The suspension is introduced at the bottom and ascends through the rotor. Solid particles are pushed to the side of the bowl, while the clarified liquid is continuously discharged from the top for collection. Periodic manual removal of solids is necessary.

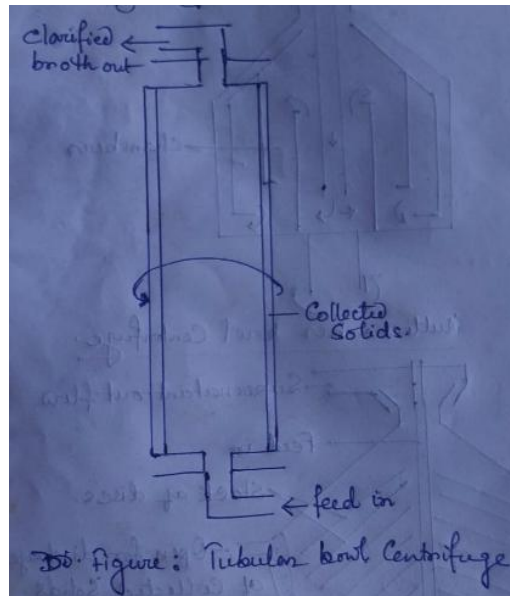


Figure 2: Tubular Centrifuge

- **Multi-Chamber Bowl Centrifuge:** This centrifuge is a variation of the tubular bowl type and is composed of multiple vertically arranged chambers interconnected to guide the feed flow in a zigzag pattern. It can function effectively at forces ranging from 5,000 to 10,000g. The centrifugal force is greater in the peripheral chambers, causing the liquid feed to pass sequentially from the center through each chamber. Consequently, smaller particles tend to settle in the outermost chamber.

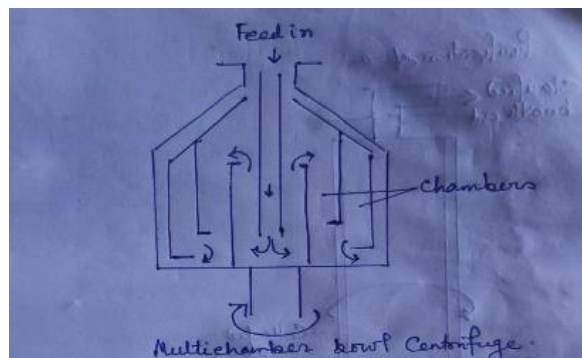


Figure 3: Multi-chamber bowl centrifuge

- **Disc Stack Centrifuge:** In this centrifuge variant, multiple discs divide the bowl into a settling zone, operating at a centrifugal force ranging from approximately 5,000 to 13,000g. Upon introducing the liquid into the centrifuge, solid particles are propelled outward toward the bowl's wall, where they accumulate, while the clarified fluid moves upward. These centrifuges typically include the capability to periodically discharge the accumulated material during operation.

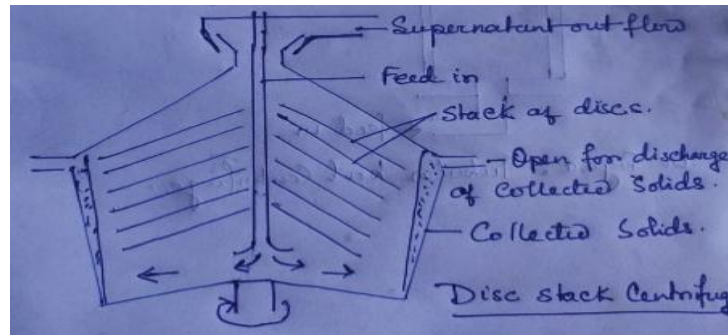


Figure 4: Disc stack centrifuge

- **Screw-Decanter Centrifuge:** This centrifuge variant is employed in continuous operations at a centrifugal force ranging from 1500 to 5000g. Its primary applications include separating sludge in sewage systems and collecting yeast and fungal mycelium.

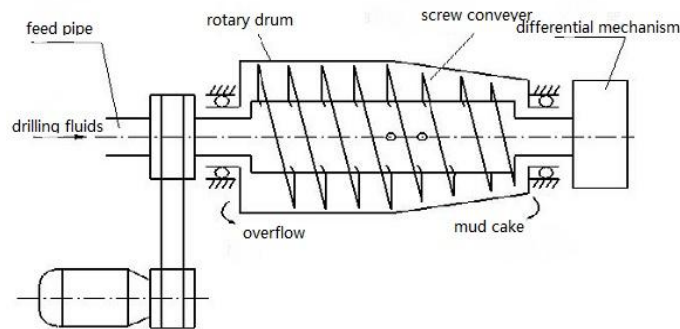


Figure 5: Screw-decanter centrifuge

5. Filtration: Filtration is a commonly used downstream procedure for segregating biomass from the culture filtrate. The effectiveness of this procedure is influenced by multiple factors, such as the size of the microorganisms' cells, the presence of other microorganisms, medium viscosity, and, notably, temperature. A variety of filters are employed in this context, encompassing depth filters, membrane filters, rotary drum vacuum filters, and absolute filters.

- **Depth Filters:** In downstream processing, depth filters are widely utilized, employing a porous filtration medium (such as glass wool, asbestos, or filter papers) to efficiently capture dispersed particles within the medium. These particles are entrapped within the matrix, permitting the fluid to pass through. Depth filters are particularly effective in eliminating filamentous fungi. This method presents several benefits, including the ability to produce substantial flocculants that can be subsequently filtered and the flexibility in selecting filter configurations to maintain high solid storage capacities while keeping energy consumption within acceptable limits.
- **Membrane Filters:** This is an advanced filtration process that involves supported membranes with specific pore sizes. The clogging of filters represents a significant

drawback in the use of membrane filters. Based on their pore sizes, membrane filters are categorized into three types: microfiltration, ultrafiltration, and reverse osmosis membranes.

- **Microfiltration:** It is very effective method of clarification of liquid. Generally, particles or microbial cells of 10^{-2} to $10\mu\text{m}$ are separated from liquid by the process. Membrane which is used to microfilter is highly cost effective which make the process expensive but the process include quite operation, lower energy requirements. The products can be easily washed using this process, and it allows for effective containment with no production of bioaerosols.
 - **Ultrafiltration:** Ultrafiltration is similar to microfiltration, but it uses smaller pore sizes in the filtering membrane to separate molecules ranging from 2000 to 50000 Da. This membrane has an anisotropic structure and is composed of a thin membrane with pores of specific diameters for selective filtering. At times, multiple ultrafiltration units can be interconnected to create an advanced purification system. Typically, it is employed for removing particulates or macromolecules in various applications such as water treatment, cheese processing, enzyme recovery, radiocarbon dating of bone collagen, and more. Another variation of the ultrafiltration system is diafiltration, which involves filtering water or another liquid to eliminate unwanted low molecular weight contaminants. This can serve as an alternative to gel filtration or dialysis for removing ammonium sulfate from a protein preparation that has been precipitated with this salt, for purposes like changing a buffer or water purification.
 - **Reverse Osmosis:** This is a dewatering process primarily used in desalinating seawater for drinking purposes. In osmosis, water flows across a semipermeable membrane when the concentration of osmotically active solutes on the salt side is higher than on the other side of the filtering semipermeable membrane. Achieving this process requires applying high pressure. To illustrate, a pressure of 30–40 bar is required to dewater a 0.6 mmol/L salt solution (please note: 1 bar equals 100 kPa, or 0.987 atm). Additionally, robust metal casings are essential to house this equipment.
- 6. Cell Disruption:** For intracellular target products like enzymes and recombinant proteins, they often aggregate into inclusion bodies within the microorganisms. As a result, it becomes essential to disrupt these microorganisms to release these products. Some cells have robust cell walls that pose challenges in breaking them open. For example, yeast cell walls require an approximate pressure of 650 bar for disruption. A notable issue with cell disruption is the release of DNA, which can increase the suspension's viscosity. This problem can be mitigated by using DNase. Cell disruption can be achieved through both mechanical and non-mechanical methods. The effectiveness of the disruption process is influenced by factors like particle size, total protein concentration, or the activity of specific intracellular enzymes released into the disruption suspension.
- **Mechanical Cell Disruption Methods:** There are several methods for cell disruption to recover products, including solid shear and liquid shear techniques. In the solid shear method, frozen cell preparations are passed through a narrow orifice at high

pressure, typically suitable for laboratory use. In contrast, the liquid shear method is more efficient and applicable in both laboratory and large-scale settings.

One device utilized in the liquid shear cell disruption process is the Manton and Gaulin homogenizer, also known as the APV-type mill. In smaller-scale operations, manual cell grinding using abrasives like alumina, glass beads, kieselguhr, and silica can be effective but may not yield optimal results. In various industries, high-speed bead mills are employed, where cell suspensions are mixed with small beads (typically ranging from 0.5 to 0.9 μm in diameter) made from materials such as zirconium oxide, glass, or titanium carbide. Cells are disrupted when they collide with these beads. Factors affecting disruption in this method include agitation speed, bead size, bead density, diameter, broth density, flow rate, and temperature.

Ultrasonic disruption is another mechanical method that creates microscopic bubbles and cavities using pressure waves and cavitation. High-frequency sound waves at around 20 kilohertz per second are produced by ultrasonic vibrators to disrupt cells. However, it's important to consider that this approach can produce heat, which may lead to the denaturation of proteins that are sensitive to temperature. Notably, rod-shaped bacteria tend to be more vulnerable to disruption than cocci, and Gram-negative organisms are typically easier to disrupt than Gram-positive cells.

- **Non-Mechanical Cell Disruption Methods:** Several non-mechanical techniques are utilized for cell disruption, such as autolysis, osmotic shock, disruption caused by ice crystal formation (freeze/thawing), or heat shock. Autolysis, for instance, is frequently employed in the extraction of yeast products, providing cost-effectiveness by leveraging the microorganism's own enzymes without introducing external substances into the product.

Osmotic shock is a valuable method for liberating products from the periplasmic space. This can be accomplished by initially exposing cells to a 20% (w/v) buffered sucrose solution, followed by swift harvesting and resuspension in cold water at 4°C. In small-scale microbial disruption, various chemicals and enzymes are utilized. Organic solvents like acetone, butanol, methanol, and chloroform have been utilized to separate enzymes and other substances from microorganisms. These organic compounds create openings in the cell membrane, facilitating the release of enzymes. Alkalis and detergents such as sodium lauryl sulfate or Triton X-100 can also effectively disrupt cells. Cell wall-degrading enzymes, like lysozymes, can readily break down cell membranes and release desired products. Snail gut enzymes, which contain a mixture of β -glucanases, are employed to disrupt yeast cell walls. Occasionally, in the case of actively growing microorganisms, antibiotics like Penicillin and Cycloserine are used in conjunction with osmotic shock to disrupt the cell membrane.

7. Product Recovery Processes:

- **Chromatography:** Several Chromatography techniques are applied to recover high value products. Choosing of particular type of chromatographic technique is very important and this selection process is depended on several factors like: molecular

weight, biological affinities, hydrophobicity, isoelectric point and chromatographic technique depends on some parameters like: capacity, recovery, resolving power (selectivity).

Some types of chromatography techniques: Basic principle and applications in down steam processing:

- **Absorption Chromatography:** This represents the most ancient chromatography technique, in which the mobile phase comprises both liquid and gas and becomes absorbed onto the solid surface of the stationary phase. The fundamental principle revolves around the analytical separation of a chemical mixture, predicated on the interaction between the absorbate and the absorbent. Typically, absorbents such as silica gel H, silica gel G, silica gel N, silica gel S, hydrated gel silica, microcrystalline cellulose, alumina, and modified silica gel are employed. This method relies on hydrogen bonding and/or van der Waals forces for separation and is proficient at eluting various types of protein molecules.
- **Affinity Chromatography:** Affinity chromatography stands as one of the oldest and exceptionally selective chromatography techniques. Its selectivity hinges on the specific affinity existing between a substance immobilized in the separation material, referred to as the adsorbent, and the desired components within the mixture, known as ligands. This method has found extensive use in large-scale industrial bioprocessing. A diverse range of affinity ligands is applied, including antibodies and their fragments, receptors and their binding agents, avidin/biotin systems, textile and biomimetic dyes, (oligo)peptides, antisense peptides, chelated metal cations, lectins and phenylboronates, protein A and G, calmodulin, DNA, sequence-specific DNA, (oligo)nucleotides, and heparin. Similarly, a variety of support materials have been developed and employed, encompassing natural, synthetic, inorganic, and composite materials.
- **Gel Filtration Chromatography:** This chromatographic technique focuses on the separation of molecules predicated on their molecular size and configuration. The stationary phase comprises porous beads made from acrylic polymers, agarose, cellulose, cross-linked dextran, and analogous substances, each possessing a distinct pore size. These materials generally incorporate ionic groups. The selection of the suitable stationary phase material holds paramount importance, as certain materials may engage in interactions with the target product. For example, matrices based on carbohydrates might interact with glycoproteins. Predominantly, this method is employed for the purification of protein substances.
- **Ion Exchange Chromatography:** Ion exchange chromatography is a highly favorable bioprocessing technique that revolves around ionic interactions as its core principle. The matrix materials predominantly rely on cellulose substitution and include both positively and negatively charged ions. A commonly used example is the anion-exchange resin diethylaminoethyl (DEAE) cellulose. Typically, molecules establish electrostatic interactions with oppositely charged sites on the stationary phase. Maintaining the pH at an appropriate level is a critical factor in this process. Ion exchange chromatography is prominently employed in the separation of antibiotics and proteins.

- **High-Performance Liquid Chromatography (HPLC):** This chromatography technique primarily serves for the isolation of organic molecules from non-aqueous solvents and proteins from aqueous solutions. The process involves the use of tightly packed columns containing small, rigid particles with diameters ranging from 5 to 50 μm , typically made from silica or cross-linked polymers. Operated under high pressure, this method is both swift and offers excellent resolution of solute molecules. It plays a pivotal role in large-scale industrial bioprocessing.
- **Dialysis and Electrodialysis:** Dialysis is a separation method designed to separate low molecular weight solutes and inorganic ions from solutions. The membranes used have size selectivity, determined by specific molecular weight cut-offs. Low molecular weight solutes move across the membrane through osmosis, traveling from areas of higher to lower concentration. Electrodialysis, on the other hand, segregates charged molecules from solutions by applying a direct electrical current carried by mobile counter-ions. This technique is primarily used in the desalination of water.
- **Distillation:** This is a recovery process used to separate fuel alcohol, acetone, and other solvents from the fermentation media. While batch distillation in pot stills is still used for producing specific whiskies (as discussed in Chapter 12), continuous distillation is the preferred method for most other applications. For example, in the case of ethanol, the continuous system produces a product with a maximum ethanol concentration of 96.5% (v/v). This azeotropic mixture represents the highest concentration achievable from aqueous ethanol, unless an additional dehydration step is introduced using a water entrainer such as benzene or cyclohexane.

8. Finishing Steps of Downstream Processing:

- **Crystallization:** Product crystallization can be achieved using techniques like evaporation, cold treatment, or the addition of a reactive chemical to interact with the solute. Solubility of the product can be reduced by adding solvents, salts, polymers (e.g., non-ionic PEG), polyelectrolytes, or by adjusting the pH.
- **Lyophilization:** Lyophilization, also known as freeze-drying, is a water removal process primarily employed for preserving perishable materials, extending their shelf life, and facilitating transportation. This technique involves three fundamental stages: freezing, primary drying (sublimation), and secondary drying (absorption).

In the initial phase, products are frozen by placement on a shelf in a freeze dryer, a freezer, or a chilled bath (shell freezer). Achieving lyophilization is simplest with the formation of large ice crystals, which can be attained through slow freezing or annealing. However, when dealing with biological materials, excessively large crystals can damage cell walls, resulting in suboptimal freeze-drying outcomes. To circumvent this issue, rapid freezing is employed. In cases where precipitation is likely, annealing comes into play. This method entails swift freezing, followed by elevating the product's temperature to facilitate crystal growth.

The second phase, known as sublimation, involves a low-pressure heating process. Approximately 95% of the water molecules within the materials are eliminated during this stage, and it may proceed at a relatively slow pace. Excessive heat application can potentially alter the product's structure.

The third and final phase is the absorption phase of lyophilization. In this step, ionically bound water molecules are removed by raising the temperature higher than during the primary drying phase. Freeze-dried materials maintain their porous structure. Following the completion of the lyophilization process, the vacuum can be replaced with an inert gas before sealing the material. Most substances can be dried to achieve a residual moisture content of 1-5%.

Problems to Avoid During Lyophilization:

- Elevating the product's temperature excessively can result in melt-back or product collapse. Overloading the condenser can occur when an excessive amount of vapor comes into contact with it.
- Excessive vapor generation.
- An excessive surface area.
- Inadequate condenser area.
- Insufficient refrigeration.
- Vapor choking, where vapor is generated at a rate that exceeds its capacity to pass through the vapor port (the connection between the product chamber and the condenser), resulting in increased chamber pressure.

Lyophilization is a widely utilized process across various bioprocessing industries, including but not limited to food processing, dairy industries, and pet food processing industries.

REFERENCES

- [1] K.G. Clarke, Downstream Processing, Science Direct, 2013.
- [2] T.R. Bansod, L.D.Hingane, Fermentation Technology, World Journal of Pharmacological Research, 2021.
- [3] U. Satyanarayana, Biotechnology, books & allied (P) Ltd, 2005.
- [4] Vyas, S.P and Dixit V.K. Pharmaceutical biotechnology, CBS publishers and distributors New Delhi, 1998.
- [5] W.Sun, M.H. Shahrajabian and M. Lin, Research Progress of Fermented Functional Foods and Protein Factory-Microbial Fermentation Technology,MDPI, 2021.
- [6] N.A.Castillo, A.L.Valdez, J. I. Fariña, Microbial production of scleroglucan and downstream processing, Frontiers, 2015.
- [7] K. Singh, S. Jha, P. Rana, R.P.Sinha, Application of Synthetic Biology Approaches to High-Yield Production of Mycosporine-like Amino Acids, Research Gate, 2023.
- [8] W.Y. Chai, K. T. K. Teo, M. K. Tan, H. J. Tham, Fermentation Process Control and Optimization, Wiley Online Library, 2022.
- [9] M. J. Waites, N. L. Morgan, J. S. Rockey, G. Higton, Industrial Microbiology: An Introduction, School of Applied Science, South Bank University, 2001.
- [10] P. B.K. Kishor, A. M. Rao, I.S. Kumar, Biotechnology, Himalaya Publishing House, 2011.