# Downstream processing

Dear Students welcome for the lecture series on Industrial Microbiology, in next two episodes we will discuss in detail about the downstream processing.

In this session we are concentrating on the following modules,

- **Downstream processing and Fermentation**
- **Differences between Downstream and Upstream Processing**
- **Steps of Downstream Processing**
- **Detailed Study of Stages of Downstream Processing**
- **Release of Intracellular Products**

## I. Downstream processing and Fermentation

Industrial fermentations comprise both upstream (USP) and downstream processing (DSP) stages. USP involves all factors and processes leading to and including the fermentation and consists of three main areas: the producer organism, the medium and the fermentation process.

DSP encompasses all processes following the fermentation. In most cases this means recovery of a product from a dilute aqueous solution. The complexity of DSP is determined by the required purity of the product which is in turn determined by its application. The products of biotechnology include whole cells, organic acids, amino acids, solvents, antibiotics, industrial enzymes, therapeutic proteins, vaccines, gums etc.

The primary objective in industrial fermentation processes is to recover the product efficiently, reproducibly and safely to its required specification, while achieving maximum product yield at minimum recovery costs.

Fermentation processes are designed to produce useful products and are carried out on an industrial scale, thus, benefitting billions of people worldwide. Not only does it provide employment to people, but also a large

number of beneficial products which were earlier unavailable in such quantities to public. Industrial fermentation is defined as the “intentional use of microorganisms, such as bacteria and fungi for profitable bulk production of metabolites useful to humans.”

A vast variety of fermented products are used as food as well as other general need items. Using fermentations, certain commodity chemicals, such as acetic acid, citric acid, and ethanol are manufactured. Almost all of the commercially produced enzymes, like lipase, invertase and rennet are produced through fermentation using genetically modified microbes. In some cases, biomass production is the sole objective of fermentation. An example of this is the commercial production of baker's yeast and lactic acid bacteria starter cultures for cheese making. A large number of important therapeutic drugs like insulin, growth hormone, interferons etc. are now being commercially produced using transgenic microorganisms on an industrial scale at an affordable cost. A number of these are life-saving drugs too.

The innumerable products synthesized using industrial fermentations can’t directly be consumed/used, rather they need to be harvested, purified, packaged and tested before they could be put to their actual use.

Downstream processing refers to “the recovery and purification of biosynthetic products, particularly pharmaceuticals, from natural sources such as animal or plant tissue or the spent fermentation broth, including the recycling of salvageable components and the proper treatment and disposal of waste.” It is an important step in the production of pharmaceuticals like the following:-

1. Antibiotics such as penicillin

2. Hormones such as insulin and human growth hormone

3. Vaccines

4. Antibodies and enzymes used for diagnostic purposes

5. Industrial enzymes

6. Natural fragrance and flavor compounds

## II. Differences between Downstream and Upstream Processing

"Upstream" and "downstream" are business terms applicable to the production processes that exist within several industries. Industries that commonly use this terminology include the metals industry, oil, gas, biopharmaceutical and biotechnology industries. Upstream, downstream and midstream make up the stages of the production process for these and other industries.

### Definition of Upstream

The upstream stage of the production process involves searching for and extracting raw materials. The upstream part of the production process does not do anything with the material itself, such as processing the material. This part of the process simply finds and extracts the raw material. Thus, any industry that relies on the extraction of raw materials commonly has an upstream stage in its production process. In a more general sense, "upstream" can also refer to any part of the production process relating to the extraction stages.

### Examples of Upstream Processes

In the petroleum industry, locating underground or underwater oil reserves characterizes the upstream process. Additionally, the upstream process in this industry involves bringing oil and gas to the surface. Extraction wells represent an example of a structure operating in this stage in the process. The upstream stage in the production process may also manifest itself as a supplier providing raw materials to manufacturers or other businesses that ultimately process the materials.

### Definition of Downstream

The downstream stage in the production process involves processing the materials collected during the upstream stage into a finished product. The downstream stage further includes the actual sale of that product to other businesses, governments or private individuals. The type of end user will vary depending on the finished product. Regardless of the industry involved, the downstream process has direct contact with customers through the finished product.

**Examples of Downstream Processes**

In the oil and gas industry, the downstream process consists of converting crude oil into other products and then selling those products to customers. Thus, oil refineries represent structures that operate within the downstream process. However, any kind of plant that processes raw materials may qualify as operating within the downstream stage of production. A company that combines both upstream and downstream processes is an integrated company.

## III. Steps of Downstream Processing

Downstream processing is generally considered to be a specialized field in biochemical/chemical engineering. It encompasses harvesting, purification and packaging of a specific industrial product synthesized in marketable quantities. Analytical bio separation refers to the separation or purification of biological products at different scales of operation, done at analytical scale, the purpose of which is to maintain the desired quality of the product.

Downstream processing involves four important steps that result in the following progressive improvements in purity and concentration of the desired product:-

**1. Removal of insoluble:** This step involves the harvest of the product in the form of solute in a particle-free supernatant. An example of this is the separation of cells, cell debris or any other particulate matter from the spent fermentation broth containing an antibiotic. This can be achieved by various

methods like filtration, centrifugation, sedimentation, flocculation, electro-precipitation, precipitation, and gravity settling. Other methods such as grinding, homogenization, or leaching, may be required for recovering products from solid sources like plant and animal tissues.

**2. Product isolation:** This step involves the removal of those components whose properties differ significantly from that of the desired product. For maximum products, the presence of water in them is the main impurity, and consequently, product isolation essentially involves drying and concentrating the product. This step involves processes such as solvent extraction, adsorption, ultrafiltration, and precipitation.

**3. Product purification:** This step is performed for separating those contaminants whose physical and chemical properties closely resemble those of the product. Since this is rather difficult to carry out, the steps involved at this stage may be expensive, and may require sensitive and sophisticated apparatus. This stage alone may use up a significant fraction of the entire downstream process expenditure. Examples of some of the methods used in this step are affinity chromatography, size exclusion chromatography, reversed phase chromatography, crystallization and fractional precipitation, all of which are very specific techniques.

**4. Product polishing:** This is the last step that involves final processing steps that end up with a stable, functional, easily transportable and convenient product. This step involves processes like crystallization, desiccation, lyophilization and spray drying. Depending on the type of product and its application, polishing might even include product sterilization and removal/deactivation of trace contaminants that may compromise product safety. It might virus removal too.

Not every product essentially requires all the above mentioned steps. Some products may be recovered by a combination of just two steps. For example, using affinity chromatography, one can isolate and purify a product in a single step.

## IV. Detailed Study of Stages of Downstream Processing

The DSP scheme normally employed for isolation and purification of bio molecules can be divided into five stages:

First Stage is,

### a. Solid-Liquid Separation:

The first step in product recovery is the separation of whole cells (cell biomass) and other insoluble ingredients from the culture broth. If the desired product is an intracellular metabolite, it must be released from the cells before subjecting to solid-liquid separation. Several methods are in use for solid-liquid separation. These include flotation, flocculation, filtration and centrifugation.

**Flotation:**

When a gas is introduced into the liquid broth, it forms bubbles. The cells and other solid particles get adsorbed on gas bubbles. These bubbles rise to the foam layer which can be collected and removed. The presence of certain substances, referred to as collector substances, facilitates stable foam formation e.g., long chain fatty acids, amines.

**Flocculation:**

In flocculation, the cells or cell debris form large aggregates to settle down for easy removal. The process of flocculation depends on the nature of cells and the ionic constituents of the medium. Addition of flocculating agents is often necessary to achieve appropriate flocculation.

**Filtration:**

Filtration is the most commonly used technique for separating the biomass and culture filtrate. The efficiency of filtration depends on many factors—the size of the organism, presence of other organisms, viscosity of the

medium, and temperature. Several filters such as depth filters, absolute filters, rotary drum vacuum filters and membrane filters are in use.

**Depth Filters:**

They are composed of a filamentous matrix such as glass wool, asbestos or filter paper. The particles are trapped within the matrix and the fluid passes out. Filamentous fungi can be removed by using depth filters.

**Absolute Filters:**

These filters are with specific pore sizes that are smaller than the particles to be removed. Bacteria from culture medium can be removed by absolute filters.

**Rotary Drum Vacuum Filters:**

These filters are frequently used for separation of broth containing 10-40% solids (by volume) and particles in the size of 0.5-10μm. Rotary drum vacuum filters have been successfully used for filtration of yeast cells and filamentous fungi. The equipment is simple with low power consumption and is easy to operate. The filtration unit consists of a rotating drum partially immersed in a tank of broth. As the drum rotates, it picks up the biomass which gets deposited as a cake on the drum surface. This filter cake can be easily removed.

**Membrane Filters:**

In this type of filtration, membranes with specific pore sizes can be used. However, clogging of filters is a major limitation. There are two types of membrane filtrations—static filtration and cross-flow filtration. In cross-flow filtration, the culture broth is pumped in a crosswise fashion across the membrane. This reduces the clogging process and hence better than the static filtration.

**Types of filtration processes:**

There are 3 major types of filtrations based on the particle sizes and other characters. These are microfiltration, ultrafiltration and reverse osmosis.

**Centrifugation:**

The technique of centrifugation is based on the principle of density differences between the particles to be separated and the medium. Thus, centrifugation is mostly used for separating solid particles from liquid phase. Unlike the centrifugation that is conveniently carried out in the laboratory scale, there are certain limitations for large scale industrial centrifugation.

However, in recent years, continuous flow industrial centrifuges have been developed. There is a continuous feeding of the slurry and collection of clarified fluid, while the solids deposited can be removed intermittently. The different types of centrifuges are depicted in and briefly described hereunder.

**Tubular bowl centrifuge:**

This is a simple and a small centrifuge, commonly used in pilot plants. Tubular bowl centrifuge can be operated at a high centrifugal speed, and can be run in both batch and continuous mode. The solids are removed manually.

**Disc centrifuge:**

It consists of several discs that separate the bowl into settling zones. The feed/slurry is fed through a central tube. The clarified fluid moves upwards while the solids settle at the lower surface.

**Multi-chamber centrifuge:**

This is basically a modification of tubular bowl type of centrifuge. It consists of several chambers connected in such a way that the feed flows in a zigzag fashion. There is a variation in the centrifugal force in different chambers. The force is much higher in the periphery chambers, as a result smallest particles settle down in the outermost chamber.

**Scroll centrifuge or decanter:**

It is composed of a rotating horizontal bowl tapered at one end. The decanter is generally used to concentrate fluids with high solid concentration (biomass content 5-80%). The solids are deposited on the wall of the bowl which can be scrapped and removed from the narrow end.

## V. Release of Intracellular Products:

As already stated, there are several biotechnological products (vitamins, enzymes) which are located within the cells. Such compounds have to be first released (maximally and in an active form) for their further processing and final isolation. The microorganisms or other cells can be disintegrated or disrupted by physical, chemical or enzymatic methods.

The selection of a particular method depends on the nature of the cells, since there is a wide variation in the property of cell disruption or breakage. For instance, Gram-negative bacteria and filamentous fungi can be more easily broken compared to Gram-positive bacteria arid yeasts.

**Cell Disruption:**

**Physical methods of cell disruption:**

The microorganisms or cells can be disrupted by certain physical methods to release the intracellular products.

**Ultra sonication:**

Ultrasonic disintegration is widely employed in the laboratory. However, due to high cost, it is not suitable for large-scale use in industries.

**Osmotic shock:**

This method involves the suspension of cells (free from growth medium) in 20% buffered sucrose. The cells are then transferred to water at about 4°C. Osmotic shock is used for the release of hydrolytic enzymes and binding proteins from Gram-negative bacteria.

**Heat shock (thermolysis):**

Breakage of cells by subjecting them to heat is relatively easy and cheap. But this technique can be used only for a very few heat-stable intracellular products.

**High pressure homogenization:**

This technique involves forcing of cell suspension at high pressure through a very narrow orifice to come out to atmospheric pressure. This sudden release of high pressure creates a liquid shear that can break the cells.

**Impingement:**

In this procedure, a stream of suspended cells at high velocity and pressure are forced to hit either a stationary surface or a second stream of suspended cells (impinge literally means to strike or hit). The cells are disrupted by the forces created at the point of contact. Micro fluidizer is a device developed based on the principle of impingement. It has been successfully used for breaking E. coli cells. The advantage with impingement technique is that it can be effectively used for disrupting cells even at a low concentration.

**Grinding with glass beads:**

The cells mixed with glass beads are subjected to a very high speed in a reaction vessel. The cells break as they are forced against the wall of the vessel by the beads. Several factors influence the cell breakage-size and quantity of the glass beads, concentration and age of cells, temperature and agitator speed. Under optimal conditions, one can expect a maximal breakage of about 80% of the cells.

A diagrammatic representation of a cell disrupter employing glass beeds is shown in Fig. It contains a cylindrical body with an inlet, outlet and a central motor-driven shaft. To this shaft are fitted radial agitators. The cylinder is fitted with glass beads. The cell suspension is added through the inlet and the

disrupted cells come out through the outlet. The body of the cell disrupter is kept cool while the operation is on.

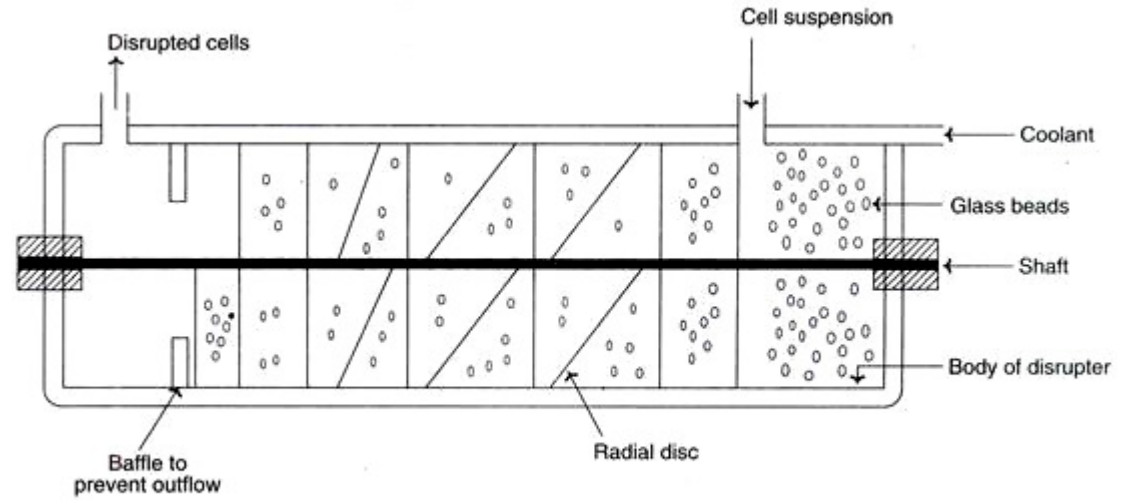


*Fig. 20.6 : Diagrammatic representation of a cell disrupter.*

**Mechanical and non-mechanical methods:**

Among the physical methods of cell disruption described above, ultra sonication, high-pressure homogenization, impingement and grinding with glass beads are mechanical while osmotic shock and heat shock are non-mechanical. The chemical and enzymatic methods are non- mechanical in nature.

**Chemical methods of cell disruption:**

Treatment with alkalies, organic solvents and detergents can lyse the cells to release the contents.

**Alkalies:**

Alkali treatment has been used for the extraction of some bacterial proteins. However, the alkali stability of the desired product is very crucial for the success of this method e.g., recombinant growth hormone can be efficiently released from E. coli by treatment with sodium hydroxide at pH 11.

**Organic solvents:**

Several water miscible organic solvents can be used to disrupt the cells e.g., methanol, ethanol, isopropanol, butanol. These compounds are inflammable; hence require specialised equipment for fire safety. The organic solvent toluene is frequently used. It is believed that toluene dissolves membrane phospholipids and creates membrane pores for release of intracellular contents.

**Detergents:**

Detergents that are ionic in nature, cationic-cetyl trimethyl ammonium bromide or anionic-sodium lauryl sulfate can denature membrane proteins and lyse the cells. Non-ionic detergents (although less reactive than ionic ones) are also used to some extent e.g., Triton X-100 or Tween. The problem with the use of detergents is that they affect purification steps, particularly the salt precipitation. This limitation can be overcome by using ultrafiltration or ion-exchange chromato-graphy for purification.

**Enzymatic methods of cell disruption:**

Cell disruption by enzymatic methods has certain advantages i.e., lysis of cells occurs under mild conditions in a selective manner. This is quite advantageous for product recovery. Lysozyme is the most frequently used enzyme and is commercially available (produced from hen egg white). It hydrolyses β-1, 4-glycosidic bonds of the mucopeptide in bacterial cell walls. The Gram- positive bacteria (with high content of cell wall mucopeptides) are more susceptible for the action of lysozyme.

For Gram-negative bacteria, lysozyme in association with EDTA can break the cells. As the cell wall gets digested by lysozyme, the osmotic effects break the periplasmic membrane to release the intracellular contents. Certain other enzymes are also used, although less frequently, for cell disruption. For the lysis of yeast cell walls, glucanase and mannanase in combination with proteases are used.

**Combination of methods:**

In order to increase the efficiency of cell disintegration in a cost-effective manner, a combination of physical, chemical and enzymatic methods is employed.

So students we will continue our discussion about this topic in the next episode. Until then take care thank you.