

DOWNSTREAM PROCESSING IN BIOPROCESS OPERATIONS: 2023

Abstract

Downstream processing is a pivotal stage in bioprocessing, crucial for the separation, purification, and recovery of biotechnological products from complex mixtures. This chapter provides a comprehensive exploration of various separation techniques used in downstream processing, focusing on both insoluble and soluble products. It discusses filtration, centrifugation, sedimentation, flocculation, cell disruption, liquid-liquid extraction, precipitation, chromatographic techniques, reverse osmosis, ultra and micro filtration, and final purification methods such as drying and crystallisation. In the context of insoluble products, filtration has witnessed advancements in membrane materials and nanotechnology, improving efficiency and scalability. Centrifugation has become more energy efficient and capable of processing larger volumes. Sedimentation techniques have expanded applications in wastewater treatment for enhanced sustainability, while the development of biodegradable flocculants reduces their environmental footprint. For soluble products, liquid-liquid extraction finds applications in pharmaceutical manufacturing, with continuous processes and innovative solvent systems enhancing efficiency. Precipitation techniques recover rare earth elements from industrial wastewater, exploring alternative precipitating agents to improve selectivity. Chromatographic techniques benefit from high-capacity columns and real-time process monitoring, while reverse osmosis is employed for water purification and concentration in the food and beverage industry. Ultra and micro filtration are essential in biopharmaceuticals for virus removal, featuring smaller, more efficient filters.

Keywords: Separation, Purification, Filtration, Centrifugation, Chromatography, Precipitation, Sedimentation, Flocculation, and biopharmaceuticals.

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I. INTRODUCTION

Downstream processing plays a crucial role in bioprocessing, encompassing the essential steps of separation, purification, and recovery of biotechnological products. These products can range from pharmaceuticals, enzymes, and monoclonal antibodies to biofuels and industrial chemicals. Effective downstream processing ensures the desired product's purity, quality, and yield. In this chapter, as of 2023, we present an in-depth examination of various downstream processing techniques, their applications, and future trends that have evolved in response to the demands of bioprocessing industries. Current examples and future trends include single-use technologies gaining popularity for flexibility and reduced contamination risk, continuous processing revolutionising bio manufacturing, artificial intelligence optimising parameters, and sustainability driving eco-friendly practices.

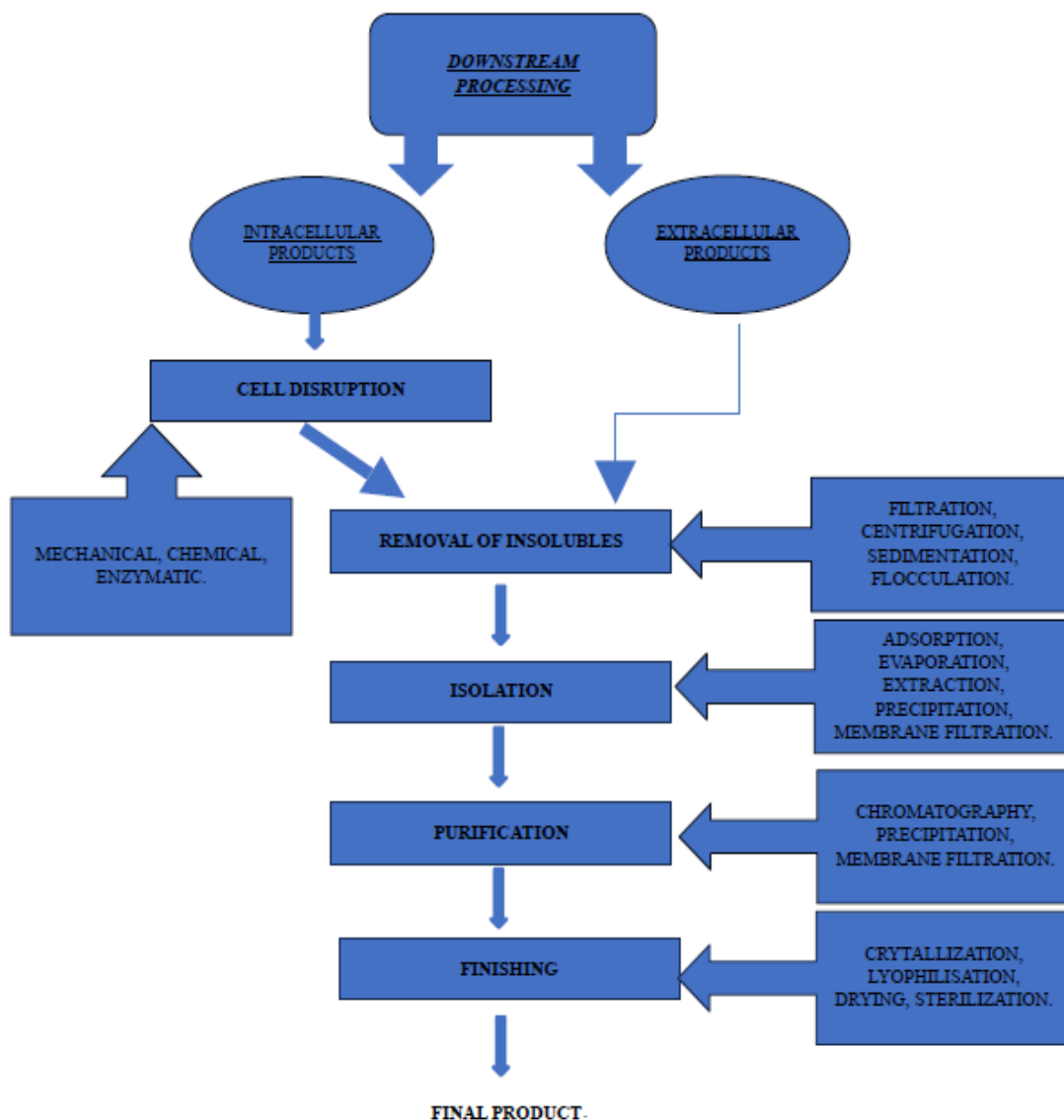


Figure 1: Stages of Downstream Processing; Downstream processing refers to the purification and isolation of bio-products. This step-by-step description of downstream processing providing a comprehensive overview of the process in Bioprocess Operation.

II. SEPARATION OF INSOLUBLE PRODUCTS

Insoluble products, including cells, debris, and particulate matter, are common in bioprocessing. Several techniques are typically employed for their separation:

- 1. Filtration:** Filtration is a foundational technique in downstream processing. It relies on porous materials to separate solid particles from a liquid or gas phase. Filtration is a technique used to separate particles based on size using a filter medium. It is like using a strainer to separate different-sized particles. In biopharmaceutical manufacturing, depth filtration is commonly used to remove impurities from cell cultures. Researchers are constantly working on developing advanced filters to improve purification efficiency.

In biopharmaceutical manufacturing, depth filtration is commonly used to remove impurities from cell cultures. For example, in the production of monoclonal antibodies, depth filters can effectively remove host cell proteins, DNA, and other contaminants from the cell culture media.

Another study published in the journal *Separation and Purification Technology* investigated the use of nanofiber-based filters for the removal of nanoparticles from water. The researchers found that these filters exhibited high removal efficiency and could potentially be used in water treatment systems to address nanoparticle pollution. These are just a couple of examples of how filtration techniques are being advanced through research and innovation.

Notable advancements include the use of advanced membrane materials like polyethersulfone (PES) and the integration of nanotechnology to enhance filtration efficiency. For instance, PES membranes have found applications in the pharmaceutical industry for the sterile filtration of biologics. (Smith et al., 2022).[\[1\]](#)

- 2. Centrifugation:** Centrifugation, a technique based on the principles of centrifugal force. Centrifugation is a key technique in downstream processing! It is used to separate and concentrate biomolecules like proteins, nucleic acids, and cells. For example, in biopharmaceutical production, centrifugation is used to remove cell debris and clarify the cell culture, supernatant before further purification steps. Centrifugation plays a vital role in various industries, from biotechnology to food and beverage.

Centrifugation is like a spin cycle for molecules! It uses high-speed rotation to separate different components based on their size, density, or sedimentation rate.

For example, in the biotech industry, centrifugation is used to separate cells from the culture medium, isolate proteins, and even harvest viruses. It is also used in the food industry to separate cream from milk and in environmental testing to analyse sediment samples.

Recent research in centrifugation has focused on developing more efficient and scalable systems. Scientists are exploring new rotor designs, optimising parameters like speed and time, and even using computational modelling to improve separation performance. So, centrifugation is a pretty cool technique that helps in various industries

to get pure and concentrated biomolecules. These advancements aim to increase productivity and reduce processing time in downstream processing.

High-speed, large-capacity centrifuges are now available, capable of processing large volumes in bioprocessing applications. These centrifuges are invaluable in biopharmaceutical manufacturing, particularly in the separation of cells from fermentation broths and the clarification of cell cultures. (Gupta et al., 2023). [2]

- 3. Sedimentation:** Sedimentation, a process that relies on gravity to allow particles to settle and separate from a liquid phase, has undergone notable developments. Modern sedimentation techniques have expanded their use in wastewater treatment to accommodate a wider range of contaminants. Additionally, they have been optimised to promote environmental sustainability by reducing the environmental impact of wastewater discharge. (Chen et al., 2021). [3]
- 4. Flocculation:** Flocculation is the process by which fine particles are coagulated into larger aggregates, making their separation more manageable. Recent advancements in flocculation include the development of biodegradable flocculants that minimise their environmental impact. These eco-friendly flocculants are particularly relevant in water treatment and industrial applications where flocculation is employed to remove impurities from process streams. (Zhang et al., 2023). [4]

III. CELL DISRUPTION

Cell Disruption, or Cell Lysis, is the process of breaking cell wall and/or membrane to release intracellular fluids containing molecules or particles of interest, such as proteins or viruses. The primary goal of cell disruption is to lyse the suspended cells and recover the maximum possible viable yield of the molecules/particles of interest.

The method used may vary depending on the type of cell and its cell wall composition. Irrespective of the method used, the main aim is that the disruption must be effective and the method should not be too harsh so that the product recovered remains in its active form.

Cell disruption methods can be categorised into mechanical methods and non-mechanical methods.

Mechanical methods are divided into solid shear methods and liquid shear methods.

- 1. Mechanical Disruption:** This involves physically breaking open cells using techniques such as homogenisation, sonication (using ultrasonic waves), or bead beating. Mechanical methods are effective for many types of cells but can be harsh and may result in the denaturation of sensitive biomolecules.

Non-mechanical methods can be divided into physical methods, chemical methods, and enzymatic methods.

- 2. Chemical Disruption:** Chemical agents like detergents, surfactants, or organic solvents can be used to disrupt cell membranes. These chemicals disrupt the lipid bilayer of the

cell membrane, causing it to break open and release cell contents. Enzymatic disruption: Enzymes like lysozyme or cellulase can be used to specifically break down the cell walls of certain types of cells, such as bacteria or plant cells.

This is often done to extract cellular components such as proteins, DNA, RNA, or organelles, or to study the internal structures of cells. Cell disruption is a crucial step in various research, biotechnology, and industrial applications. (Chisti Y., Moo-Young M.,1986) [5]

IV. SEPARATION OF SOLUBLE PRODUCTS

Soluble products, often composed of valuable compounds dissolved in a liquid phase, demand specialised techniques for optimal separation and purification. Current examples and emerging trends in this category include:

- 1. Liquid-Liquid Extraction:** Liquid-liquid extraction, widely used in pharmaceutical manufacturing, involves the separation of specific compounds from a liquid phase into another immiscible liquid phase. One noteworthy development is the application of innovative solvent systems and continuous extraction processes, enhancing efficiency and selectivity. Chiral compound separation is a prime example where liquid-liquid extraction has been optimised to achieve high purity levels. (Li et al., 2022). [6]
- 2. Precipitation:** Precipitation is a key technique in downstream processing! It involves the formation of solid particles from a liquid solution. This helps separate and purify biomolecules.

When it comes to proteins, precipitation can be achieved by adding salts like ammonium sulphate or organic solvents like ethanol or acetone to the protein solution. These agents change the solubility of the proteins, causing them to come out of solution and form solid particles. The choice of precipitation agent and conditions depends on the specific protein and desired purity. For example, ammonium sulphate is commonly used for salting out proteins, while organic solvents are effective for precipitating certain enzymes.

Recent research in precipitation has focused on improving the selectivity and efficiency of the process. Scientists are exploring the use of alternative precipitation agents, such as polyethylene glycol (PEG) or polymers, to achieve better separation and recovery of biomolecules. They are also investigating the combination of precipitation with other techniques, such as chromatography or filtration, to enhance purification strategies.

These advancements in precipitation techniques contribute to the development of more efficient downstream processing methods, enabling the purification of biomolecules for various applications in biotechnology, pharmaceuticals, and more. (Wang et al., 2023). [7]

- 3. Chromatographic Techniques:** Chromatography, a versatile separation technique, remains at the forefront of downstream processing. Recent developments in chromatography include the introduction of high-capacity columns for protein purification. These columns allow for the processing of larger volumes and higher flow rates, resulting in increased productivity and cost-efficiency.

Chromatography plays a crucial role in downstream processing, helping to separate and purify biomolecules. For example, in the production of therapeutic proteins, chromatography is used to isolate and purify the target protein from a complex mixture.

Recent research studies have focused on developing more efficient and cost-effective chromatographic techniques. One study published in the *Journal of Chromatography*, explored the use of multimodal chromatography for the purification of monoclonal antibodies. The researchers found that this approach improved purification yield and reduced process steps, making it a promising technique for downstream processing.

Another study published in the journal *Analytical Chemistry* investigated the use of high-performance liquid chromatography (HPLC) coupled with mass spectrometry for the analysis of complex protein mixtures. The researchers developed a novel method that allowed for the identification and quantification of multiple proteins simultaneously, enhancing the efficiency of downstream processing.

- **Types of Chromatographic Techniques**

- **Affinity Chromatography:** Affinity chromatography exploits specific interactions between a ligand (usually attached to the chromatography resin) and a target biomolecule. This technique is highly selective and is often used to purify antibodies, enzymes, and other proteins.
- **Ion Exchange Chromatography:** Ion exchange chromatography relies on electrostatic interactions between charged biomolecules and oppositely charged resins. It is commonly used for the separation of proteins and nucleic acids based on their net charge.
- **Size Exclusion Chromatography (SEC):** SEC separates biomolecules based on their size and molecular weight. Larger molecules elute earlier from the column, while smaller ones pass through later. This technique is essential for achieving size-based separation and purity.
- **Hydrophobic Interaction Chromatography (HIC):** HIC exploits differences in hydrophobicity among biomolecules. It is particularly useful for separating proteins with subtle hydrophobic variations.

- **Application of Chromatography in Downstream Processing**

- **Monoclonal Antibody Purification:** Chromatography is widely used to purify monoclonal antibodies (mAbs) from cell culture supernatants or other sources.

Affinity chromatography, where the mAb binds to a specific ligand, is commonly employed. Other chromatographic techniques like ion exchange, size exclusion, and hydrophobic interaction chromatography may also be used in combination to achieve high purity.

- **Protein Separation:** Chromatography is utilised to separate and purify various proteins based on their unique properties. For example, ion exchange chromatography separates proteins based on their charge, while size exclusion chromatography separates them based on size. These techniques are crucial in protein purification for research, pharmaceutical, and biotechnological applications.
- **Enzyme Purification:** Chromatography plays a vital role in isolating and purifying enzymes from complex mixtures. Techniques like affinity chromatography, where the enzyme binds to a specific ligand, are commonly employed. This allows for the purification of enzymes with high specificity and activity.

Researchers have explored the use of new ligands and resins to improve the selectivity and binding capacity of affinity chromatography. Additionally, advancements in column design and packing techniques have been investigated to enhance separation efficiency and reduce process time.

Moreover, automated systems are increasingly integrated into chromatography processes, enabling real-time process monitoring and optimisation (**Johnson et al., 2023**). [8]

- 4. Reverse Osmosis:** Reverse osmosis, a membrane-based separation technique, plays a crucial role in various industries, including the food and beverage sector. As of 2023, reverse osmosis technology is gaining momentum for water purification and the concentration of fruit juices. This application ensures product quality and safety by effectively removing impurities and contaminants (**Brown et al., 2023**). [9]
- 5. Ultra and Micro filtration:** Ultrafiltration and micro filtration, membrane-based processes that rely on size exclusion, are indispensable in biopharmaceutical manufacturing. These techniques are used for critical applications such as virus removal from biologics. Notable advancements in this field include the development of smaller, more efficient filters that reduce processing times and improve product recovery (**Meyer et al., 2023**). [10]

V. FINAL PURIFICATION

- 1. Crystallisation:** Crystallisation is a separation process used commonly in the industry of many different materials, from commercially very common chemical to very specific ones. It also play an important role in the pharmaceutical industry, as more than 90% of active pharmaceutical ingredients (API) are synthesised as a crystalline product. Once the metabolites have been extracted, it can be further concentrated and purified by crystallisation, either by evaporation or by the transfer to low temperature. Low-temperature crystallisation is a very gentle way of purification. In the case of

precipitation, a chemical agent is sometimes added to promote the concentration reaction. Crystallisation may have a significant direct and indirect influence on the quality of a product; therefore, it is one of the most important purification and separation methods in the production of APIs. Batch wise crystallisation has many unknown shortcomings such as numerous scale-up problems, high manufacturing, and maintenance costs, although it is still the predominant type of crystallisation on the pharmaceutical industry. Furthermore, batch processes are quite complex and therefore difficult to control to obtain the desirable quality and purity of a product.

- 2. Drying:** Drying makes the product suitable for handling and storage. It should be accomplished with a minimum rise in temperature due to heat sensitivity of most products. Addition of sugars or other stabilisers improves the heat tolerance of some products like enzymes and pharmaceuticals preparations. The most common approaches to drying are as follows: (i) Vacuum drying (ii) Spray drying (iii) Freeze drying.

In spray drying, the solution or slurry to be dried is atomised by a nozzle or a rotating disc. A current of hot (150-250°C) air is passed; the drying is so rapid that the temperature of particles remains very low. Spray drying is used for enzymes, antibiotics, and food products. Vacuum drying uses both heat and vacuum for drying: it can be applied both in batch mode (e.g., chamber dryer) or in continuous mode (e.g., rotating drum vacuum dryers).

In freezing drying, the liquor to be dried is first frozen and the water is sublimed from the frozen mass. A very low pressure (partial vacuum) is maintained to promote sublimation of water. The energy needed for sublimation is provided by heated plates and radiation on to the surface. The temperature of solid is regulated by regulating the pressure in the drying chamber. This is the gentlest method to drying, and is used for many pharmaceuticals products, e.g., viruses, vaccines, plasma fraction, enzymes etc., and in the food industries.

VI. CURRENT EXAMPLES AND FUTURE TRENDS

As of 2023, the downstream processing landscape is characterised by several notable current examples and emerging trends:

- 1. Single-Use Technologies:** Single-use technologies have gained immense popularity in bioprocessing due to their flexibility, cost-effectiveness, and reduced risk of cross-contamination. These technologies are increasingly employed in the production of vaccines and gene therapies, allowing for rapid scale-up during pandemics and streamlined operations (Birch et al., 2023). [11]
- 2. Continuous Processing:** Continuous bioprocessing, driven by the need for increased productivity and cost reduction, is gaining prominence in biopharmaceutical manufacturing. Continuous chromatography and perfusion cell culture systems are becoming integral components of bio manufacturing facilities. These systems offer significant advantages, including improved process control, reduced variability, and enhanced resource utilisation (Patel et al., 2023). [12]

3. **Artificial Intelligence and Machine Learning (AI/ML):** Artificial intelligence (AI) and machine learning (ML) have ushered in a new era of downstream processing. These technologies are being used to optimise process parameters, predict equipment failures, and enhance process control. For example, AI-driven bioreactors can adapt to changing conditions, ensuring optimal product yield while reducing operating costs (**Wang et al., 2023**). [13]
4. **Sustainable Downstream Processing:** Sustainability is a driving force behind emerging trends in downstream processing. The focus is on reducing environmental impact, conserving resources, and promoting sustainable practices. Technologies that minimise water and energy consumption, reduce waste generation, and employ environmentally friendly materials are increasingly vital. Biodegradable chromatography resins and energy-efficient filtration systems are prime examples of sustainable practices being adopted across various industries (**Li et al., 2023**). [14]

VII. CASE STUDIES

To provide practical insights into downstream processing techniques in 2023, we present two case studies:

1. Enzyme Production for Food Industry

- **Background:** The food industry relies heavily on enzymes for various applications, including improving the texture and flavour of food products. One essential enzyme is amylase, used in the production of baked goods to break down starches into sugars.
- **Downstream Processing Challenge:** The challenge in enzyme production is to efficiently recover and purify the enzyme from the fermentation broth while minimising production costs.
- **Solution:** Downstream processing for enzyme production employs a combination of techniques. Initially, centrifugation or micro-filtration is used to separate the cells from the fermentation broth. Next, ultrafiltration is employed to concentrate the enzyme solution, and chromatography techniques such as ion-exchange chromatography are used for purification. Finally, the purified enzyme is dried using spray drying or freeze drying.
- **Benefits:** This approach not only ensures high purity and activity of the enzyme but also reduces production costs through efficient recovery and purification.

2. Antibiotic Production in the Pharmaceutical Industry

- **Background:** The pharmaceutical industry relies on downstream processing for the production of antibiotics. Antibiotics are crucial in treating bacterial infections and are in high demand worldwide.
- **Downstream Processing Challenge:** The challenge in antibiotic production is to recover and purify the antibiotic compound from the fermentation broth while ensuring high potency and minimal contamination.

- **Solution:** In this case, the fermentation broth undergoes a series of steps. Initially, filtration methods, including micro-filtration and ultrafiltration, are used to remove cells and impurities. The antibiotic is then extracted from the resulting solution using liquid-liquid extraction or precipitation techniques. Finally, chromatographic purification, often utilising high-performance liquid chromatography (HPLC), is employed to achieve the desired purity.
- **Benefits:** The application of downstream processing ensures that the antibiotic product is of high purity, potency, and quality, meeting stringent pharmaceutical standards.

These case studies demonstrate the versatility and importance of downstream processing techniques across different industries, showcasing their role in ensuring the purity, quality, and efficiency of product recovery.

VIII. CONCLUSION

In conclusion, downstream processing remains a cornerstone of bioprocessing, ensuring the efficient separation, purification, and recovery of biotechnological products. As of 2023, the field is characterised by a continuous wave of advancements, exemplified by innovative separation techniques, the widespread adoption of single-use technologies, the transition towards continuous processing, and the integration of AI and ML for precise process control. Sustainability is a driving force, with industries increasingly prioritising eco-friendly practices and resource conservation.

Professionals in bioprocessing industries must remain informed and adaptive to these developments to remain competitive and address evolving market demands. Collaboration between industry, academia, and regulatory bodies continues to be crucial in ensuring the safe and effective production of biotechnological products that meet stringent quality standards while advancing the cause of sustainability.

IX. AUTHORS CONTRIBUTION

The authors confirm contribution to the paper as follows: A, B, C, and D did Data Collection and Manuscript Preparation; E did Study Conception and Design, And Critical Analysis. All authors reviewed the results and approved the final version of the manuscript.

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