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REVIEW PAPER

TITLE:

Exploring Downstream Processing Strategies for Active Pharmaceutical Ingredients (APIs) Derived from Industrial Fermentation

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Exploring Downstream Processing Strategies for Active Pharmaceutical Ingredients (APIs) Derived from Industrial Fermentation

ABSTRACT

Industrial fermentation is a commercial process involving the use of microbes to produce various goods with significant economic importance. The downstream processing of these products involves separating required metabolites and products from the fermentation broth and purifying them to the required level. Product recovery can cost 30-60% of the entire production cost. Factors such as product placement, chemical and physical characteristics of the culture broth, impurities, metabolite concentration, intentional application, required purity, consumer expectations, and finished product cost influence recovery method development. The article explores the potential of a bio-based economy, focusing on the efficient use of biomass in agriculture to create end goods like medicines and active pharmaceutical components. It discusses sustainable bioenergy production methods, bio-based goods, and biofuels, aiming to meet local needs and provide opportunities for larger markets. The study also discusses biotechnological alternatives, innovative fermentation methods, cultivation conditions improvement, enzyme synthesis, and metabolite extraction and purification procedures. It also discusses recent developments in downstream processing of active pharmaceutical ingredients and their fundamental and biotechnological features.

KEYWORDS: Industrial fermentation, Upstream processing, Downstream processing, Chromatography, Crystallization. APIs,

1. INTRODUCTION

Active pharmaceutical ingredients (APIs) are chemical substances primarily manufactured in China, USA, India and Europe. These active components are used to diagnose, cure, alleviate, and manage diseases when combined with other substances. Over 100,000 metric tonnes of pharmaceutical goods are used worldwide, with Europe accounting for approximately 24% of the total consumption. The creation of APIs has led to the emission of chemicals into the environment, resulting in pollution. Pharmaceutical firms are now approving the use of microbial-based fermentation using bacteria or yeast instead of chemical channels that produce API due to their negative effects **(Kumar et al., 2022)**. In the last decade, there has been a growing trend towards using microbial-based production for recombinant products, leading to FDA clearances for human use. Microbial-based biopharmaceuticals generated around one hundred billion dollars with the industry growing at a significant rate of six percent CAGR. In 2020, the global demand for fermentation-based protein medicines is projected to increase from forty-four billion dollars to sixty billion dollars, while the

demand for peptide hormones and vaccines is projected to increase to $18–28 billion **(Kumar et al., 2021)**. Pharmaceutical firms like Bayer, GlaxoSmithKline, AbbVie, Biocon, Sanofi, Eli Lilly, and Merck rely on microbial systems and structures to produce biopharmaceutical products. This study focuses on recent advancements in the chemical processing of biomass compared to conventional approaches, with a specific emphasis on the production of APIs. Chemical-based active substances pose a significant risk to human health, water sources, and other organisms due to their unrestricted exposure to the environment **(Savairam et al.,2023)**. Chemical compounds are formed from various components, often originating from a single intermediate, and are transformed into Active Pharmaceutical Ingredients (APIs) through extensive purification processes. Carbohydrates, such as starch, cellulose, hemicellulose, pectin, and lignin, are a significant biomass used to produce chemicals, constituting about 95% of the planet's organic molecules. To create different products, such as chemicals and polymers, changes to the fermentation process are necessary **(Park et al., 2021)**. The focus has shifted towards using cellulosic biomass, particularly lignocellulosic material, for fuel generation rather than converting it into xylose and glucose. Lignocellulosic materials are transformed into sugars, chemicals, or ethanol through chemical, thermal, or biological processes. Pretreatment methods such as hydrolysis, alkaline treatment, delignification using sulfuric acid, high-temperature steam treatment, and pressure-based homogenization are typically necessary to break down these materials and disrupt their structured plant-based composition **(Park et al., 2021)**. To use the potential applications of lignocellulosic materials while maintaining environmental safety and reducing manufacturing stages or costs, technical and economic constraints must be overcome. Several studies emphasize the production of ethanol above other products, but there are few life cycle assessments (LCAs) capable of producing various goods. Forestry-based biorefinery systems are an example of environmentally friendly production of bioethanol and biodiesel **(Mujtaba et al., 2023)**.

Approximately 192,000 tonnes of propionic acid and its related esters are produced annually, used in various applications in the chemical industry, such as thermoplastics, solvents in paints and resins, and animal feeds **(Kumar et al., 2023)** . It is anticipated that the economic conversion of glucose to propionic acid will be 15% less than that of glycerol. Glycerol fermentation can also produce 3-hydroxypropionic acid, a crucial chemical building component, and further convert it into acrylic acid, offering improved environmental functionality. Researchers from the National Renewable Energy Laboratory have developed a novel pre-treatment method using an organic solvent and water to separate lignin and sugars from their chemical source **(Ranaei et al., 2020)**. Sugar alcohols are essential chemical building blocks for low-calorie or non-caloric sweeteners, replacing sugar in the food sector. Currently, most sugar alcohols are

produced using refined sugars, but a shift towards sustainable, non-food sources is expected. Various yeasts can transform sustainable raw materials like lignocellulosic substrates, glycerol, and molasses into sugar alcohols. However, these bioconversions face challenges in achieving high yields and productivities. Recent research focuses on using yeasts to convert renewable feedstocks into sugar alcohols like xylitol, erythritol, mannitol, and arabitol, and addressing limitations in sugar alcohol synthesis **(Erian and Sauer, 2022)**.

Another study focuses on creating a consolidated bioprocessing organism (CBP) to transform biomass into lycopene. The organism possesses two genes from the zeaxanthin biosynthesis pathway and a synthetic ten-gene pathway that includes enzymes xylose conversion, xylitol oxidase, and D-xylulokinase. The maximum amount of zeaxanthin produced from xylan was 21. The study found that the biosynthesis of commercially important carotenoids was 0.74 mg/liter, with the expression of NADPH and ATP-supplying enzymes changed due to the deletion of genes encoding zeaxanthin glucosyltransferase and lycopene-cyclase. The biosynthesis of commercially important carotenoids was found to be 0.74 mg/liter. The pathways involved in the methylerythritol phosphate (MEP) provide precursors for the production of naturally occurring carotenoids, as illustrated in Figure 1.

The study reveals that altering five pathways in *E. coli* led to a 3.5-fold increase in β-carotene accumulation, enhancing the entire β-carotene production mechanism. TCA and ATP modules did not increase β-carotene synthesis, but TCA and PPP modules did. Increased NADPH accessibility was more beneficial for β-carotene synthesis than ATP. Mutant strains resistant to oxidative stress produced 30% more β-carotene. Increasing substrate channeling of β-carotene 3-hydroxylase and CLY β-produced more zeaxanthin compared to *D. salina*.

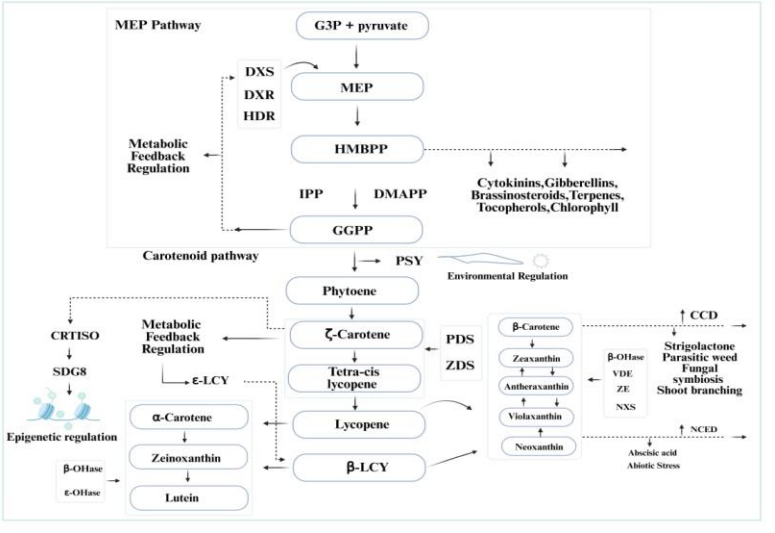


Figure 1 shows the metabolic pathways The pathways involved in the methylerythritol phosphate (MEP) provide precursors for the production of naturally occurring carotenoids.

Industrial fermentation is the large-scale production of commercial products or significant chemical transformations using living organisms, particularly microbes. The goal is to enhance physiological or biochemical processes in microbes to provide better quality and quantity of a specific product. This process involves understanding disciplines such as biochemistry, microbiology, genetics, cell biology, chemical and bioprocess engineering, chemistry, and computer science. The main microorganisms used in industrial fermentation are bacteria and fungi, primarily yeast. Large bioreactors and fermenters are used for thousands of liters of reactions. Industrial fermentation is now carried out in various industries, including microbiology, pharmaceutical, food, chemical, and biotechnology **(Sharma et al., 2020)**. In biology, fermentation is defined as the practice of harvesting energy by breaking down organic molecules in anaerobic conditions. Sugars are primarily used for this purpose. Two classic ways to categorize fermentation are the type of substrate metabolized and the product produced in the reaction. Industrial fermentation is referred to as any significant microbiological process in industry, but it has a distinct meaning in industry. Most commercial fermentations require oxygen or aerobic conditions. Various organisms, including plants, animals, fungi, and bacteria, are employed in industrial fermentation **(Siddiqui et al., 2023)**.

Fermentation is a process where microorganisms develop a desired product by incorporating specific nutrients into their media, which are typically broth or liquid. Major media components include carbon, oxygen, nitrogen, and hydrogen, while minor or trace elements like phosphorus, iron, or sulfur are supplied in adequate amounts. Fermenters or bioreactors are closed vessels used to offer controllable conditions for microbial growth and maintenance. Engineers construct these vessels to optimize parameters such as temperature, pH, fluid flow, and nutrients. There are two types of fermenters: aerobic (with oxygen) and anaerobic (without oxygen). Aerobic fermenters are complex and require aeration, with stirring using oxygen sparging or impellers for easy oxygen transfer and dispersion. Controllable conditions are crucial for microbes to carry out reactions properly. Computers are often used for monitoring and controlling fermentation processes, controlling pH, temperature, oxygen concentration, cell density, nutrient levels, product concentration, and nutrient levels **(Liebetrau.et al., 2018)**

Advancements in technologies like genetic and metabolic engineering and enzymatic engineering have created opportunities to develop innovative industry-based goods like APIs using renewable biomass as raw materials. Renewable resources serve as excellent substrates for creating green chemicals, bioengineered APIs, and key starting materials (KSMs) within this extensive field **(Fernández-Cabezón and Nikel, 2020).**

1.1. Steps Involved in Bioprocessing

Upstream and downstream are two major steps utilized in the development of active pharmaceutical ingredients (API), which are the building blocks of biopharmaceuticals. Little amounts of modified microbial or

mammalian cell culture are used in upstream processing to increase their volume in bioreactors under controlled environment **(Stocker et al., 2020)**. Cells or products that are produced during upstream process are purified and separated by various methods like chromatography, filtration, or centrifugation in the downstreamenvironments process step. API manufacture and processing are hampered by rising manufacturing costs and regulations. This necessitates adaptable solutions for upstream and downstream bioprocessing, that's why more and more manufacturing processes are using single-use systems to make each process step saleable from the lab to the industrial level. To produce large quantity of cells from little amount of various cell lines, however, is the primary goal of upstream processing **(Chandra et al., 2020; Olughu et al., 2019)**. Titers serve as the principal benchmark to classify upstream production efficiency, with relatively high titers typically suggesting that more required product is developed using the same or less quantity of fluid or packed bioreactor volume. The needed volume can fluctuate, and single-use techniques are an efficient method to meet various requirements and volumes. During the whole process development, it is necessary to keep an eye on certain characteristics, including glycosylation sequences for monoclonal antibody (mAb) molecules, which are predominantly affected by the upstream bioprocess **(Somasundaram et al., 2018)**.

In a bioreactor, there are two different forms of upstream bioprocessing. The continual removal of cell culture from the bioreactor and replacement with new cell culture medium is known as perfusion, also known as upstream continuous processing. It was used to make contemporary bioproducts, when speed to market is important. Because of its great flexibility, effective utilization of resources, and associated cost savings, perfusion is used as efficiently as possible **(Chen et al., 2018)**. In contrast, in fed-batch mode, the bioreactor is supplied with nutrients while it is cultivating. Reactions from fed batches might persist for 14 days **(Schulze et al., 2022)** .

The initial step in bioprocessing, where microorganisms or cells are cultivated either through microbial or mammalian cell cultures in bioreactors, is referred to as the upstream phase of a bioprocess. By providing nutrients, cell culture medium or growth hormones to the fermenter, cell growth and cultivation can be promoted **(Yew.et al., 2019)**. Mammalian cells cannot cultivate as quickly as microorganisms and might take hours or even days to grow. Cells are retrieved after they are prepared for harvesting, before proceeding further in the downstream processing phase. The collected bioreactor material is cleaned up for further refining in the clarifying and filtering processes by removing contaminants and particles **(Tan et al., 2018).** The cells need to be collected and purified in order to be used for biomanufacturing procedures once they have been grown and harvested. Downstream processing involves assembly of a decontaminated final product comprising hormones, antibiotics and enzymes-typically acquired in large scales, whereas analytical bio separation (accomplished by downstream processing) corresponds to the purification method for primarily measuring the

components of a formulation. The latter might involve smaller sample sizes like a single cell, whereas large sample size is required for authorized vaccines or gene editing products. For this reason, scaling up is not only a desired alternative, but is also seen as a crucial component of the entire development process **(Janghorban et al., 2023)**.

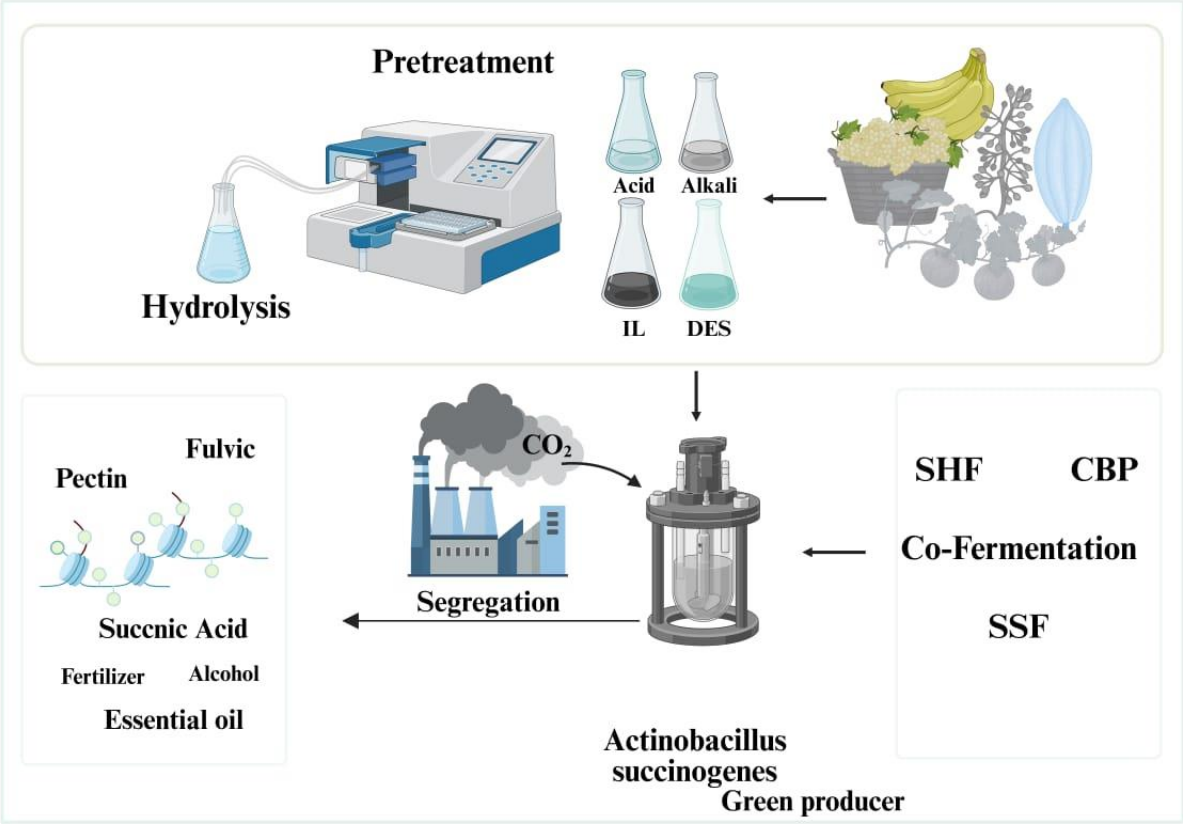
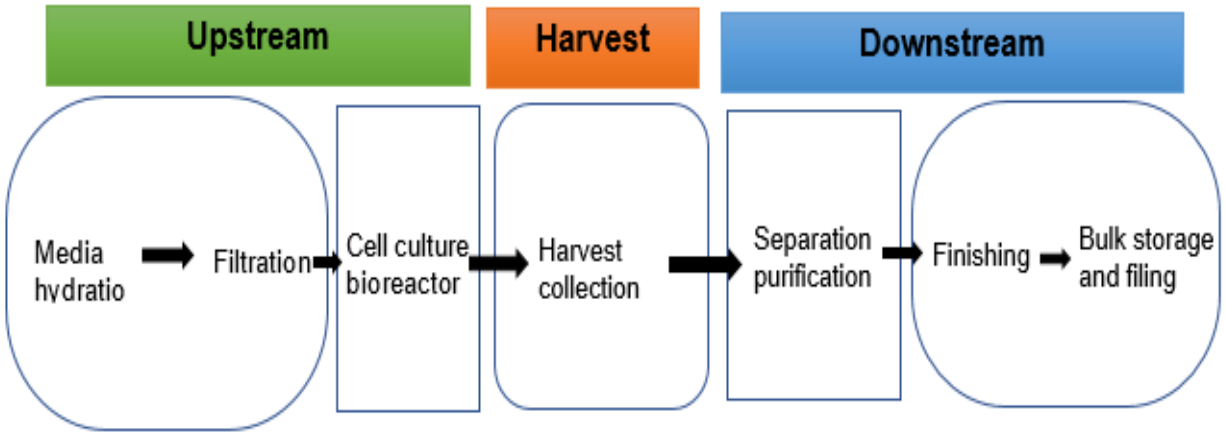


Figure 2. A schematic representation of *Actinobacillus succinogenes* as green producer.

Downstream bioprocessing comprises of processes that generate a pure product from biological components like cells. The series of steps involved in downstream processing are separation, cell lysis, extraction of product, isolation, refinement, removal of contaminants, virus inactivation and concentration etc. Filtration, sedimentation, centrifugation, or precipitation are common procedures to remove insoluble materials **(Díaz-Montes and Castro-Muñoz, 2019)**. The elimination of components whose qualities significantly differ from those of the desirable final product is known as product isolation. Solvent extraction, ultrafiltration and adsorption are typical methods in the isolation process that eliminate contaminants. A wide range of equipment for specialized applications and functions is included in bioprocessing equipment. In broad spectrum and in relation to a flow diagram of process (Fig. 03), the equipment's can be divided in three groups: upstream, harvesting and downstream. Upstream equipment pertains to

the development of a host species to create a product **(Chisti and Moo-Young, 2020).** The final product might be the organism itself, it can be hold inside of organisms, or they can defecate it into the growing media. The downstream machinery purifies the harvest that results from the upstream operation, for instance, by filtering and chromatography. Support equipment includes other items required in biomanufacturing include incubators, liquid mixers, utility carts, bead mills, holding tanks and various cell disruptors. Autoclavable, chemically subitizable or sterilize-in-place (SIP) systems maintain a sterile environment and sanitary design with considerable challenges. Single-use apparatus enables the development of a fresh, innovative subset of bioprocess equipment that is mostly free from the burden of sanitary design [24].

Figure 03: Diagram of a simplified typical bioprocess flow (Chisti & Moo-Young, 2020)



For equipment that cannot be made entirely single-use, disposable flow channels and components give a comparable level of flexibility. Components, functionality, and structural concerns must change swiftly as applications of industrial equipment expand and the advantages of single-use technology are still sought after dedicated production facilities depending on recyclable equipment were typical in the past, but today's biomanufacturers need more flexibility and versatility **(Mussatto et al., 2021)** odern, new facilities are anticipated to be more effective, efficient, and able to manage many products and change production levels as needed by the market.

2. Downstream processing

The downstream processes and purification strategies are a crucial step in the manufacturing process and have a sizable impact on the process costs as a whole. The composition of the substance and its amount in the fermenter at harvest determine how much the downstream purification program actually contributes to the intricacy and expense of the overall operation **(Straathof, 2011 ; Qin et al., 2023)**. Diluted products, in particular, frequently have substantial

purifying costs, which might be between 50% and 90% of the overall manufacturing expenses. Low concentration products, including vitamins (like vitamin B12, which has a few mg/L concentration), must be processed in huge quantities per mass of product, requiring big process equipment with correspondingly high capital and running costs.

Due to the low concentrations, additional recovery stages are required and result in persistent losses. Moreover, processing of particular items could demand more money and specialized machinery, such chromatography, to reach the necessary level of purification **(Mukherjee, 2019)**. As many of these specific products are fragile, there are only a few possibilities for purifying processes, and less expensive alternatives might not be appropriate. Many unit activities that are relevant to bioproduct accumulation and purification are presented in this review and include their usefulness in relation to fragile bioproducts. The difficulty in preserving the integrity of fragile products (such physiologically active enzymes) was specifically mentioned. Conceptual plans for recovery strategies of cell-associated metabolites and expelled cell products are described. Therefore, in this review, recovery programmed for items requiring various steps of purification are covered.

2.2. Potential Recovery Methods

Bioproduct manufacturing uses multiple unit operations to facilitate particle disintegration, solute molecule recovery, and segregation of liquids and solids, including crystallization and drying techniques. These processes are crucial for the recovery of valuable bioproducts and are essential for sustainable production methodologies. The upstream stage is crucial for optimizing yield and quality. The downstream operations process and recovery costs decrease when a large amount of product enters the recovery procedure. The initial physical stage involves separating cells from the extracellular environment, allowing the appropriate stream to be exploited for product recovery **(Teke et al., 2022)**.

Based upon whether a solid stream or a liquid stream enters in the process, different program steps will follow. If such product is able to dissolve in the liquid stream, unit operations created to condense and filter a soluble molecule are used to restore the liquid stream. The unit procedures that allow for some amount of concentration should be carried out initially so that the succeeding purification stages may be completed with little material **(Dimitrijević et al., 2023)**. This is crucial for recovering specialized biomolecules that need expensive machinery (such certain kinds of chromatography) to achieve the high purity requirements. Prior to purification, first operations to remove the product which is linked to a solid stream, whether it is inside the cytoplasm or adherent to the cell-surface, must be carried out. This calls for the cell to be broken down and the extracted cell to be separated from the broken cell. After which, the cell extract goes through one or even more procedures meant to concentrate and clean a soluble component **(Wang et al., 2021b)**. Naturally, only finishing procedures like drying are necessary if the product is the cell itself (such as *S. carlsbergensis* and *S. cerevisiae* intended for the beverage and food sectors) (Figure 4).

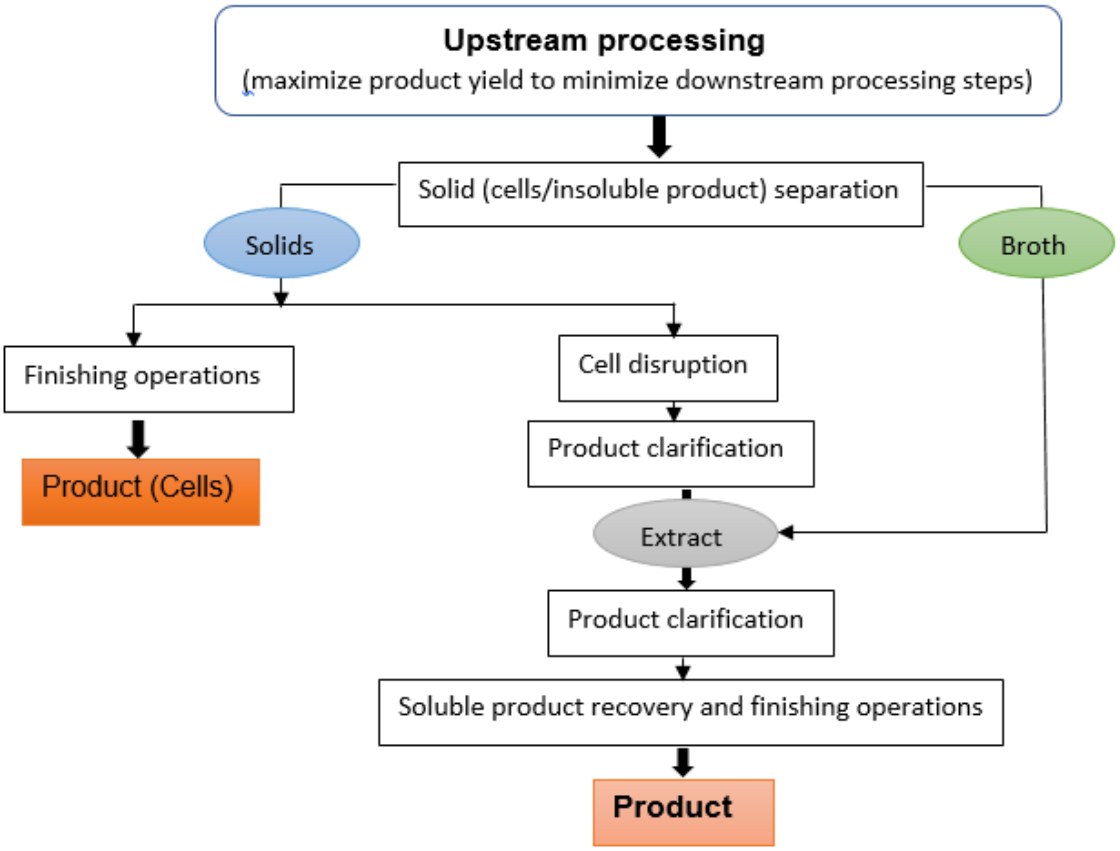


Figure 04: Overview of microbial product recovery (Arun et al., 2020).

3. Isolation of cells, extracellular fluids and Metabolites

After fermentation, a series of methodical and orderly actions must be taken to obtain significant products out from cultured broth **(Kostelac et al., 2023)** . Two types of fermentation are; submerged fermentation and solid state fermentation. For intracellularly produced products, isolation pertains cell disruption is the first step in solid substrate fermentation's. Then, prefiltration is used to remove any insoluble material, and concentration comes last. Only separation of cells, followed by filtering and concentration, constitutes isolation in submerged fermentation. For each of these phases, several industrial processes are used, including centrifugation, ultra sonification and homogenization, flocculation and precipitation, filtration using membrane filters, ultrafiltration, and microfiltration **(Mukherjee et al., 2019)**.

3.1 Separation Techniques

The following methods are covered in detail for isolation: (1) flocculation (2) filtration and (3) aqueous two-phase segregation.

3.1.1 Flocculation Technique

Large clusters of cells (or cell detritus) occur during flocculation. These aggregates are simple to remove and progressively cluster together. The characteristics of the cells and

the ionic components of the medium affect the flocculation process. Agents like organic polyelectrolyte, mineral hydrocolloids and inorganic salts are provided in the form of high molecular weight polyelectrolytes (natural or synthetic) to achieve appropriate flocculation. These polyelectrolytes connect and engross the colloidal particles in the form of enormous flocks used for flocculation and measure 10 mm in size **(Maćczak et al., 2020)**. Throughout the last three decades, flocculating agents had already undergone tremendous development, which has considerably improved the usage and functionality of several categories of separation equipment. The pH, charge and size distribution of the particles, conductivity of the medium, molecular mass of the polymer, the charge density and other variables all have an impact on how flocculants function. These specific characteristics have an impact on the procedure' safety profile as well as the total recovery of the desired protein. Charged flocculants make dispersed particles seem larger by aggregating them, which makes it easier to separate solids from liquids and lowers process costs (**(Maćczak et al., 2020; Lee et al., 2014)**

A wide variety of flocculants were tried to increase filter capacity above the typical optimal limit (Table 11). These high-molecular-mass polymers have substantial positive or negative charges (such as polyacrylamides or modified polyethyleneimine), which encourage clustering by charge-neutralization and bridging of dispersed particles **(Ajao et al., 2021)**. Since aggregation raises the average magnitude of the scattered particles, this process is referred to as "flocculation," which leads to the creation of flock-like aggregates. To increase the longevity and performance capacity of the refined filters (such as depth filters), flocculants promote the separation of solids using less costly coarse filters (such as bag filters). Because of this, less expensive filter material is required to provide a feed stream with a very low turbidity threshold. Occasionally, flocculants stop ta rget proteins from recovering because they interact with charged polymers and cause them to cluster **(Djaani and Baba, 2023)**.

Table 01: Flocculants employed for particulate precipitation before depth filtration **(Hadpe et al., 2020)**.

Flocculants	**Charge**	**Nature**	**Charge density (meq g^{-1})**
Sedipur CL 950	Cationic	Polyamine	7.0
Praestol 822 BS	Cationic	Polyacrylamide	1.0
Lupamin 9095	Cationic	Polyvinylamine	12
ZETAG 7109	Cationic	Dimethylaminoethyl acrylate methylchloride	5.5
Praestol 2350	Anionic	Polyacrylamide	6.9

Magnafloc LT 37	Cationic	Polydiallyl dimethylammonium chloride	4.7

As a result, selecting the ideal dosage and polymer is a crucial step that involves thorough experimentation. Many variables, including distribution of particle size, concentration of solids, surface composition, pH level and electrolyte content influence the choice of best type of flocculant to use and their dosage. When the polymer covers 50% of the particle's surface area, the dosage equivalent is optimal. High levels of hydrogen bonding and calcium ions create salt linkages that allow polymer anionic groups to adhere to the coal surface. Anionic flocculants, for instance, are known to work effectively on coal slurries **(El-taweel et al., 2023; Marichamy et al., 2021)**.

3.1.2 Filtration

The bioprocessing industries make extensive use of filtration, with both continuous filtration and batch filtration having several applications. For separating cells on a large scale, like when producing Saccharomyces species, continuous filtration is used, often using suction drum filters **(Raina et al., 2022)**. This entails drawing cells onto drum surface while maintaining consistent pressure using a rotating drum in a trench of suspended cells (vacuum). In this approach, a cake/sheet of cells is produced on the surface of drum as it exits the trench. A string or a blade 2 is employed to extract the filter cake, depending on whether it has to be processed further or discarded. Batch filtration is often practiced, however on a smaller scale application, and the chamber press is the filter of choice most often. The chambers of this filter press are splitted by filter plates and are held within a single frame **(Mahato et al., 2019)**.

When the filtrate passes onto the filter press, the pressure-fed cell suspension is delivered to the chambers, where the cells are lodged as a cake filter upon the filter media. Batch filtration could be done with constant pressure (where the rate of filtration drops over time) and constant rate (where the rate of filtration rises over time), or these parameters can be variable **(Kaškonienė et al., 2017)**. During cell culture procedures, liquid or gas filters guarantee the quality of the air flow and nutrients, decontaminates the circulating medium during perfusion procedures, help with harvest clarifying, and eliminate chromatographic eluate from buffers after chromatography columns. They are utilized in process waters, environmental monitoring, fill-finish, and formulation work. Filters' usefulness has increased recently due to the advancement in chromatographic membranes.

3.1.2.1. Types of filtrations

The most useful method for separating the culture filtrate and biomass is filtering them. The effectiveness of filtration is influenced by variables such as organism size, incorporation of different organisms, temperature and viscosity of medium **(Souza et al., 2022)**. For these uses, a variety of filters are employed, including depth filters,

membrane filters, surface filters, and rotary vacuum filters. The removal of particles of a certain size out of a fluid depicts the quality of various filters. Pore size evaluations reflect the size of an organism or particular particle that is efficiently trapped on the filter medium. The clarifying stage is preceded by filtration using 0.45- or 0.2-m membranes, to lessen bio-burden and minimize contamination of downstream chromatographic sections **(Droppo, 2014)**. Efficiency of filtration is determined by its absolute ratings. For example, the average filtration rate of a ceramic filter is 0.9 m This indicates that the greatest pore size of the filter is 0.9 μm. Hence, the filter will probably entirely retain microorganisms bigger than 0.9 μm. Defined pore sizes of these filters are shorter than the target particles to be eliminated. Absolute filters are effective in removing bacteria from culture media **(Dev et al., 2022)**.

The high precision of protein selectivity is achieved by the filtering process, which takes advantage of variations in size as well as in charge. For instance, the two-steps ultrafiltration (UF) method employs two different sized UF membrane filters, the first of which has a larger pore size (stage 1) and the second of which has a smaller pore size (stage 2) than the desired protein **(Vicente et al., 2021)**. Efficient implementation of scaling up the ultrafiltration techniques for recovery of medicines created through DNA recombination took place after significant research work. In the biotechnology sector, linear scale-up of these recovery procedures is very crucial since it directly affects protein quality, product economics and process yield **(Nor et al., 2018)**. Due to its cheap initial cost as well as simplicity of validation, depth filtration is frequently employed for the purification of cultured cells harvests (compared with TFF devices and centrifugation) **(Parau et al., 2022)**. Depth filtration, sometimes known as a "workhorse filter," provides a useful and reasonably priced substitute by eliminating contaminants and avoiding clogging of the downstream membrane filter. In order to offer the required cationic surface property and wet resistance, the majority of depth filters used in biopharmaceutical processes are composed of cellulose fibres and filter additives, such as diatomaceous earth, bound together with a polymeric glue.

Several types of depth filters include asbestos, filter paper and glass wool. The fluid exits while the particles are kept inside the matrix. The matrix of a filter, made of cellulose fibers, is used to separate filamentous fungus **(Khanal et al., 2018)**. The diameters of those fibers and how thoroughly they are compressed in a filter bed define a filter's porosity, particle-retention rating and void volume. The penetration and stability properties of a filter are closely associated with its degree of compression. The tighter compaction can hold small particles in a better way which ultimately lowers the permeability **(Mikyška et al., 2019; Chen et al., 2021)** .

Filtering of culture broths in bioprocess with tangential-flow filter (also known as cross-flow filtration or CFF) using microfiltration membrane filter has become more common. Using a TFF (Tangential flow filtration) technique has the benefit of producing a stream that is comparatively clean and

requires little filtering before stacking on a protein **(Musumeci et al., 2018)**. TFF (or cross-flow microfiltration's) have a large starting flux, which is a significant disadvantage. As a consequence, if such transmembrane pressure is not adequately regulated during startup, a fouling layer quickly forms, causing the flux to decline to a small portion of its original value. Rotary barrel hoovers filters work well for separating slurries with particulates between 0.5 and 10 μm in size and 10% to 40% solids by volume. These screens are effective for filtering filamentous fungi and yeast cells **(Madsen et al., 2022)**. The apparatus is simple to use and consumes little electricity. The filtering system comprises of a rotating cylinder that is submerged in a broth vessel to some extent. When the liquid is drawn within the rotating cylinder, the biomass adheres to the surface in the shape of a cake. Solid particulates made of this filter cake are expelled.

A membrane filter act as a barrier which has the ability to redistribute the constituents in the fluid stream without causing a phase change, by utilizing a driving force of pressure differential method **(Bamforth and David, 2019)**. When a differential pressure (1-100 bar) is applied, the components undergo a molecular sieving based on the size of pores in the membrane. Reverse osmosis (RO), microfiltration (MF), ultrafiltration (UF), and conventional filtration (CF) are the different types of membrane separation methods based on the size of pores (Figure 10In contrast to UF and MF membranes, which have pores with diameters between 0.01-2.0 mm and 0.2-10 mm, respectively, pore size of membrane is larger in CF that is 10 mm. UF membranes are typically available in the molecular size of 1-500 kDa and are defined by means of cutoff values **(Cavero-Olguin et al., 2019)**.

In the initial phases of recovery, UF and MF are more frequently employed than RO. The strategy of two-stage UF has been effectively used to separate a variety of proteins including whey protein isolate, surfactant from fermentation broth, α-lactalbumin and β-lactoglobulin and separation of bromelain **(Md Saleh et al., 2021)**. While bromelain has a molecular mass range of 23.4 to 35.73 kDa. Another study found that using the two-phase UF process, which consisted of membranes with larger MWCO (in the UF stage 1) and smaller one (in the UF stage 2) from the bromelain molecular mass, increased the purity of the compound by 2.5 times **(Nor et al., 2018)**. Polymeric membranes such polyvinyl fluorite, cellulose acetate and polysulfide have been employed in the majority of earlier investigations.

The generation of proteins from transgenic plants frequently involves a number of processes intended to increase the expression of specific proteins, from gene editing to breeding. Trypsin, laccase, avidin, and β-glucuronidase are a few examples of these typical proteins. These proteins exhibit a variety of molecular weights, localizations and functions, revealing the system's flexibility **(Valiulahi et al., 2018)**. The application of transgenic plant engineering for commercial enzyme production has several benefits, including the substitution of chemicals that pollute the environment. According to research, amylopullulanase (APU), which is derived from the bacterium

Thermoanaerobacter thermohydrosulfuricus, was expressed heterologously in maize seeds. The stored starch was then fermented, which immediately increased the amount of bioethanol that could be produced from maize grains. This study demonstrated how a biotechnology technique can simplify the starch-based synthesis of bioethanol utilizing maize grains **(Niu et al., 2023)**.

It is well established that the combination of transgenic proteins with elastin-like peptides (ELPs) increases the concentration of recombinant proteins in plants, leading to a better yield and less complicated recovery. According to research, the ELP tag made it easier for heterologous proteins to accumulate in tobacco leaves. The membrane-based inverse transition cycling approach (cITC), or centrifugation-based methods including heating, salting, and resolubilization without salt at a reduced temperature, further assisted in the deposition of ELPylated proteins **(Guo et al., 2023)**. This procedure has been further improved by applying microfiltration to separate the precipitate in a pure form, or "the good". Anion exchange chromatography, Ni-column chromatography, Fc fusions, and other protein-based affinity chromatography techniques have already been used to isolate industrial enzymes derived from plants as well as genetically modified antigens from the plant matrix. Another method involves combining two-stage separations, such as multiple gel filtration and membrane filtration **(Phan et al., 2020)**. Consequently, an effective DSP is attainable, with superior product quality, by methodically characterizing the host cell protein criteria. This classification may be employed in a USP-DSP strategy to avoid the creation or buildup of "bad" and "ugly" contaminants, resulting in an effective manufacturing process.

3.1.3 Aqueous two-phase system

In general, "solvent extraction" refers to either solid-liquid separation (leaching) or the separation of constituents based on changes in their absorption in two phases. Intensive contact between both the culture medium and an appropriate solvent, in which any of the target components are often more soluble, is required for liquid-liquid extraction **(Jong et al., 2017).** The two phases are then physically separated by centrifugation or settling. The terms "extract" and "raffinate" refer to the solvent-rich solution that contains the extracted substance and the residue, which comprises of less solvent, respectively. Through distillation, solvent from the extracts is usually recovered. Extraction might be a continuous process, a single stage, or a series of phases. Liquids typically flow in a countercurrent direction, while different flow patterns are conceivable. To facilitate separation, continuous countercurrent differential-type contactors backflow the residue after removing the solvent out from extract **(dos Santos et al., 2020)**. The key factors that influence the choice of solvent are mainly cost, the partition (or dispersion) coefficient (i.e., the ratio of quantity of the component in the extract to the raffinate) and toxicity.

By adjusting the temperature, pH, or by adding salts, the partition coefficient might be changed. The solid (biomass) is incorporated into close contact with the solvent during solid-liquid separation. After

separating the remaining biomass, the product is extracted from the mixture (Shi et al., 2022). There are several flow schemes that may be employed, including concurrent, continuous-column extraction, and others. The most well-known liquid extraction process is solvent extraction, which depends on the product's preferential solubility in an auxiliary organic phase that is immiscible with aqueous phase. The effectiveness of this extraction method is significantly evaluated by a distribution coefficient that links the solute concentrations in the various phases mainly depending on adequacy of solvent. Optimal solubility in the solvent and hence effective separation are indicated by a high distribution coefficient **(Phong et al., 2018).** Penicillin may be transferred between the organic and aqueous phases by only changing the pH since the acid is selectively soluble in the organic phase (amyl acetate) while salt is generally soluble in the aqueous phase. Acid is first supplied to the incoming crude aqueous stream in the penicillin recovery stage to encourage separation into amyl acetate on interface. Alkali is then introduced into the amyl acetate extract, and the penicillin salt is removed into water since frequent contact with amyl acetate is hazardous to the penicillin product **(González-Valdez et al., 2018).** Centrifugal extractors, which reduce the amount of time that the penicillin is in contact with the amyl acetate, are used in this method to limit penicillin degradation. Less than two minutes may be needed to complete the solvent extraction procedure from the beginning to the penicillin's re-entry into the final aqueous phase.

Examples of solvent extraction are the recovery of acetic acid, beta-carotene and lactic acid. Bacterial proteins, heavy metals and calcium are first taken out of the fermented broth before recovering the lactic acid. Afterwards, isopropyl ether is used in a countercurrent flow, to extract the lactic acid from the aqueous solution **(Ma et al., 2019)**. The derived aqueous extract is decolored, exposed to ion exchange, and then concentrated by the evaporation into food-grade production of lactic acid after additional extraction with distilled water in such a countercurrent flow. The acetic acid is recovered by using ethyl acetate and isolated by distillation in the extraction of acetic acid from submerged vinegar fermentation including ethanol. The *Blakeslea trispora* mycelium is first dried before being processed with methylene chloride, that removes the b-carotene, in order to recover the pigment. By evaporating the extract at a low temperature, the extract is refined, and purified beta-carotene is precipitated from solvents like chloroform and acetone. Water-based enzyme extraction from mouldy bran and hexane-based fat extraction from Rhodotorula cells are two examples of this. theExamples of solid-liquid extraction include the extraction of enzymes from the mouldy bran employing water as well as the extraction of lipids from Rhodotorula cells utilizing organic solvents like hexane **(Roukas and Mantzouridou, 2001)**.

3.1.4. Salting (Precipitation)

Salting out is the most popular precipitation method for concentration and purification of proteins. In this method salts are added to create an imbalance between the electrostatic

and hydrophobic forces that tend to keep proteins in solution and produce protein aggregates. (Fan et al., 2022). For this purpose, neutral salts like citrates, phosphates, and sulfates are preferred. But the most widely used salt is ammonium sulphate which is selected due to its multiple characteristics like cost effectiveness, capable to stabilize enzymes and high solubility. At the end of the procedure the residual salts may contaminate the precipitated proteins, therefore, to remove the residual salts gel filtration or infiltration techniques are used. Depending upon some characteristics of the protein the method of salting out is selected. Before applying the technique of salting-out these parameters should be kept in mind i.e. the selection of the salt, method of contact, characteristics of the proteins and cost.

Recovery process of alpha-amylases from the fermented broth is shown in Figure 5.

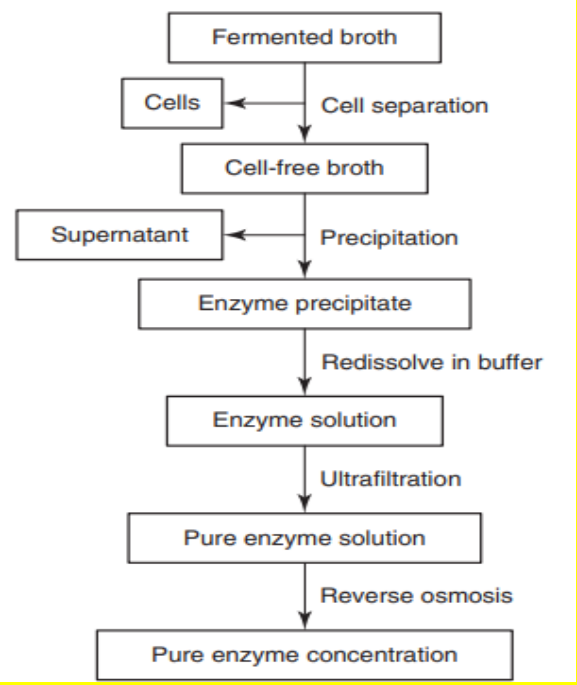


Figure 05: A method for extracting a-amylases enzyme from fermented broth **(Sewalt et al., 2018)**.

The addition of salts increases the ionic strength of the solution leading to increase the solubility of the salt. This process is named as salting in. But if this increase in salt concentration is continued then the protein solubility decreases leading to protein precipitation. This is known as 'Salting-Out'. This process decreases the proteins solubility and increase the occurrence of stabile native confirmation which is opposite to the process of Salting-in where salts act as denaturants leading to unstable the structure of proteins **(Hanke et al., 2022)**.

Protein purification can be done easily and effectively with salt precipitation. The most popular salt used in this method is ammonium sulphate because it is inexpensive, extremely soluble in water, and easily interacts with water. To precipitate the appropriate proteins, ammonium sulphate is either supplied directly as a solid or added as a (typically) saturated solution **(Wingfield, 2016).** The solubility of proteins tends to increase with the addition of more salt during salting-in at low salt concentrations (0.15 M). When the concentration of salt beyond the threshold, a phenomenon referred to as "salting-out," protein solubility decreases due to an excessively high ionic strength.

This occurs because the proteins and dissolved salt compete for decreasing water molecules, raising the water's surface tension and pushing the protein to fold more tightly. There are protein-water interactions as the protein's surrounding surface area shrinks **(Tola & Missihoun, 2023).** Protein aggregation and protein precipitation are both

brought on by an increase in hydrophobic interactions between protein molecules. Because they will precipitate out depending on the salt concentration, proteins in the solution can also be separated out. Undesirable proteins can be precipitated out by adding a specified amount of ammonium sulphate. Then recover the supernatant, and add a small amount more of ammonium sulphate for desired protein precipitation. Precipitated protein pellet then preserves. It is possible to purify particular proteins in this manner. Since salt precipitation merely modifies protein solubility rather than denaturing it. The recovered fraction can be kept in the salt solution for lengthy periods without fear of bacterial contamination. This is because the high salt content inhibits any microbial growth or protease activity **(Pei et al., 2020)**.

Protein solubility is decreased during ammonium sulphate precipitation, although proteins are not denatured. Proteins can therefore be concentrated by eliminating the residual ammonium sulphate solution, and the protein pellet can subsequently be resolubilized in conventional buffers or with ammonium sulphate at a reduced concentration. Hydrophobic Interaction Chromatography (HIC) or Gel-Filtration chromatography can be used for protein purification. By gradually raising the concentration of ammonium sulphate, some proteins that have been denaturized by substances like urea can be guided back to their correct natural conformations **(Keshavarz et al., 2021)**.

4. Chromatography

Column chromatography is a key component of downstream processing strategy because it allows for quick and precise capture. Purification quality is improved by adding extra chromatographic steps like hydrophobic interaction or ion exchange chromatography. It has been acknowledged in theory that protein crystallization, that has primarily been used in protein structural studies, is a final process of protein precipitation **(Bernau. Et al., 2022)** Possible cost considerations for the manufacturing of therapeutic proteins include the chromatographic procedures employed throughout downstream bioprocessing again for purification and separation of multicomponent systems. The need for versatile and affordable procedures for the purification step has grown over the past ten years in the pharmaceutical industry. It is being tried to use new conventional biopharmaceutical methods. Chromatography, main workhorse of the separation process, continues to be the primary cost-determining source, thus it is crucial to optimize it in order to lower production costs **(Carta & Jungbauer, 2020)**. To successfully develop and optimize chromatographic purifying procedures, it is important to have a solid understanding of their complexity.

By separating molecules according to variations in their composition or structure, metabolites can be recovered via chromatography. Chromatography typically uses a variety of stationary supports through which the target metabolite is passed. The metabolite interacts differently with the stationary support, resulting in the molecule separation that identical to one another **(Quirino et al., 2020)**. Stronger contacts between test molecules and the assistance

will cause them to pass through the support more slowly versus test molecules having weaker connections. By doing this, as molecules pass through the material of support, they may be kept apart from one another. There are several different substrates that may be used for chromatographic separations, such as thin layer chromatography (in which silica is immobilized on glass plates), gas chromatography (volatile gases are used), liquid chromatography (insoluble molecule is used), and paper chromatography. The most used method is liquid chromatography, which is additionally readily scaled up (De Luca et al., 2021).

When better purification is demanded for expensive items, affinity chromatography, a highly specialized method, is utilized. It is frequently used for the recovery of enzyme since it may be 100 times purified in a single process **(Ccki & Riske, 2020).** In this method of chromatography, the ligands are strongly attached to a solid support via covalent linkage, including cellulose, agarose, or polyacrylamide, and the column is filled with these ligands. These ligands possess a highly particular affinity for the molecules of solute, and by chemically bonding with them, the solute is maintained in the column. An antibody might be maintained by connecting with its enzyme or an antigen, could be held by associating itself with inhibitor or its co-enzyme. The solute molecules are added at various instances which may favor the coupling, such as ionic strength, temperature, pH etc. The solute is then removed by adding a different molecule, such as substrate of enzyme, because the solute seems to have a stronger affinity compared to that of the ligand **(Arora et al., 2017)**.

Gel chromatography, also known as gel filtering, uses size exclusion to segregate solutes. Smaller molecules can enter the pores of porosity gel beads having regulated pore diameters manufactured from polysaccharides like polyacrylamides or dextran **(Brocklebank, 2020).** The biggest solutes are transported to the most distant end of column because they enter the pores fewest. Smallest solutes that enter pores the deepest are held in place the longest. As a consequence, the column has a gradient of sizes. The exclusion threshold and distribution of pore sizes of each gel make them distinct. While a tighter distribution of pore size improves the identification of molecules with comparable sizes, a larger distribution of pore size mechanism to achieve a wider variety of sizes **(Wang et al., 2018a)**. This approach uses hydrated sponge-like beads with holes that are the size of molecules and have a limited range of diameters as the stationary phase. Molecules that appear larger than the filtration medium's pores pass quickly through the column. This technique is also called ‘molecular sieving' strategy for separation of molecules. The order of molecules that eventually eluted was decreasing in molecular size. It appears that the molecular weight of the smallest substances that are unable to pass through a certain gel's pores is its "exclusion limit." **(Ohata et al., 2016)**.

Apart from traditional chromatographic methods, there have been significant advancements in this sector, notably affinity chromatography, immobilized metal affinity chromatography, reverse-phase

chromatography, and hydrophobic interaction chromatography to mention a few **(Begum et al., 2021)**.

5. Crystallization

For many ages, crystallization has been acknowledged as an effective method of producing pure proteins. It has been used in numerous enzyme production techniques, however on just a few occasions in pharmacologically active protein synthesis processes **(Siew & Zhang, 2021)**. Throughout the last twenty years, the emphasis of protein crystallization has changed from purifying procedure to the creation of excellent single-crystal diffraction. Insulin is a perfect illustration of a crucial pharmacological protein that is frequently refined. Insulin is a tiny, very stable peptide that can readily rearrange into its original form after it has been exposed to chemical solvents. Eventually in the purification process, after the majority of the contaminants have been completely eliminated, it crystallizes. Crystalline formulations are more stable and can be stored for longer periods of time than protein solutions **(Pu et al., 2022)**.

The particular protein and technique have a significant impact on when protein crystallization may be employed in a purifying procedure. It would be good to use fermentation broth for direct specific crystallization without treatment. This goal seems impossible to achieve practically. Hence, the effective strategy is to concentrate the protein that may theoretically crystallize **(Ouranidis et al., 2021)** Impure product crystallization often exhibits a significant alteration in the impact parameters' multidimensional domain. This generally results in a noticeable reduction in the dimensions of this zone, which weakens the process and might lead to reduced yields. Nucleation might occasionally be entirely inhibited. But, by utilizing seed crystals as well as a good seeding approach, this challenge may be resolved **(Hohmann et al., 2008)**. A phase transition during which a product of crystalline is produced from a solution is known as crystallization. We need a supersaturated solution—a solution with much more dissolved particles inside the solvent than it can typically be supported at a certain temperature at equilibrium—before crystallization can start. Two-phase process of crystallization that is referred to as fellow. Primary nucleation involves simply the formation of new crystals. To begin this fundamental stage, a significant supersaturation generating force is necessary. The uncontrolled crystal production and smashing primary nucleation process commences and continues until the residual solution concentration reaches equilibrium. Crystal growth is a phase 2 procedure that starts with ‘seeding' and takes place at a decreased supersaturation level **(Hülsewede et al., 2019)**. The introduction of tiny crystals to such a solution inside a metastable region causes connections among previous crystals and crystal interaction with the crystallizer walls. Crystals will form on such crystals till the solvent concentration achieves solubility equilibrium. While crystals are naturally clean, capillary attraction and sorption allow contaminants first from mother liquid to accumulate upon their surfaces and inside the spaces of the particulate aggregate. As a result, the crystals should be cleaned as well as pre-dried in a solvent that is somewhat

insoluble to them. The mother solvent and this solvent ought to be miscible. After washing, the crystals were dehydrated by vacuum drying, spray drying, and lyophilization. To preserve its chemical and biological function and to guarantee that it keeps a high degree of functioning following drying, crystals should be dried with extraordinary care **(El-taweel et al., 2023); Marichamy] et al., 2021)**.

The most common approach for polishing medicines, particularly penicillin G type, is batch crystallization. Penicillin G crystallizes as a colorless or odorless, crystalline powder or white crystal. Batch crystallizers are basically tanks containing stirrers that are occasionally confounded **(Cote et al., 2020)**. They are gradually chilled to achieve supersaturation. Seeding induces growth and nucleation, which is then cooled progressively till the required crystals are formed.

6. Electrophoresis

Lastly, electrophoresis may be employed to differentiate molecules with various charges, but it is only utilized on a limited scale to differentiate various proteins **(van der Burg et al., 2023)** . An electric potential is given to the terminals of a column that contains charged macro-biomolecules (for example, proteins) inside a gel or fluid (for example polyacrylamide or agar). Even though proteins possess net charges, they would migrate inside the gel at a pace proportionate to the amount of inherent charge. As a result, the proteins would collect in various locations throughout the gel. This technique is a top choice for the tiny-scale resolving of compositions of charged biomolecules and provides a really better resolution of protein fractionation. However, the excellent voltage resistance of a buffer and the considerable current needed to achieve migration cause the buffer to heat up chemically **(Boulos et al., 2023 ; Meleady, 2019)**. This results in convection currents that reduce separation effectiveness and desaturate proteins. Ohmic heating possess so far prohibited this technique from being effectively scaled up.

API Chemical Production through downstreaming Process:

Shikimic acid, also known as butanedioic acid or amber acid (3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid), is a widely used in biosynthesis of different compounds in plants, animals, and microbes. It is used in the pharmaceutical sector for the production of chiral building blocks, such as Oseltamivir, an antiviral medication used to treat and prevent influenza infections **(Priyanka et al., 2020)**. Shikimic acid can also be used to evaluate aromatic amino acids and aromatic vitamins by using strains of *E. coli* that inhibit the first three stages of the Pittard and Wallace aromatic amino acid pathway. Swiss pharmaceutical company Roche uses fermentative methods on E. coli to create shikimic acid. Succinic acid is a prevalent metabolite in plants, animals, and microbes and is used in various fields such as plastics, food, textiles, radiation dosimetry, agriculture, medicine, cosmetics, plating, waste gas cleaning and photography. It serves as a raw material for the esterification reactions that create a number of fundamental chemicals, including polybutyrate succinate (PBS) and polyamides. The most recent application of succinic acid is in the biodegradable plastic Bionelle, which is

produced by Showa Highpolymer Co., Ltd. in Tokyo, Japan, using succinic acid and 1,4-butanediol ester **(Kumar et al., 2021)**.

The cost of chemicals needed to produce succinic acid from maleic anhydride has significantly decreased, leading to a reduction in its industrial use. Additionally, hydrolysis of petroleum products and lignocellulosic biomass are used for commercial production of succinic acid, which also leads to environmental concerns. Green technology is becoming a significant factor to manage polution in the chemical industry from petrochemical processing and address supply challenges by shifting hydrocarbon production to a sustainable, environmentally friendly carbohydrate-based economy. Erythritol is a sugar alcohol known for its reputation as a low-calorie sweetener worldwide **(Yang et al., 2024)**. It is naturally found in a variety of fruits and fermented foods. Erythritol is typically produced from glucose using basidiomycetous fungi (*Moniliella pollinis*) and food-grade osmophilic yeast in fermentation method. It is purified after being separated from the fermentative broth, resulting in a final crystalline product with 99% purity. It does not cause any gastric-related negative effects due to its unique digesting metabolism. In United States of America, erythritol per capita intake is estimated to be 80 mg/person/day due to its presence in foods. In conclusion, sugimic acid, succinic acid, and erythritol are essential components of the pharmaceutical industry. These compounds have various applications and are crucial for various industries, including medicine, agriculture, and food production **(Erian and Sauer, 2022)**.

Clavulanic acid is a cost-effective pharmaceutical used to combat bacterial resistance to β-lactam antibiotics. It is derived from 7-aminocephalosporanic acid, which is a heterocyclic component of cephalosporins that have led to the development of various medications such as ceftriaxone, Cefdinir, cefotaxime and cefuroxime. Cephalosporin C starts the production of 7-amino-cephalosporanic acid (7-ACA) which is the most appropriate starting material for microbial fermentation **(Rusu et al., 2023)**. Rifampicin, a versatile antibiotic, is in high demand for human medicinal treatment and inhibits the development of several diseases. Inexpensive agro-industrial by-products such as ragi bran were used for the production of rifamycin SV (1310 mg/100 gds). a mutant strain of Amycolatopsis mediterranei OVA5-E7 in optimized solid-state fermentation (SSF) was used for rifamycin production. **(Nagavalli–et al., 2015 ; Muteeb et al., 2023)**. Pregabalin (anticonvulsant drug) is effective to treat neuropathic pain, seizure disorders and fibromyalgia. The (S)-enantiomer of pregabalin is recognized for its pharmacological efficacy, making its asymmetric synthesis significant in the pharmaceutical sector. A crucial precursor of (S)-pregabalin, (S)-3-cyano-5-methylhexanoic acid, has been synthesised asymmetrically using both biocatalytic and chemocatalytic methods. However, both routes were deemed inappropriate due to environmental concerns and economic factors **(Verma et al., 2014 ; Martínez et al., 2023)**. Ectoine, a premium substance extensively used in the cosmetics and pharmaceutical industries, is a cyclic amino

acid. Halophilic bacteria are used for its synthesis. Ectoine production is costly due to elevated substrate expenses, but researchers are working on synthesizing it cost-effectively by employing inexpensive substrates. Ectoin was effectively recovered by fermentation broth using *Halomonas elongata* by bacterial milking procedures, achieving a productivity of 7.4 g/L at a rate of 0.22 g/L/h after at least nine cycles. Halophilic bacteria, *H. elongata* (DSM 142T) was used for the production of ectoin on an industrial scale by using batch fermentation with NaCl (15% w/v) **(Ng et al., 2023 ; Liu et al., 2021)**.

Future Prospects

The Active Pharmaceutical Ingredients Committee (APIC) is utilizing biomass and its conversion to create valuable bioproducts in the circular bioeconomy. However, this approach faces challenges in meeting consumer demand and economic potential. To overcome these challenges, it is essential to focus on good biomass management and implement stringent waste recycling policies. Recent biotechnology methods, such as genetic engineering and bioengineering, are being used to create new microbial strains and improve models for increasing biomass yield, CO2 utilization, lipid accumulation, and bioremediation capabilities. Integrating different technologies and computer engineering methods is crucial for achieving a circular bioeconomy and producing valuable bioproducts like APC and biofuels from biomass.

Currently, there are concerns about the effective use of biomass due to the large amount of agricultural waste generated and the need for sustainable and cost-effective renewable energy resources. Recycling biomass and converting it to APC is a crucial method for using biological waste and transforming it into useful end goods. However, there is a lack of comprehensive information on the various methods used for understanding biomass resources and their conversion in the context of air pollution control. Extensive study using molecular and biochemical methods is necessary to understand the repair process and prioritize cost-effective and sustainable commercial applications.

CONCLUSION

The biotechnology industry uses transgenic animals, plants, mammalian cells, and recombinant bacteria to produce bioactive molecules. Currently, around 300-350 recombinant protein-based pharmaceuticals are under clinical testing, with several already approved for human use. However, the challenge lies in developing technological frameworks to efficiently remove contaminants and byproducts, resulting in high-purified products. The main constraints in production-purification strategy are downstream operations, which contribute significantly to the overall manufacturing cost of finished goods. Biomass generated from agricultural activities and food processing poses environmental risks. Sustainable methods are being explored for recycling these materials, with potential applications in medicine, biopolymers, bio-solar cells, specialty chemicals, and lubricants. Technological advancements could create a bio-based economy while reducing environmental risks. This review summarizes the purification and separation methods commonly used in industrial

settings, focusing on essential ones like filtration and chromatography. It also summarizes both traditional and contemporary purification techniques used for commercial purposes. The completion of the human genome is a significant achievement in biomedical sciences, allowing researchers to develop novel therapeutics. The biotechnology sector faces increased expectations in creating innovative biotherapeutics and pharmaceuticals, primarily containing biomolecules and value-added products. The field of biotechnology involves cultivating mammalian and microbial cell cultures for producing high-value pharmaceuticals. Downstream processing varies depending on the product and expression system used for product generation. Biotechnology-derived peptides are often found within complex product matrices, posing challenges in their isolation and purification. Further research is needed to integrate different technologies and minimize pollution levels by efficiently using bioresources in a structured manner, leading to a profitable company.

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