BIO SEPARATION-A REMARKABLE PROGRESS FROM PREMATURE TO ADVANCED TECHNIQUE, AN UNPRECEDENTED ADVANCEMENT IN THE FIELD OF BIOTECHNOLOGY

Abstract

Bioseparation is the large-scale purification of biological products utilising fundamental engineering and biological ideas. This abstract presents an overview of the remarkable expands and exceptional advancement in bioseparation strategies, which play a crucial role in medicine, biotechnology, and in food industry. The bioseparation uses has spread across numerous sectors, notably in biotechnology. Bioseparation applications were formerly simple, time-consuming, and poor yielding. However, tremendous progress has been made in recent years via the integration of analytical tools, computerization, and automation, resulting in improved yields. AI and machine learning technologies have optimised process parameters, resulting in improved- quality products and higher yields. Emerging bioseparation techniques target specific biomolecules based on variables such as size, charges, and hydrophobicity, having a substantial influence in biotechnology and related sectors. Bioseparation is critical for the purification of biopharmaceuticals. Membrane-based technologies, chromatography techniques, sophisticated filtering procedures, and other approaches make it possible to easily bioseparate biological components. Bioseparation has seen a rapid shift in recent decades, driven by innovative methods and novel materials. Rising demand for biotechnological goods, together with continuous bioseparation research and innovation, offers revolutionary applications that will send biotechnology into unknown areas of development and discovery.

Keywords: Food industry, Biomolecules, Biotechnological goods, Biopharmaceuticals.

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I. INTRODUCTION

Bioseparation procedures for life sciences necessitate techniques that differ from those employed in standard chemical businesses[13].All bioseparation approach should be capable of removing, purifying, or recovering desired product. The number of factors influences the bioseparation processes which includes the fundamental properties of temperature constancy, solubility, diffusion, shipping, isoelectric pH, and others[1].The biotechnology sector evolves continually to increase product throughput, purity, yield, and profitability while consuming less resources[8].Biotechnological techniques are increasingly being used to manufacture valuable goods. The expense of the entire procedure is heavily weighed down by the requirement to isolate the relevant biological macromolecule. It is suggested that80% of overall production costs is required expensesfor the recovery and purification process [23]. Van Brunt (1985) indicates that bioseparation processes include, but are not limited to, cell disruption, centrifugation, chromatography, drying, evaporation, extraction, filtration, membrane separation, and precipitation.

In biotechnology, there are several viable bio separation strategies.

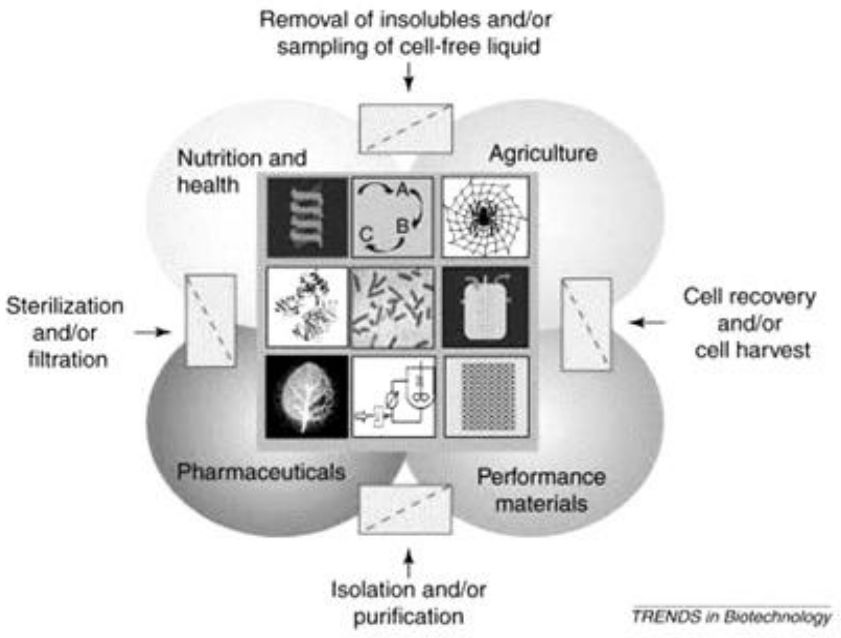

Figure 1: Various Bioseparation Technologies will be required in the Future for an Extensive Spectrum of Business Domains.

II. BIO SEPARATION TECHNIQUES

1. **Celldistruption Method:** In the processing of intracellular biomolecules cell lysisis a necessary step and the initial stage of the separation process, with the goal of achieving a high amount of yield with less loss rate along less product contamination. As a result, recovering intracellular biomolecules raises downstream costs, and selecting the optimum disruption strategy is considered a problem, particularly for large-scale systems. The main

goals for the above process are high selectivity, energy efficiency, operational costs, and ease of scaling-up [7].

2. **Drying Method:** Drying of pharmaceutical and biotechnological products is used for a wide assortment of chemical and biochemical materials produced in quantities from large tonnages to very small batches[17].

3. **Centrifugation:** Centrifugation is a significant and most adapted research technique in biochemistry, cellular and molecular biology, suspension and emulsion assessment in pharmacy, and medicine. There are different types of centrifugation techniques such as: Differential Centrifugation, Density Gradient Centrifugation-Rate-Zonal Centrifugation, Selection of Suitable Density Gradient Medium, Polyhydric Alcohols, Polysaccharides, Inorganic Salts (Ionic Metal Salts), Nonionic Iodinated Density Gradient Media[15].Centrifugal microfluidic technologies have successfully demonstrated their capacity to handle liquids in a durable, high-performance manner, enabling flexible, multi-purpose lab-on-a-chip platforms for a variety of life-science applications[3].

4. **Crystallization:** Crystallisation is used in industry to recover and purify a broad spectrum of inorganic and organic compounds. Crystallisation is a well-established commercial process that provides unique hurdles when used to purify bio-macromolecules. It normally requires high concentrated and highly pure target-containing solutions, and its operation might take some time even some days. The resultant crystals can be extremely pure, therefore being appropriate for usage as drug products, drug storage, or even drug-delivery colloids in some situations[31].

5. **Nanofiber Membranes:** Highly effective protein purification is becoming increasingly relevant in bio separation engineering, notably for pharmaceutical areas, food industries, and medical applications. Protein separation may become more easier with the development of well established membranes with small pore-size distributions. Many novel strategies for improving the efficiency of membrane-based procedures have been developed. For the further improvement in membrane performance, electrospun fibres of polymers or hybrids have been employed among unique structural membrane materials. Membrane filtration is an advanced separation technique that has the capability to be used for concentration, desalting, clarifying and fractionation [12].

6. **Chromatography:** Chromatographic stages dominated bioseparation procedures. Primary recovery is oftenly performed using chromatographic separation, which employs a fluidized bed instead of a fixed bed. The rapid operation is utilised by every available chromatography media. There are cases where a residence duration of less than 3 minutes is adequate to fully use the adsorbent[11].

    - **TLC (Thin Layer Chromatography) :** TLC is a cost-effective, user-friendly planar chromatographic technology that has been used in general chemistry laboratories for decades to separate chemical and biological compounds. Chemical and optical methods have historically been used to visualise the analyte spots on the TLC plate. It may also be used to identify impurities in a substance[14].

    - **Affinity Chromatography:** Bioaffinity techniques dependents on the interaction of an antibody and an antigen so it have a capacity to detect and identify substances. The

initial aim of an environmental research is successful separation of an analyte of interest from a complex matrix, the strength and specificity of biological separations are well adapted to the analytical techniques. Affinity chromatography may be used to purify larger molecules such as enzymes, antibodies, even for the smaller substances before detection and also for cell separations process[32] .The application of molecular recognition is no longer limited to affinity chromatography modes.

- **Bio Affinity Method:** Affinity based separations include precipitation process, membrane based purifications and two-phase/three-phase extractions. Affinity chromatography is also used in proteomics to reduce the complexity of the system prior to electrophoresis and mass spectrometry analysis. The bioseparation of proteins is one of the most significant applications of the affinity principle-Creating an affinity macroligand by conjugating an affinity ligand with a polymeric substance. This comprises matrix activation and/or affinity ligand coupling to the matrix, capture of the target protein by the affinity macroligand, isolation of this complex from the rest of the environment, and elution of the target protein from its combination with the affinity macroligand [16].

- **HPLC (High-Performance Liquid Chromatography):** The biospecificity of affinity chromatography is paired with the strong support materials of HPLC in high performance liquid affinity chromatography (HPLAC). In chromatography, HPLAC can provide selectivity, speed, and high resolution on both analytical and preparative scales[19].

- **AXC (Anion Exchange Chromatography):** At the time of monoclonal antibody purification, anion exchange chromatography in product flow through mode is capable of successfully removing probable viral contamination. It has been demonstrated that mammalian cell lines used to produce biopharmaceutical products produce endogenous retrovirus-like particles and host adventitious viruses. In order to ensure product safety and regulatory compliance, recovery processes must be capable of removing or inactivating any viral impurities or contaminants that may be present. AEXis a common technique for recovering monoclonal antibody products that has been proven effective for virus eradication[28].Membrane filtering, aqueous two-phase extraction, three-phase partitioning, precipitation, crystallisation, monoliths, and membrane chromatography are some alternate procedures for chromatography.

7. **Precipitation Method:** By concentrating on isolating the required protein, targeted precipitation techniques can greatly speed up the process of getting purified products. The selectivities attained by "selective precipitation" are compared to those attained by other downstream purification techniques like liquid chromatography[18]. Ayazi-Shamlou and co-workers have developed ‘ultra-scale-down’ equipment that mimics the shear environment of process-scale precipitations, enabling predictive small-scale process development work. Work is on-going to understand the impact of shear history on apparent protein solubility and precipitate properties [22].

    - **Salting in and Out:** To determine the efficiency limits within which this precipitation method can be used to produce fines (mean particle size 10 m) with

acceptable quality and productivity, a comprehensive investigation of salting-out precipitation is conducted [30]. Salting-out is the process of removing a less soluble chemical from a solution where it has been infused with other substances. The use of the idea in biological and medical fields is a relatively recent development. The term "salting effect" describes how the presence of an electrolyte alters the solubility of a nonelectrolyte in a water-based solution. As a result, the solubility of a nonelectrolyte can be increased or decreased as more electrolyte is introduced. The terms "salting-out" and "salting-in" are used to describe them, respectively [10].

- **Ethanol / Acetone Precipitation:** Ethanol precipitation is a process commonly used in molecular biology and biochemistry to extract and concentrate nucleic acids from a solution, such as DNA or RNA. It entails adding ethanol to a nucleic acid-containing solution, which causes the nucleic acids to precipitate and be collected for further processing. Because of its simplicity and efficacy, ethanol precipitation is a popular technique for purifying nucleic acids. It may be used to eliminate impurities from nucleic acid samples such as proteins, salts, and residual reagents, leading in improved purity and concentration of the target molecule. To minimise shearing or deterioration, it is critical to treat nucleic acids carefully throughout the precipitation process [25].Protein precipitation is a typical technique for protein precipitation and concentration. Because acetone precipitation is such a popular method in protein sample preparation for proteomics, considerable care is advised[27].A synergistic method of protein precipitation in acetone with salt that is suitable with a model of ion pairing in organic solvent is established as a better method for recovering proteins and proteome combinations in high yield [5].

- **Organic Solvent Precipitation:** An easy approach that can selectively deplete high molecular weight abundant proteins and concentrate for low molecular weight less abundant proteins is needed to improve protein coverage in proteomics research. Proteins were initially extracted from solutions using isoelectric point precipitation.To separate the soluble protein fractions (supernatants) from the soluble protein fraction, the known optimum solvent was utilised[9].

- **pH Induced Precipitation:** Precipitation was the most significant carbonate sink, and it also compensated denitrification's alkalinity generation. Although alkalinity rose in most cases, systems with a large carbonate buffer and high pH intensified precipitation, resulting in a negative net change in alkalinity. The interaction between fluctuations in calcium concentration, total carbonate concentration, pH, and alkalinity could determine how well the hollow-fiber membrane-biofilm reactor (HFMBR) performs in the field over the long term [20].

- **Co Precipitation:** Coprecipitation is an increasingly essential technology for distributing ingredients and precursors utilised in a process to make a necessary substance. Coprecipitation is used to manufacture multicomponent materials by forming intermediate precipitates, mainly hydrous oxides or oxalates, such that an intimate combination of components forms during precipitation and chemical homogeneity is preserved during [2].

8. **Magnetic Separation Method:** In recent years, there has been a lot of interest in the use

of functionalized magnetic adsorbent particles in conjunction with magnetic separation methods. By employing magnetic separation in this manner, numerous phases of sample preparation (particularly centrifugation, filtering, and membrane separation) that are ordinarily required to condition an extract before it is applied to packed bed chromatography columns can be avoided[6].The encapsulation of magnetic nanoparticles generated using coprecipitation techniques into organic polymers has been proven to be an appropriate way for providing the nanomagnets with a variety of advantages such as chemical stability, dispersability, and usefulness [29].

9. **Filtration Methods:** Filtration is an essential aspect of the multi-barrier technique used to eliminate microorganisms. Granular filtration's potent efficacy in particle removal can improve disinfection efficiency. Although sand is one of the most used filter media, other media are currently being developed and employed [4].

- **Mechanical Filteration Method**: Utilisation of mechanical washing approaches, specifically filter cake washing, on calcium-ion removal from peat, a natural substance applied in a variety of industries such as agriculture, medicine, cosmetics, and so on. Peat's intriguing features, such as its porous structure and sorption behaviour, impact the distribution of liquid within the bulk as well as the flow behaviour of liquid by means of the porous structure [24].

- **Absorption Filteration Method**: Superhydrophobic and superoleophilic filter paper was successfully created by treating commercially available filter paper with a combination of hydrophobic silica nanoparticles and polystyrene solution in toluene for the filteration process for an effective separation [33].

- **Ion Exchange Filteration Method:** This approach has been employed for water softening on an unprecedented scale of applications, and it has become an essential component of new technical and industrial processes. Ion exchange is a reversible stoichiometric chemical reaction that maintains overall electroneutrality by exchanging an ion from a solution, electrolyte, or molten salt for an identically charged ion connected to an immobile and insoluble solid substance [21].

- **Reverse Osmosis Filteration Method:** One of the most significant and generally recognised methods for producing fresh water from saltwater water is reverse osmosis (RO).The most important factors to consider when selecting a membrane module are cost, concentration polarisation, operating parameters (especially pressure), and fouling resistance[26].

III. CONCLUSION

In the downstream stages, where separation as well as purification play crucial roles in commercial development, engineering solutions will be primarily responsible for the effectiveness of biotechnology in large-scale manufacturing. The development of effective bioseparation technologies is critical for a variety of industries, including medicines, nutrition and health products, bio-based materials, and crop protection chemicals. Depending on the finished product's worth and the volume of manufacturing, different amounts of processing

are necessary. When choosing a separation strategy, it is crucial to take process throughput, particle size of the product and contaminants, and required end-product concentration into account. Many improvements in bio separation methods are propelling advancements in a variety of disciplines, including pharmaceuticals, biotechnology, and biomedical research, allowing for exact characterisation and purification of biomolecules for therapeutic uses and scientific studies. A tremendous array of diligence has been performed and is still being carried out in many parts of the world to simplify bio separation procedures even more than they are now. In order to handle the complex challenges of growing life science processes, a strategic strategy that recognises the need of separation as a key element within unit operations is required. Successful commercialization of bioprocesses will rely on the development of efficient, economically viable, and selective separation advances in technology.

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