EMERGING TRENDS AND INNOVATIONS IN BIOPROCESSING TECHNIQUES

Abstract

This chapter explores the latest advancements and novel approaches in the field of bioprocessing techniques. Bioprocessing plays a vital role in the production of biopharmaceuticals, biofuels, and various bioproducts. The rapid progress in biotechnology and engineering has led to the emergence of innovative strategies that enhance process efficiency, product quality, and cost-effectiveness. The chapter begins by discussing the application of advanced cell culture techniques such as perfusion and continuous processing, which enable higher cell densities, longer culture durations, and improved product yields. It explores the utilization of bioreactor technologies, including single-use systems, microfluidics, and miniaturized platforms, that offer advantages in terms of flexibility, scalability, and process control. Furthermore, the chapter delves into cutting-edge approaches in downstream processing, such as continuous chromatography, membrane separations, and advanced filtration techniques. These innovations streamline purification processes, reduce product losses, and enhance productivity. The chapter also highlights the emerging trends in process analytical technology (PAT), data analytics, and artificial intelligence (AI) applications in bioprocessing. These technologies enable real-time monitoring, process optimization, and predictive modelling, ultimately leading to improved process understanding and control. Moreover, the chapter explores the utilization of alternative and sustainable feedstock for bioprocessing, including lignocellulosic biomass and waste streams. It discusses the development of bioconversion technologies and metabolic engineering strategies to enhance the production of biofuels, bioplastics, and other value-added chemicals. Lastly, the chapter emphasizes the need for collaboration between academia, industry, and regulatory bodies to facilitate the adoption and implementation of these innovations.

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I. INTRODUTION

Bioprocessing techniques are a set of methods used in the production and manufacturing of biological products. This term is often associated with the use of living cells or their components (e.g., bacteria, enzymes, chloroplasts) to manufacture desired products, frequently in pharmaceutical and biochemical industries. The key techniques used in bioprocessing include - Fermentation, Cell culture, Bioreactors, Downstream processing, Protein Engineering, Genetic Engineering and Genetic Engineering. Fermentation is a process where microorganisms like yeast or bacteria are used to convert sugars into other useful products such as alcohols, organic acids, and gases. It's one of the most traditional and commonly used processes in biotechnology. Cell Culture is a technique where cells are grown in controlled conditions, often outside of their natural environment [1]. This is used in cell biology, drug discovery, and in the production of therapeutics, among other areas. Bioreactors are large vessels where biological reactions take place, often used in industry for large-scale cell culture or fermentation. They are designed to support a biochemically active environment that sustains life and promotes growth or product formation [2]. Downstream Processing involves the purification and recovery of the biosynthetic products from natural sources like an organism's tissues or fermentation broth, including stages like separation, concentration, and formulation of the product. In protein engineering, proteins are modified to improve or alter their functionality. This can be done through techniques like direct genetic manipulation or using computational techniques for design, often used in areas like enzyme technology or therapeutic protein discovery. Genetic Engineering is a practice where the genetic material of an organism is directly manipulated, which can result in the biological entity producing new substances or performing new functions. Bioprocessing is a rapidly evolving field due to advancements in genetic engineering, synthetic biology, and process technology. It forms the core of many industries including biopharmaceuticals, food and beverages, biofuels, and environmental management[3].

In recent years, the field has witnessed remarkable advancements and innovations that are reshaping the landscape of biotechnology and bioengineering. Cell culture techniques such as perfusion and continuous techniques enable higher cell densities, longer culture durations, and improved product yields .Traditionally bio processing has been based on batch production. The shift towards continuous bio processing allows for ongoing production without intermediate steps, potentially enhancing efficiency, reducing costs, and aiding in sustained production of products. Additionally, bioreactor technologies, including single-use systems, and other devices are becoming increasingly popular. These can significantly minimize the risks of cross- contamination, reduce cleaning, setup times, and facilitate flexible, modular bioprocess designs [4]. This flexibility can be especially useful for companies producing multiple products in small quantities. Downstream processing, a crucial stage in bioprocessing, has also witnessed significant innovations. Continuous chromatography, membrane separations, and advanced filtration techniques have streamlined purification processes, reduced product losses and enhancing productivity [5]. The conversion from traditional to intelligent bioprocesses encompasses features like connectivity, real-time monitoring, automation, artificial intelligence, and machine learning. The complex and dynamic nature of bioprocesses, which are influenced by a multitude of factors and interactions, presents challenges in managing the process, ensuring stability, and continually optimizing the output. Key determinants such as organic loading rate, oxygen levels, temperature, pH, and other components, together with microorganism composition, metabolic interactions, and enzymatic activity, collectively shape the overall result. Digitizing

bioprocesses calls for specific control tools for precise regulation, including process categorization, soft sensors, fault identification, and instant monitoring. Data-driven predictive control and optimization is vital to handle bioprocesses effectively, as they can be susceptible to sudden operational changes and require tight regulation[6]. Inclusion of image recognition, blockchain, and internet of things (IoT) in smart bioprocessing can also leverage state-of-the-art machine learning and computational power. In a broader viewpoint of sustainable economic growth, machine learning-supported circular bioeconomy is key to efficiently handle various waste streams. The integration of process analytical technology (PAT), data analytics, and artificial intelligence (AI) has revolutionized bioprocessing. Real-time monitoring, process optimization, and predictive modelling are now achievable, leading to improved process control and a deeper understanding of complex bioprocesses. The chapter also discusses the utilization of alternative and sustainable feedstock for bioprocessing. Lignocellulosic biomass and waste streams are being harnessed, employing bioconversion technologies and metabolic engineering strategies to produce biofuels, bioplastics, and other value-added chemicals.
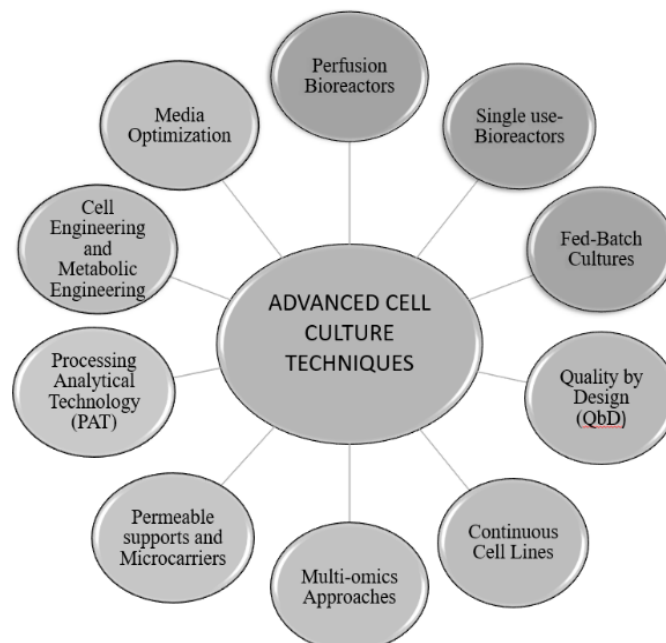

Figure 1: Types of Advanced cell culture techniques

II. ADVANCED CELL CULTURE TECHNIQUES IN BIOPROCESSING

Cell culture technologies have transformed bioprocessing, a technique used to convert raw materials into valuable pharmaceutical products [7].Traditional bioreactors, which have served as the workhorse for cell culture, are increasingly being overtaken by novel methods, offering improved yields and more controlled conditions. Traditionally, bioprocessing utilizes large, stirred-tank bioreactors for cell culture. These bioreactors provide a controlled environment for cells, considering factors like pH, oxygen, temperature, and nutrients [8]. However, due to limitations related to scalability, control over microenvironments and cell damage due to shear stress, newer cell culture technologies are being explored.

Microcarrier technology, a significant development in cell culture, allows cells to adhere to small beads suspended in the culture medium. This technology increases the surface area available for cell growth, crucial for anchorage-dependent cells[9]. Additionally, it allows an accurate adjustment of the fluid dynamic conditions, reducing the risk of damaging the cells. Perfusion systems have shown to improve cell health and longevity, permitting a continued supply of nutrients and removal of metabolites. The advancements like tangential flow filtration and alternating tangential flow (ATF) have enhanced cells growth in perfusion processes [10]. 3D cell culture systems have revolutionized cell culture techniques. These systems have manufactured realistic models of human tissues, enabling precise drug testing and research studies [11]. Bioprinting, an offshoot of 3D cell culture, permits cells, extracellular matrices (ECM's), and bioinks to print 3D printed tissues and organs[12]. A significant advancement in cell culture techniques is the utilization of single-use bioreactors. These types offer lower contamination risks, increased flexibility, and faster setup times, decreasing the cost involved in cleaning and sterilization activities[13]. The Chinese hamster ovary cells (CHO) are the gold standard of mammalian cell lines used in bioprocessing. The introduction of engineered CHO cells that ensure high yield and quality of biopharmaceuticals represents an essential advancement [14].

1. **Perfusion and Continuous Processing:** Perfusion Bioreactors and Continuous Processing have revolutionized the biotechnology industry with their efficient and cost-effective production methods. Both systems allow for the continuous filtration and cultivation of cells, leading to high-productivity bioprocesses[15].Perfusion bioreactors enhance volumetric productivity by maintaining a high cell density for an extended period. They operate by continually feeding fresh culture medium and removing spent media while maintaining a high cell concentration[16]. This technology also reduces production costs by decreasing the use of consumables and media components[17]. As a result, perfusion-based technologies optimally balance productivity and cost-effectiveness, thereby, promoting sustainable biomanufacturing.

   Continuous Processing employs a similar mechanism as Perfusion Bioreactors but extends it through the entire bioprocess. This platform integrates various process stages such as cell growth, protein expression, and purification into a single, uninterrupted operation[18]. This approach improves product quality by maintaining constant optimal process conditions, reducing manufacturing footprint, and decreasing batch-to-batch variability[19].Both Perfusion Bioreactors and Continuous Processing have also found significant roles in biotherapeutic production. For instance, they have streamlined the production of monoclonal antibodies (mAbs), enabling high volumetric productivity, extended cultivation times, and low overall production costs[20]. Coupled with advancements in cell-line engineering and media optimization, these technologies have potential for a range of applications, including the production of therapeutic proteins and vaccines.Despite the advantages, several challenges persist in the full-scale implementation of these technologies. Technological intricacies, such as system sterility, monitoring, and control, can hamper the operational efficiency of these systems. Therefore, further research and technological advancements are crucial to addressing these challenges and promoting large-scale industrial applications.

2. **Enhanced Bioreactor Technologies:** Bioreactor technology has undergone immense progress over the years, with various types of systems now available for optimizing various biological processes. The primary types of enhanced bioreactor technologies

include Hollow fibre bioreactors, aerated-stirred tank bioreactors, airlift bioreactors, packed-bed bioreactors, and fluidized-bed bioreactors. Hollow fibre bioreactors have a significant impact on high cell density culture methods. The field has seen recent progress, leading to enhanced therapeutic production. A new type, the microfiltration based hollow fibre bioreactor, has been developed. This system includes two hollow fibres inserted into a vessel, enabling the elimination of inhibiting low molecular weight metabolites while keeping the cells intact [21]A crossed hollow fibre membrane bioreactor incorporates two varieties of fibres, each having a distinct molecular weight cut-off (MWCO) and completely different physicochemical characteristics [22]. Aerated-stirred tank bioreactors are considered the "workhorse" of industrial fermentation. This technology, which allows for meticulous environmental control, is commonly used in the pharmaceutical sector. The aerated-stirred tank bioreactors excel in providing a well-mixed environment, high oxygen transfer, and robust control systems. Oxygen is used as a metabolic regulator of cellular activity and is essential for growth in aerobic conditions[23]. Airlift bioreactors are another common technology. These systems operate by injecting air at the bottom of the reactor, which then rises, creating an upward flow that moves the culture medium[24]. Buoyancy forces generated by the air bubbles circulate the liquid in the airlift reactors. This approach provides important advantages, such as simplicity of design and operation, greater efficiency, and lower shear forces, which are vital for sensitive cell types.

Packed-bed bioreactors form the backbone of biofilm-based reactor technologies. They are characterized by a solid phase (the packing material) and a liquid phase. This design aims to offer increased surface area for cell colonization, leading to high cell densities [25]. Packed-bed reactors perform exceptionally well in long-term, continuous cultures due to biofilm formation, which provides critical protection against environmental variations and hostile conditions, such as substrate or product inhibition. Fluidized-bed bioreactors are unique in terms of operating principles. They employ small particles as growth support for microbial cells. These particles are kept suspended by the upward motion of the culture medium, which allows high mass and heat transfer rates[26]. Fluidized-bed bioreactors are particularly used in treating wastewater due to their high removal efficiency and tolerance of variable loading rate. The demand for cost-effective bioreactors in both experimental and industrial settings has sparked discussion around the use of disposable bioreactor systems. The Cryogen bioreactor, a respected and disposable high cell density perfusion system, offers cells a prolonged and impactful lifespan. This bioreactor has been used for the steady, long-term manufacture of therapeutic proteins [27], [28]Cryogens are three-dimensional polymeric scaffolds formed at sub-zero temperature by polymerization of monomers or by polymeric precursor by the phenomenon of cryogelation, which consists of an interconnected network of macropores. Cryogelation technique has an advantage that the cryogels can be made in different sizes and shapes like disc, sheets, or monoliths with varying dimensions. The polymer-based cryogel has several advantages over other kinds of gels i.e., simple approach by which they can be synthesized, use of aqueous solvent for their synthesis and unique combination of high porosity with adequate mechanical strength and osmotic stability. The size of these macropores varies from few micrometres to 100 μm,which allows the unhindered convectional mass transfer. This makes cryogels an ultimate support for cell immobilization and proliferation. The hybridoma cell lines are adsorbed to the inner pore walls of cryogel matrices which are covalently immobilized with gelatin. The coating with gelatin enhances the adherence of cells to the matrices. The cells are situated in such

a microenvironment which makes sure that there is virtually no barrier that arises for easy diffusion of substrates and metabolites [29], [30]Modern advances in biotechnology have also sprung novel types like wave-mixed bioreactors and CELL-trainer bioreactors. Numerous attempts have been made with wave bioreactors as well. Specifically, a perfusion culture technique utilizing a wave bioreactor was shown to yield human monoclonal antibodies from Drosophila Schneider 2 cells. When operating the wave bioreactor in perfusion mode and implementing alterations in microfiltration or ultra filtration through alternating tangential flow (ATF) or tangential flow filtration (TFF), CHO cell line growth was observed to reach up to 108 cells/ml after twelve days, and monoclonal antibody (mAb) production was six times higher. Additionally, the use of low-intensity pulsed ultrasound on wave bags boosted the production of mAb. A five-minute ultrasound treatment was seen to elevate the antibody yield by as much as 25% [31]. In a different instance, a novel 2D rocking bioreactor, based on fresh technology, has been developed and employed to produce recombinant proteins. This setup, known as the CELL-tainer bioreactor system, serves as an alternative to the traditional stirred-tank reactors for bioprocessing activities anchored on microbial platforms [32].

III. INNOVATIONS IN DOWNSTREAM PROCESSING

1. **Continuous Chromatography:** Downstream processing (DSP) constitutes a crucial segment of the biotechnology industry, particularly in the manufacture of bio-based products[33]. It involves several stages, including the recovery, concentration, purification, and formulation of biosynthetic products. DSP's most critical and challenging stage is the purification of proteins, which can affect product quality and account for 40-70% of total manufacturing costs[34]. Therefore, technological innovations have focused significantly on improving efficiency and reducing costs in this crucial area. A standout innovation in this regard is the transition from batch processing to continuous processing. Continuous processing offers several advantages in downstream operations. It optimizes productivity by minimizing the duration and number of batch handling steps, ensures a consistent product quality through real-time monitoring and control, and reduces capital and operational expenses through smaller equipment sizes and lower buffer usage[35].Several technologies enable continuous downstream processing, but the most prominent is probably multi-column continuous chromatography (MCC), especially simulated moving bed (SMB) chromatography, originally developed for the sugar industry but now used to purify therapeutic proteins. MCC, which uses multiple chromatographic columns operating in parallel, allows for the deconvolution of the capture step and results in higher productivities, better resin utilization, lower buffer usage, and lower capital costs[36]. The operational scheme moves resin between columns during processing, contributing to efficient use of chromatography columns and streamlining productivity.

SMB has revolutionized continuous chromatography with its ability to maximize separation efficiency through counter-current movement of the solid phase, which leads to sharper separation and higher productivity rate [37]. This technique is particularly effective for separating chiral, isomer or closely related substances in the pharmaceutical industry. An important extension of SMB is the VariCol process, an advancement that offers increased versatility through modulating flow rates in individual columns to optimize separation performance. This continuous chromatography system uses less hardware compared to traditional SMB, and its flexible configuration has allowed

operators to adapt to various product-specific conditions, enhancing efficiency.

Another game-changing innovation is single-pass tangential flow filtration (SPTFF) for continuous concentration/diafiltration[38]. Unlike conventional TFF, which recirculates the retentate back to the feed tank, SPTFF eliminates the need for recirculation, allowing operators to handle increasing concentrations continuously, and converse buffer and facility space.

2. **Advanced Filtration Techniques and Membrane Separations:** Filtration is an increasingly important part of a diverse range of operational sectors, and the development of new and more technologically advanced methods is driving significant progress. One such method is Crossflow filtration, a technique that filters liquid solutions by separating them into two streams — product and retentate[39]. By allowing the feed solution to flow across the filter membrane, instead of being forced through, this method reduces clogging and allows for more efficient and long-term operations. Other advanced filtration techniques include adsorptive filtration, where certain compounds adsorb onto the media, thus removing them from the liquid. This technique is beneficial in wastewater treatments and is gaining attention [40]. Likewise, nanofiltration, a relatively new technology, employs membranes with extremely thin pore sizes that allow for the selective filtering of solutions. These membranes filter more effectively, removing even the tiniest of particles or ions from the liquid[41]. Lastly, ceramic filtration is noteworthy, given its high resistance to harsh processing conditions such as extreme pH, high temperature, and abrasive particles[42].

Membrane separations are a cornerstone of downstream processing, acting as a vital part in the harvesting and purification of biomolecules. These techniques emerged due to the need for reliable, scalable, and efficient separation methods to isolate valuable products from complex mixtures [43]. The commonly used types of membrane separation include microfiltration, ultrafiltration, and diafiltration. Microfiltration separates particulate matter and suspended solids, while ultrafiltration separates macromolecules and concentrates solutions[44]. On the other hand, diafiltration utilizes dilution and concentration cycles to remove low molecular weight solutes from macromolecules. Furthermore, the rapid development of membrane materials, such as ceramic and polymeric membranes, further expand applications in pharmaceutical, food, and emerging sectors like biotechnology[45]. Membranes, in such processes, are not just static barriers but are now tuned to provide transport selectivity, increased fouling resistance, enhanced chemical stability, and potentially even catalytic activity, thus transforming the very essence of separation processes and downstream processing.

IV. PROCESS ANALYTICAL TECHNOLOGY (PAT) AND DATA ANALYTICS

Process Analytical Technology (PAT) and data analytics play crucial roles in bioprocesses, enhancing the efficiency, reliability, and scalability of operations in life sciences and biotechnology industries. PAT, a system instituted by the FDA in 2004, aims to promote understanding and control manufacturing processes through feasible measurements of critical process parameters (CPPs) and key performance indicators (KPIs) [46]. It introduces the concept of Quality by Design (QbD), suggesting that quality is built into products via thorough echo-understanding of formulation and manufacturing. Conversely, data analytics – incorporating statistical process control, multivariate analysis, and machine

learning techniques – offer substantial gains in the process efficiency and quality of bioproducts. They offer benefits like online monitoring and real-time quality control[47]. The integration of PAT and data analytics can generate dynamic models to elucidate complicated bioprocess mechanisms and scale-up processes, ensuring consistent product quality and minimizing waste [48].

Moreover, advancements in bioprocess monitoring tools, sensors, and data capturing have led to the accumulation of extensive datasets or "Big Data"[49], offering enormous potential for data analytics in bioprocessing. Various AI and machine learning techniques, such as deep learning and neural networks, can discover hidden patterns, trends, or unexpected correlations within data [50]. These techniques help build powerful predictive models, offer insights into CPPs and their influences on product quality and yield, thus aiding in optimization and control [51].However, despite their potential, a more thorough understanding of these methodological approaches and the development of regulatory guidelines for their application are yet to be achieved. It involves secure data management, integration of appropriate algorithms within PAT frameworks, and the training of scientific personnel to leverage these technologies to their full potential[52].By harnessing the power of PAT and data analytics, industries can achieve superior quality control, efficient utilization of resources, reduced downtime, and improved processes in biopharmaceutical manufacturing.

1. **Real-Time Monitoring:** Incorporating physical sensors into the bioprocess flow is a crucial element that allows for immediate collection of data. The Process Analytical Technology (PAT) framework, published by the US Food and Drug Administration, provides guidelines on the development and various methods of integrating analytical sensors at the Critical Control Points (CCPs) of the bioprocess.In-line sensors are frequently used, where the sensors are embedded within the bioprocess flow, allowing for data collection without having to extract samples from the unit operation[53], [54]. Vibration spectroscopic probes like Raman and Fourier Transform Infra-Red (FT-IR) are renowned for capturing real time data from production bioreactors and other units involved in the bioprocess.Biologic medicine continues to lead the pharmaceutical sector by introducing unparalleled mechanisms of action and therapeutic results. Consequently, the production process must embrace technological advancements to enhance productivity, efficiency, and consistent quality. It is crucial to monitor bioprocesses in real time to achieve Real-Time Release Testing (RTR) and align with Industry 4.0 principles. Essential for real time data collection from various unit operations are analytical sensors such as spectroscopic or chromatographic PAT tools, along with automated sample capabilities.

   Methods for analysing both multivariate and univariate data, coupled with visualization, facilitate real time tracking of quality attributes. The analysed data can be utilized for feedback/feedforward process control. Techniques like machine learning and deep learning, crucial for comprehensive process understanding, should be incorporated into the real time monitoring framework. A successful pathway to real-time process monitoring involves precise identification of critical quality attributes (CQAs), critical process parameters (CPPs), and the deployment of suitable PAT tools at critical control points (CCPs)[53]. It's crucial to employ the right tools, understanding both their inherent analytical capabilities and limitations, to collect precise real-time process information. Advanced data-driven modelling can also be employed for comprehensive monitoring and prediction of the complete process (not on a unit operation basis), followed by

advanced process controls (APCs)[55]. Real-time bioprocess monitoring promotes RTR and Industry 4.0 goals, aiding in the production of biopharmaceuticals with consistent quality and effectiveness for patients[56].

2. **Process Optimization and Predictive Modelling:** Process optimization and predictive modelling have become increasingly important tools in various industries including manufacturing, healthcare, retail, and a host of others. These methodologies help enterprises optimize resource utilization by predicting future outcomes.Process optimization chiefly involves maximizing system efficiency and minimizing costs[57]. One common example is in the manufacturing sector where process optimization ensures the precise allocation of resources, reduces waste, and thereby enhances profitability. Methods such as linear programming, simulation, process mapping, and six sigma methodologies provide systematic procedures to review operations, identify problems, devise solutions and track improvements [58]. Predictive modelling, a key component of data analytics applied in bioprocessing, goes a step further. It leverages the extensive multi-parametric data obtained during bioprocesses to build models predicting the process outcome. Traditional empirical models can often not account for the complexity and non-linearity of the biological system, thus advanced machine learning algorithms have been proposed to create predictive models with enhanced accuracy [59]. For example, leveraging neural network-based approaches or ensemble learning methods, predictive modelling can provide real-time predictions with appreciable precision, thereby bridging the gap between process understanding and control [60].

The rigorous combination of process optimization, PAT, and predictive modelling through data analytics propels significant benefits for bioprocessing. While there are challenges related to data handling, integration, and interpretation, the improved process understanding, enhanced control and predictive power stand to accelerate product development, improve product consistency and reduce manufacturing costs, thereby making a substantial contribution to the evolution of the bioprocessing industry.

3. **Artificial Intelligence (AI) Applications:** Artificial Intelligence (AI) has found numerous applications in bioprocessing, revolutionizing the way biological products are developed, manufactured, and monitored. Some of the key AI applications in bioprocessing include:

- **Process Optimization:** AI algorithms can analyse large volumes of data from bioprocessing operations to identify patterns and optimize process parameters[61]. By optimizing factors such as media composition, temperature, pH, and agitation, AI can enhance productivity and yield in bioreactor-based processes.
- **Bioprocess Control:** AI-powered control systems can continuously monitor and adjust bioprocess parameters in real-time. These systems can respond to dynamic conditions, reducing the need for manual intervention and ensuring consistent product quality.
- **Automated Bio Manufacturing:** AI-driven automation is used to streamline various aspects of bio manufacturing. From cell line development to downstream purification, AI systems can handle repetitive tasks efficiently and with reduced human intervention.
- **Data Analytics and Predictive Modelling:** AI techniques such as machine learning

and deep learning can analyse large datasets generated during bioprocessing[62]. These models can predict outcomes, detect anomalies, and identify potential areas for process improvement.

- **Quality Control and Process Monitoring:** AI can monitor critical quality attributes and process parameters to ensure the bioprocess remains within desired specifications. Real-time monitoring helps identify deviations and enables prompt corrective actions.
- **Drug Discovery and Design:** AI is being increasingly used in drug discovery to identify potential drug candidates and optimize their properties. AI algorithms can predict molecular interactions, screen compounds, and design novel drugs more efficiently.
- **Personalized Medicine:** AI can analyse patient data, genetic information, and treatment outcomes to develop personalized treatment plans, especially in areas like cancer therapy.
- **Biomarker Discovery:** AI can help identify relevant biomarkers that indicate disease status or response to treatment. This is valuable in diagnosis, prognosis, and patient stratification in clinical trials.
- Genomics and Proteomics: AI is used to analyse and interpret large-scale genomics and proteomics data [63]. It aids in identifying genetic variants, understanding gene function, and predicting protein structure and function.
- **Image Analysis:** In cell culture and microscopy, AI can be employed for automated image analysis, cell counting, and identification of cellular structures, accelerating research processes and reducing manual labour.
- **Drug Repurposing:** AI algorithms can mine existing databases of approved drugs and compounds to identify potential candidates for repurposing in treating new diseases or conditions.
- **Clinical Trial Optimization:** AI can optimize clinical trial design and patient recruitment, leading to more efficient trials with reduced costs and timelines.

V. UTILIZATION OF ALTERNATIVE AND SUSTAINABLE FEEDSTOCK

The increasing environmental concerns and dwindling fossil resources have prompted researchers to look for sustainable and alternative feedstock's in bio processing[64]. The primary challenge lies in identifying affordable, environmentally friendly feedstocks that do not compete with food sources or lead to other environmental issues. A significant breakthrough in the field came with the exploration of non-food plant biomass, like lignocelluloses materials. E.g., agricultural residues (straw, stover), forest residues, grasses, or the waste fractions in the paper industry. Lignocelluloses biomass is abundant, renewable, and does not compete with food supply [65]. Bio processing technologies have evolved, encompassing various pre-treatment methods, enzymatic hydrolysis, and fermentation to produce bioethanol from lignocelluloses biomass[66].Subsequently, some research focused on microalgae –another promising feedstock for sustainable bio processing. Microalgae grow quickly, absorb CO_2, and can generate lipids (for biodiesel), carbohydrates (for ethanol), and proteins (for animal feeds), thereby making a significant leap toward achieving circular bioeconomy objectives [67].

The exploration doesn't end here. One of the recent advancements in this context has been the adoption of organic wastes and waste gases as feedstock's. Targeting an altogether new aspect of sustainability, these feedstocks seek to mitigate the burden of waste

management while producing value-added products. Organic waste streams (like food or agricultural waste) can be an aerobically digested to produce biogas consisting mainly of methane, which can then be used as a feedstock for microbial fermentation to produce a range of products like bioplastics, biofuels, or other industrial products[68]. Industrial gas waste streams (like CO, CO_2) are also being explored as potential feedstock's for gas fermenting microbes like Clostridia and acetogens, leading to the production of biofuels, platform chemicals, etc. These advancements tie waste stream utilization to bioprocessing, thereby giving a new perspective to the concept of the circular economy. Nonetheless, significant challenges prevail, more notably in the commercial viability of these alternative feedstocks. Operating costs, scalability of operations, feedstock availability, technoeconomic feasibility are some of the issues that need comprehensive research attention. Government regulations and policy support also play a crucial role in making these alternatives mainstream [69]. It is therefore essential to keep pushing the boundaries of innovation and research in sustainable feedstock's for bio processing while addressing the bottlenecks for commercial viability.

1. **Lignocellulosic Biomass and Waste Streams:** Lignocellulosic biomass comprises the largest heterogeneous organic resources on Earth, providing a substantial and renewable raw material for sustainable biofuels and bioproducts production. Essentially, it is plant biomass that comprises cellulose, hemicelluloses, and lignin. Its global importance is underscored by its availability, renewability, and potential to mitigate carbon dioxide emissions by replacing petroleum-based fuels and chemicals. Lignocellulosic biomass can be derived from various sources, including agricultural residues, such as crop straws and hulls, forestry residues, such as wood chips and bark, and dedicated energy crops like switch grass and poplar trees [70]. However, the plenteous lignocellulosic waste generated from tanneries, pulp and paper industries, and municipal waste streams are becoming increasingly attractive feedstocks. This is due to the appeal for waste valorisation, resource recovery, and waste reduction associated with the circular bio-economy framework [71].The industrial utility of lignocellulosic biomass in bioprocessing is facilitated through pre-treatment methods [72]. These integrate mechanical, chemical, enzymatic, and/or thermal processes to break down the rigid lignocellulosic structure into fermentable sugars, making them accessible for microbial bioconversion. Bioconversion processes employ bacteria, yeast, or fungi for the fermentable sugar's metabolic exploitation into various value-added products [73]. These include biofuels like bioethanol and biogas, bio-based materials like bioplastics, and biochemical like lactic acid, citric acid, and xylitol. However, the broad-scale application of lignocellulosic biomass faced several challenges such as feedstock variability, recalcitrance, microbial inhibition by pre-treatment-derived and lignin-derived compounds, and the capital intensity of the required bioprocesses. Thus, to enhance the economic viability and environmental sustainability of lignocellulosic bioprocessing, the research is focusing on integrated biorefinery concepts to exploit the individual components of biomass (cellulose, hemicellulose, lignin) and waste streams for multiple value-added products.Moreover, advances have been made in synthetic biology and metabolic engineering to develop robust microbes tolerant to pre-treatment-derived inhibitors with improved fermentation performance[74]. Also, efforts are directed towards valorising lignin, the most underutilized biomass component due to its complex and heterogeneous structure, into high-value materials and chemicals.Conclusively, lignocellulosic biomass and derived waste streams offer a renewable and sustainable feedstock pathway for alternative fuels and products. By adopting circular economy principles, bioprocessing technologies are pushing forward sustainable development.

2. **Bioconversion Technologies:** Bioconversion technologies are rapidly becoming the cornerstone of using alternative and sustainable feedstock in bioprocessing. This has profound implications for realizing a more sustainable and circular economy, where waste or low-value feed stocks like agricultural residues, organic wastes, and non-food crops are converted into valuable bio-based products ranging from biofuels, biochemicals to biomaterials[75].

Bioconversion technologies, including biochemical and thermochemical conversion processes, enable the transformation of these diverse feedstocks into a variety of biofuels or bio-based products. These methods include anaerobic digestion, fermentation, gasification, pyrolysis, hydrolysis and more[76]. Each feedstock requires a specific set of pre-treatment steps followed by conversion and refining processes to yield the desired product. For instance, lignocellulosic biomass, an abundant and renewable feedstock, often requires pre-treatment processes like chemical, physical, or biological methods to disrupt its complex structure and increase access for conversion enzymes[77].During fermentation, microorganisms such as bacteria, yeast, or fungi are used to convert sugars derived from these feedstocks into ethanol. Genetic engineering has allowed for a modification of these microorganisms, enhancing fermentation efficiency, and enabling the production of a wider range of products. Additionally, a promising characteristic of some bioconversion processes, such as anaerobic digestion, is the ability to produce bioenergy (methane) from waste streams while converting them into valuable fertilizer. Moreover, thermo chemical bioconversion processes, including gasification, pyrolysis, and liquefaction, often serve as effective ways to convert less easily degradable feed stocks, such as wood, into gases, liquids or solid biofuels or bio-based chemicals[78]. These bioconversion technologies offer a wide range of possibilities in terms of feedstock flexibility and product spectrum. As these technologies continue to improve, it is crucial to consider the environmental impact and sustainability of these processes. Life cycle assessment (LCA) is often used for this evaluation, considering the environmental footprint of the entire process from feedstock production to end-product utilization. This assessment considers numerous aspects such as greenhouse gas emissions, fossil energy use and land use, thus guiding the development towards more sustainable bioconversion processes[79]

3. **Metabolic Engineering Strategies:** Metabolic Engineering has been recognised as a crucial method for the modification and optimisation of microbial systems to facilitate the utilisation of alternative and sustainable feedstock in bioprocessing[80]. These feed stocks include cellulose, lignocellulosic biomass, carbon dioxide (CO_2), carbon monoxide (CO), municipal solid waste, agricultural waste, etc. Different strategies can be employed based on the type of feedstock and desired end-product.For lignocellulosic feedstock, pre-treatment methods such as acid hydrolysis are used to breakdown complex substrates, and then genetically engineered microorganisms (e.g., Saccharomyces cerevisiae) are utilised to ferment sugars into products such as bioethanol [81]. Synthetic biology, a derivative of metabolic engineering, has been employed further to construct artificial metabolic pathways in organisms for the conversion of lignocellulosic biomass into higher-value chemicals such as bioplastics, antibiotics, and biofuels[82].

On the other hand, the use of CO_2 as feedstock for microbial bioconversion has gained popularity due to the global need to mitigate greenhouse gas emissions.

Engineered cyanobacteria and algae have been used for this purpose as they can directly utilise CO_2 via photosynthesis and produce biofuels and chemicals. For instance, the cyanobacterium *Synechococcus elongatus PCC 7942* has been engineered to produce isobutanol directly from CO_2[83].The engineering of metabolic pathways is a multidimensional approach that involves various aspects such as the improvement of precursor availability, the enhancement of enzyme activity, the elimination of by-product formation, regulation of gene expression, and the modulation of cellular transport. With the advent of high-throughput omics technologies and advanced computational tools, new strategies such as dynamic metabolic engineering and systems metabolic engineering are being developed to combine traditional metabolic engineering with systems biology, synthetic biology, and evolutionary engineering, allowing a more holistic manipulation of metabolic networks to optimize metabolic pathways [84].

Given the remarkable progress in metabolic engineering and multilevel integrative approaches, it is expected that improved microbial systems can be developed for efficiently processing diverse types of alternative and sustainable feedstocks. However, the challenge lies in the scalability of these methods for industrial levels, requiring well-designed bioprocesses and overcoming economic constraints for a feasible green bioprocessing industry.

VI. CHALLENGES

1. **Regulatory Considerations***:* Bio processing, or biotechnological manufacturing, is a rapidly expanding field that harnesses biological systems to create a vast array of products, from pharmaceuticals to organ replacements. However, with these advances come significant regulatory challenges and considerations, necessitating regular updates to existing frameworks to ensure safety and efficacy [85].One of the main regulatory challenges in bio processing is assuring the safety of bio manufactured products. These products stem from complex biological systems and can be influenced by subtle changes in manufacturing conditions, potentially affecting their quality and safety. Authorities like the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have stringent regulations, necessitating close collaboration between the bio processing industry and regulatory bodies to ensure safety compliance. Standardization of bio processing techniques is another major challenge. Inconsistencies in processing measures across different sites can lead to significant variability in the outcomes, compounding the challenges in bio-safety evaluation[86]. Central to this is the establishment of clear Good Manufacturing Practices (GMPs) and the enforcement of these regulations, preserving a high standard of bio-production.

A growing area of concern is the regulation of novel bioprocessing techniques. Technologies like CRISPR gene editing and bio-printing have fundamentally changed bioprocessing[87]. However, due to their relatively recent emergence, these technologies currently lack comprehensive regulatory frameworks, creating uncertainty for both the industry and consumers. Regulatory bodies must seek to expand their regulatory scope to accommodate new technologies while balancing innovation and safety. Another challenge includes potential environmental and ethical considerations. Bioprocessing can produce waste that contains genetically modified organisms or hazardous chemicals, necessitating stringent regulations to manage disposal and prevent harm to the environment [88]. On the ethical front, areas such as organ/tissue bio-printing present highly complex and

emotional ethical issues those regulatory bodies must address. Regulatory bodies hence need to adopt a proactive stance, reassessing existing industry practices and enabling the safe introduction of new technologies. Importantly, international collaboration is essential for the creation of universally applicable standards. For example, the International Alliance for Biological Standardization is fostering global communication and the harmonization of bioprocessing standards.

Bioprocessing presents a wealth of opportunities for healthcare and biotechnology. However, it also carries significant regulatory challenges that must be addressed. Regulatory frameworks need to evolve alongside biotechnological advancements, ensuring product safety, ethical integrity, and environmental stewardship. This challenging but necessary process will maximize the benefits of bioprocessing and assure its place in the future of manufacturing.

2. **Technology Transfer and Commercialization***:* While bioprocessing techniques have positioned themselves at the forefront of the biotechnological industry due to their deep involvement in producing pharmaceuticals, biofuels, and specialty chemicals, the technology transfer and commercialization of these techniques pose significant challenges. This is critically reflected in industry-academia partnerships where the feasibility of a technique established in a research lab does not always ensure its business viability. The crux of the challenge lies in scaling up techniques from laboratory-scale production to industrial-scale manufacturing, as this encompasses greater process complexity and risk. One key challenge is the generation of consistent, reproducible results upon scaling-up. This standardization difficulty resides in the diversity of biological systems, and the inherent variability can lead to unpredictable outcomes when scaled up due to changes in bioreactor geometry, microbial productivity, and process parameters [89]. As bioprocesses are intrinsically multifactorial, even a minor modification can induce a significant change in product yield or quality, posing a barrier to technology transfer [90].

Another challenge that hinders the commercialization of bioprocessing techniques is the stringent regulatory environment. Regulatory agencies require robust documentation and data management for every stage of process development and production. Firms must adhere to regulations on Good Manufacturing Practices (GMPs) to ensure product safety and efficiency. Hence, resource-constrained firms may find it overwhelming to comply with legal requirements, constraining the technology transfer process.Intellectual property rights (IPR) also impose a significant challenge. Universities and research institutions often protect their innovations through patents. Still, the high costs associated with patenting, licensing, and litigation processes may inhibit start-ups and smaller firms from transferring and commercializing these technologies[91]. These institutions further state that they are not equipped to interact with industry, citing a lack of skillets and business acumen among their academic staff [92].

The upfront capital required to establish industrial bioprocesses is substantially enormous and may act as a bottleneck for technology transfer. Necessary infrastructure, including bioreactors, downstream processing equipment, and quality control laboratories, demands significant monetary investment. Furthermore, market acceptance and lack of customer awareness about bioproducts add to the financial stress, thereby impeding commercialization. Addressing these challenges requires robust strategic and

technical responses. Standardization and rigorous process control must be pursued to mitigate risks related to scale-up and variability. Regulatory guidelines must be incorporated from the initial design phase to expedite process approval. Collaborative platforms can be established to streamline universities' interaction with industry and secure IPR. Encouraging investors by demonstrating the profitability and sustainability of bioproducts can attract the necessary capital. Future research should focus on reducing the capital and operating costs of these processes to improve their commercial viability, fostering an atmosphere conducive to global bio-sustainability.

3. **Collaboration in Bio processing**: Bio processing is a field that encompasses various interdisciplinary approaches to efficiently harnessing biological resources to produce valuable products. With its growing importance in areas such as medicine, agriculture, and industry, collaboration in bio processing has become essential for advancing research and development in this field. Collaboration fosters the sharing of knowledge and expertise among scientists, engineers, and industry professionals, enabling them to work together towards common goals. This cooperation not only promotes innovation but also accelerates the discovery of novel techniques, technologies, and processes for optimizing bio processing. By combining different skill sets and perspectives, collaborative efforts in bio processing pave the way for breakthroughs in areas such as biopharmaceuticals, biofuels, bioplastics, and more. Ultimately, collaboration in bio processing serves as a catalyst for sustainable and economically viable solutions that can address some of the world's most pressing challenges. These collaborations often involve joint development, licensing, and commercialization of intellectual property, with all parties sharing in the profits or losses. Given the high stakes involved, including investor expectations and valuable resources, it's essential for companies to carefully navigate collaboration agreements. Despite time constraints and pressure to deliver results quickly, taking the following factors into consideration can create significant long-term value for all parties involved:

- **Clear Objectives and Expectations:** All parties involved should have a shared understanding of the project's objectives, timelines, and expectations. Define the scope of the collaboration, including specific milestones and deliverables, to ensure everyone is on the same page. Intellectual Property (IP) Rights and Licensing: Determine how the ownership of IP will be handled during and after the collaboration. Address issues related to licensing, royalties, and exclusivity to avoid potential disputes later.

- **Resource Allocation and Responsibilities:** Clearly define each party's responsibilities and contributions, including financial, technological, and managerial aspects. It's crucial to establish a fair and equitable distribution of costs and risks.

- **Governance and Decision-making:** Establish a governance structure to facilitate decision-making during the collaboration. Consider how conflicts will be resolved and how modifications to the agreement can be made if needed.

- **Data Sharing and Confidentiality:** Address the handling of sensitive information and data sharing between the collaborating parties. Protecting confidential information is crucial to maintaining trust.

- Exit Strategy: Anticipate the possibility that the collaboration may not work out as intended. Establish an exit strategy that outlines how parties can terminate the agreement in case of unforeseen circumstances or disagreements.

- **Regulatory and Compliance Considerations:** Be aware of regulatory requirements that might impact the collaboration and ensure that all parties are compliant with applicable laws and regulations.
- **Cultural Fit and Communication:** Consider the cultural fit between the collaborating companies. Effective communication and a shared vision can significantly contribute to the success of the partnership.
- **Due Diligence:** Take the time to conduct thorough due diligence on potential collaborators to understand their track record, financial stability, and reputation.
- **Legal and Financial Expertise:** Engage legal and financial advisors who have experience in collaboration agreements to help navigate complex issues and protect your interests.

While there might be pressure to move quickly, rushing into a collaboration agreement without considering these factors can lead to significant challenges down the line. Taking the time to negotiate and analyse the agreement can provide long-term value by mitigating risks and ensuring a successful partnership.

VII. CONCLUSION

The chapter highlights the exciting developments in the field of Bioprocessing Techniques. Advanced cell culture techniques, bioreactor technologies, and innovative downstream processing methods enhance productivity and efficiency. The integration of process analytical technology, data analytics, and artificial intelligence enables real-time monitoring and optimization. The utilization of alternative and sustainable feedstock, along with bioconversion and metabolic engineering strategies, contributes to the production of biofuels and valuable chemicals. Collaboration is crucial for addressing challenges and ensuring successful implementation. Overall, these emerging trends and innovations hold significant promise for advancing bioprocessing and its impact on various industries.

REFERENCES

[1]   C.-P. Segeritz and L. Vallier, "Cell Culture," in Basic Science Methods for Clinical Researchers, Elsevier, 2017, pp. 151–172. doi: 10.1016/B978-0-12-803077-6.00009-6.

[2]   S. J. Allan, P. A. De Bank, and M. J. Ellis, "Bioprocess Design Considerations for Cultured Meat Production With a Focus on the Expansion Bioreactor," Front Sustain Food Syst, vol. 3, Jun. 2019, doi: 10.3389/fsufs.2019.00044.

[3]   S. Mitra and G. S. Murthy, "Bioreactor control systems in the biopharmaceutical industry: a critical perspective," Systems Microbiology and Biomanufacturing, vol. 2, no. 1, pp. 91–112, Jan. 2022, doi: 10.1007/s43393-021-00048-6.

[4]   E. S. Langer, "Single-Use Technology in Biopharmaceutical Manufacture and Beyond," ChemieIngenieur Technik, vol. 94, no. 12, pp. 1892–1901, Dec. 2022, doi: 10.1002/cite.202200095.

[5]   S. Zobel-Roos, D. Stein, and J. Strube, "Evaluation of Continuous Membrane Chromatography Concepts with an Enhanced Process Simulation Approach.," Antibodies (Basel), vol. 7, no. 1, Mar. 2018, doi: 10.3390/antib7010013.

[6]   S. J. Reyes, Y. Durocher, P. L. Pham, and O. Henry, "Modern Sensor Tools and Techniques for Monitoring, Controlling, and Improving Cell Culture Processes," Processes, vol. 10, no. 2, p. 189, Jan. 2022, doi: 10.3390/pr10020189.

[7]   T. Scheper et al., "Digitalization and Bioprocessing: Promises and Challenges," 2020, pp. 57–69. doi: 10.1007/10_2020_139.

[8]   S. S. Ozturk, "Engineering challenges in high density cell culture systems," Cytotechnology, vol. 22, no. 1–3, pp. 3–16, 1996, doi: 10.1007/BF00353919.

[9]   O.-W. Merten, "Advances in cell culture: anchorage dependence," Philosophical Transactions of the Royal

Society B: Biological Sciences, vol. 370, no. 1661, p. 20140040, Feb. 2015, doi: 10.1098/rstb.2014.0040.

[10] M. Clincke, C. Mölleryd, Y. Zhang, E. Lindskog, K. Walsh, and V. Chotteau, "Very high density of CHO cells in perfusion by ATF or TFF in WAVE bioreactorTM. Part I. Effect of the cell density on the process," Biotechnol Prog, vol. 29, no. 3, pp. 754–767, May 2013, doi: 10.1002/btpr.1704.

[11] R. Edmondson, J. J. Broglie, A. F. Adcock, and L. Yang, "Three-Dimensional Cell Culture Systems and Their Applications in Drug Discovery and Cell-Based Biosensors," Assay Drug Dev Technol, vol. 12, no. 4, pp. 207–218, May 2014, doi: 10.1089/adt.2014.573.

[12] G.-J. Kim, L. Kim, and O. S. Kwon, "Application of 3D Bioprinting Technology for Tissue Regeneration, Drug Evaluation, and Drug Delivery," Applied Science and Convergence Technology, vol. 32, no. 1, pp. 1–6, Jan. 2023, doi: 10.5757/ASCT.2023.32.1.1.

[13] Z. Fang et al., "Application of bioreactor technology for cell culture-based viral vaccine production: Present status and future prospects.," Front BioengBiotechnol, vol. 10, p. 921755, 2022, doi: 10.3389/fbioe.2022.921755.

[14] J. Y. Kim, Y.-G. Kim, and G. M. Lee, "CHO cells in biotechnology for production of recombinant proteins: current state and further potential.," Appl MicrobiolBiotechnol, vol. 93, no. 3, pp. 917–30, Feb. 2012, doi: 10.1007/s00253-011-3758-5.

[15] M. Mehrian, T. Lambrechts, I. Papantoniou, and L. Geris, "Computational Modeling of Human Mesenchymal Stromal Cell Proliferation and Extra-Cellular Matrix Production in 3D Porous Scaffolds in a Perfusion Bioreactor: The Effect of Growth Factors.," Front BioengBiotechnol, vol. 8, p. 376, 2020, doi: 10.3389/fbioe.2020.00376.

[16] R. Eibl and D. Eibl, Eds., Single-Use Technology in Biopharmaceutical Manufacture, Chap. Hoboken, NJ, USA: John Wiley & Sons, Inc., 2010. doi: 10.1002/9780470909997.

[17] J. Pollock, S. V. Ho, and S. S. Farid, "Fed-batch and perfusion culture processes: Economic, environmental, and operational feasibility under uncertainty," BiotechnolBioeng, vol. 110, no. 1, pp. 206–219, Jan. 2013, doi: 10.1002/bit.24608.

[18] J. Pollock, J. Coffman, S. V Ho, and S. S. Farid, "Integrated continuous bioprocessing: Economic, operational, and environmental feasibility for clinical and commercial antibody manufacture.," Biotechnol Prog, vol. 33, no. 4, pp. 854–866, Jul. 2017, doi: 10.1002/btpr.2492.

[19] K. B. Konstantinov and C. L. Cooney, "White Paper on Continuous Bioprocessing May 20–21 2014 Continuous Manufacturing Symposium," J Pharm Sci, vol. 104, no. 3, pp. 813–820, Mar. 2015, doi: 10.1002/jps.24268.

[20] F. Li, N. Vijayasankaran, A. Y. Shen, R. Kiss, and A. Amanullah, "Cell culture processes for monoclonal antibody production.," MAbs, vol. 2, no. 5, pp. 466–79, 2010, doi: 10.4161/mabs.2.5.12720.

[21] O. F. Restaino, D. Cimini, M. De Rosa, A. Catapano, M. De Rosa, and C. Schiraldi, "High cell density cultivation of Escherichia coli K4 in a microfiltration bioreactor: a step towards improvement of chondroitin precursor production," Microb Cell Fact, vol. 10, no. 1, p. 10, Dec. 2011, doi: 10.1186/1475-2859-10-10.

[22] L. De Bartolo et al., "Human hepatocyte functions in a crossed hollow fiber membrane bioreactor," Biomaterials, vol. 30, no. 13, pp. 2531–2543, May 2009, doi: 10.1016/j.biomaterials.2009.01.011.

[23] H. M. Van Sonsbeek, H. H. Beeftink, and J. Tramper, "Two-liquid-phase bioreactors," Enzyme MicrobTechnol, vol. 15, no. 9, pp. 722–729, Sep. 1993, doi: 10.1016/0141-0229(93)90001-I.

[24] J. M. Rawat, A. Bhandari, M. Raturi, and B. Rawat, "Agrobacterium rhizogenes Mediated Hairy Root Cultures: A Promising Approach for Production of Useful Metabolites," in New and Future Developments in Microbial Biotechnology and Bioengineering, Elsevier, 2019, pp. 103–118. doi: 10.1016/B978-0-444-63504-4.00008-6.

[25] F. Meuwly, P.-A. Ruffieux, A. Kadouri, and U. von Stockar, "Packed-bed bioreactors for mammalian cell culture: Bioprocess and biomedical applications," Biotechnol Adv, vol. 25, no. 1, pp. 45–56, Jan. 2007, doi: 10.1016/j.biotechadv.2006.08.004.

[26] J.-J. Zhong, "Bioreactor Engineering," in Comprehensive Biotechnology, Elsevier, 2011, pp. 165–177. doi: 10.1016/B978-0-08-088504-9.00097-0.

[27] V. Bansal, P. K. Roychoudhury, B. Mattiasson, and A. Kumar, "Recovery of urokinase from integrated mammalian cell culture cryogel bioreactor and purification of the enzyme usingp-aminobenzamidine affinity chromatography," Journal of Molecular Recognition, vol. 19, no. 4, pp. 332–339, Jul. 2006, doi: 10.1002/jmr.785.

[28] S. Nilsanget al., "Monoclonal Antibody Production Using a New Supermacroporous Cryogel Bioreactor," Biotechnol Prog, vol. 23, no. 4, pp. 932–939, 2007, doi: 10.1002/bp0700399.

[29] A. Kumar, V. Bansal, J. Andersson, P. K. Roychoudhury, and B. Mattiasson, "Supermacroporous cryogel matrix for integrated protein isolation," J Chromatogr A, vol. 1103, no. 1, pp. 35–42, Jan. 2006, doi:

10.1016/j.chroma.2005.08.094.

[30] V. I. Lozinsky, I. Yu. Galaev, F. M. Plieva, I. N. Savina, H. Jungvid, and B. Mattiasson, "Polymeric cryogels as promising materials of biotechnological interest," Trends Biotechnol, vol. 21, no. 10, pp. 445–451, Oct. 2003, doi: 10.1016/j.tibtech.2003.08.002.

[31] I. Jyothilekshmi and N. S. Jayaprakash, "Trends in Monoclonal Antibody Production Using Various Bioreactor Syst.," J MicrobiolBiotechnol, vol. 31, no. 3, pp. 349–357, Mar. 2021, doi: 10.4014/jmb.1911.11066.

[32] A. Westbrook, J. Scharer, M. Moo-Young, N. Oosterhuis, and C. Perry Chou, "Application of a two-dimensional disposable rocking bioreactor to bacterial cultivation for recombinant protein production," Biochem Eng J, vol. 88, pp. 154–161, Jul. 2014, doi: 10.1016/j.bej.2014.04.011.

[33] A. A. Shukla and J. Thömmes, "Recent advances in large-scale production of monoclonal antibodies and related proteins," Trends Biotechnol, vol. 28, no. 5, pp. 253–261, May 2010, doi: 10.1016/j.tibtech.2010.02.001.

[34] S. Zobel-Roos, D. Stein, and J. Strube, "Evaluation of Continuous Membrane Chromatography Concepts with an Enhanced Process Simulation Approach.," Antibodies (Basel), vol. 7, no. 1, Mar. 2018, doi: 10.3390/antib7010013.

[35] K. B. Konstantinov and C. L. Cooney, "White Paper on Continuous Bioprocessing May 20–21 2014 Continuous Manufacturing Symposium," J Pharm Sci, vol. 104, no. 3, pp. 813–820, Mar. 2015, doi: 10.1002/jps.24268.

[36] J. Pollock, J. Coffman, S. V Ho, and S. S. Farid, "Integrated continuous bioprocessing: Economic, operational, and environmental feasibility for clinical and commercial antibody manufacture.," Biotechnol Prog, vol. 33, no. 4, pp. 854–866, Jul. 2017, doi: 10.1002/btpr.2492.

[37] A. Rajendran, G. Paredes, and M. Mazzotti, "Simulated moving bed chromatography for the separation of enantiomers.," J Chromatogr A, vol. 1216, no. 4, pp. 709–38, Jan. 2009, doi: 10.1016/j.chroma.2008.10.075.

[38] O. Shinkazh, D. Kanani, M. Barth, M. Long, D. Hussain, and A. L. Zydney, "Countercurrent tangential chromatography for large-scale protein purification.," BiotechnolBioeng, vol. 108, no. 3, pp. 582–91, Mar. 2011, doi: 10.1002/bit.22960.

[39] R. V Levy and M. W. Jornitz, "Types of filtration.," Adv Biochem Eng Biotechnol, vol. 98, pp. 1–26, 2006, doi: 10.1007/b104242.

[40] M. Nageeb, "Adsorption Technique for the Removal of Organic Pollutants from Water and Wastewater," in Organic Pollutants - Monitoring, Risk and Treatment, InTech, 2013. doi: 10.5772/54048.

[41] N. A. A. M. Amin, M. A. Mokhter, N. Salamun, M. F. bin Mohamad, and W. M. A. W. Mahmood, "Anti-fouling electrospun organic and inorganic nanofiber membranes for wastewater treatment," S Afr J Chem Eng, vol. 44, pp. 302–317, Apr. 2023, doi: 10.1016/j.sajce.2023.02.002.

[42] M. W. Hakami, A. Alkhudhiri, S. Al-Batty, M.-P. Zacharof, J. Maddy, and N. Hilal, "Ceramic Microfiltration Membranes in Wastewater Treatment: Filtration Behavior, Fouling and Prevention," Membranes (Basel), vol. 10, no. 9, p. 248, Sep. 2020, doi: 10.3390/membranes10090248.

[43] J. Chen, B. Yu, H. Cong, and Y. Shen, "Recent development and application of membrane chromatography," Anal Bioanal Chem, vol. 415, no. 1, pp. 45–65, Jan. 2023, doi: 10.1007/s00216-022-04325-8.

[44] M. Cheryan, Ultrafiltration and Microfiltration Handbook. CRC Press, 1998. doi: 10.1201/9781482278743.

[45] M. Kotobuki, Q. Gu, L. Zhang, and J. Wang, "Ceramic-Polymer Composite Membranes for Water and Wastewater Treatment: Bridging the Big Gap between Ceramics and Polymers.," Molecules, vol. 26, no. 11, Jun. 2021, doi: 10.3390/molecules26113331.

[46] S. Gnoth, M. Jenzsch, R. Simutis, and A. Lübbert, "Process Analytical Technology (PAT): Batch-to-batch reproducibility of fermentation processes by robust process operational design and control," J Biotechnol, vol. 132, no. 2, pp. 180–186, Oct. 2007, doi: 10.1016/j.jbiotec.2007.03.020.

[47] Q. P. He and J. Wang, "Statistical process monitoring as a big data analytics tool for smart manufacturing," J Process Control, vol. 67, pp. 35–43, Jul. 2018, doi: 10.1016/j.jprocont.2017.06.012.

[48] A. S. Rathore and H. Winkle, "Quality by design for biopharmaceuticals.," Nat Biotechnol, vol. 27, no. 1, pp. 26–34, Jan. 2009, doi: 10.1038/nbt0109-26.

[49] C. L. Gargaloet al., "Towards smart biomanufacturing: a perspective on recent developments in industrial measurement and monitoring technologies for bio-based production processes," J Ind MicrobiolBiotechnol, vol. 47, no. 11, pp. 947–964, Nov. 2020, doi: 10.1007/s10295-020-02308-1.

[50] I. H. Sarker, "Deep Learning: A Comprehensive Overview on Techniques, Taxonomy, Applications and Research Directions," SN Comput Sci, vol. 2, no. 6, p. 420, Nov. 2021, doi: 10.1007/s42979-021-00815-1.

[51] X. Xu and Q. Hua, "Industrial Big Data Analysis in Smart Factory: Current Status and Research Strategies," IEEE Access, vol. 5, pp. 17543–17551, 2017, doi: 10.1109/ACCESS.2017.2741105.

[52] M. Jenzsch, S. Gnoth, M. Kleinschmidt, R. Simutis, and A. Lübbert, "Improving the batch-to-batch reproducibility in microbial cultures during recombinant protein production by guiding the process along a predefined total biomass profile.," Bioprocess Biosyst Eng, vol. 29, no. 5–6, pp. 315–21, Dec. 2006, doi: 10.1007/s00449-006-0080-1.

[53] A. S. Rathore, "Roadmap for implementation of quality by design (QbD) for biotechnology products," Trends Biotechnol, vol. 27, no. 9, pp. 546–553, Sep. 2009, doi: 10.1016/j.tibtech.2009.06.006.

[54] H. Wu et al., "Real time monitoring of bioreactor mAb IgG3 cell culture process dynamics via Fourier transform infrared spectroscopy: Implications for enabling cell culture process analytical technology," Front Chem Sci Eng, vol. 9, no. 3, pp. 386–406, Sep. 2015, doi: 10.1007/s11705-015-1533-3.

[55] G. Shao, H. Latif, C. Martin-Villalba, and P. Denno, "Standards-based integration of advanced process control and optimization," J Ind Inf Integr, vol. 13, pp. 1–12, Mar. 2019, doi: 10.1016/j.jii.2018.10.001.

[56] D. P. Wasalathanthriet al., "Technology outlook for real-time quality attribute and process parameter monitoring in biopharmaceutical development—A review," BiotechnolBioeng, vol. 117, no. 10, pp. 3182–3198, Oct. 2020, doi: 10.1002/bit.27461.

[57] A. Kusiak, "Smart manufacturing," Int J Prod Res, vol. 56, no. 1–2, pp. 508–517, Jan. 2018, doi: 10.1080/00207543.2017.1351644.

[58] H. Lal Bhaskar, "Lean Six Sigma in Manufacturing: A Comprehensive Review," in Lean Manufacturing and Six Sigma - Behind the Mask, IntechOpen, 2020. doi: 10.5772/intechopen.89859.

[59] A. Galal, M. Talal, and A. Moustafa, "Applications of machine learning in metabolomics: Disease modeling and classification," Front Genet, vol. 13, Nov. 2022, doi: 10.3389/fgene.2022.1017340.

[60] V. Brunner, M. Siegl, D. Geier, and T. Becker, "Challenges in the Development of Soft Sensors for Bioprocesses: A Critical Review," Front BioengBiotechnol, vol. 9, Aug. 2021, doi: 10.3389/fbioe.2021.722202.

[61] S. K. Khanal, A. Tarafdar, and S. You, "Artificial intelligence and machine learning for smart bioprocesses," BioresourTechnol, vol. 375, p. 128826, May 2023, doi: 10.1016/j.biortech.2023.128826.

[62] P. P. Mondal et al., "Review on machine learning-based bioprocess optimization, monitoring, and control systems," BioresourTechnol, vol. 370, p. 128523, Feb. 2023, doi: 10.1016/j.biortech.2022.128523.

[63] M. Mann, C. Kumar, W.-F. Zeng, and M. T. Strauss, "Artificial intelligence for proteomics and biomarker discovery," Cell Syst, vol. 12, no. 8, pp. 759–770, Aug. 2021, doi: 10.1016/j.cels.2021.06.006.

[64] P. S. Nigam and A. Singh, "Production of liquid biofuels from renewable resources," Prog Energy Combust Sci, vol. 37, no. 1, pp. 52–68, Feb. 2011, doi: 10.1016/j.pecs.2010.01.003.

[65] V. Ashokkumar et al., "Recent advances in lignocellulosic biomass for biofuels and value-added bioproducts - A critical review," BioresourTechnol, vol. 344, p. 126195, Jan. 2022, doi: 10.1016/j.biortech.2021.126195.

[66] S. Periyasamy et al., "Recent advances in consolidated bioprocessing for conversion of lignocellulosic biomass into bioethanol – A review," Chemical Engineering Journal, vol. 453, p. 139783, Feb. 2023, doi: 10.1016/j.cej.2022.139783.

[67] R. H. Wijffels and M. J. Barbosa, "An outlook on microalgal biofuels.," Science, vol. 329, no. 5993, pp. 796–9, Aug. 2010, doi: 10.1126/science.1189003.

[68] F. Liew, M. E. Martin, R. C. Tappel, B. D. Heijstra, C. Mihalcea, and M. Köpke, "Gas Fermentation—A Flexible Platform for Commercial Scale Production of Low-Carbon-Fuels and Chemicals from Waste and Renewable Feedstocks," Front Microbiol, vol. 7, May 2016, doi: 10.3389/fmicb.2016.00694.

[69] C. O. Tuck, E. Pérez, I. T. Horváth, R. A. Sheldon, and M. Poliakoff, "Valorization of biomass: deriving more value from waste.," Science, vol. 337, no. 6095, pp. 695–9, Aug. 2012, doi: 10.1126/science.1218930.

[70] A. Blasi, A. Verardi, C. G. Lopresto, S. Siciliano, and P. Sangiorgio, "Lignocellulosic Agricultural Waste Valorization to Obtain Valuable Products: An Overview," Recycling, vol. 8, no. 4, p. 61, Jul. 2023, doi: 10.3390/recycling8040061.

[71] A. Haile et al., "Pulp and paper mill wastes: utilizations and prospects for high value-added biomaterials," Bioresour Bioprocess, vol. 8, no. 1, p. 35, Apr. 2021, doi: 10.1186/s40643-021-00385-3.

[72] J. Baruah et al., "Recent Trends in the Pretreatment of Lignocellulosic Biomass for Value-Added Products," Front Energy Res, vol. 6, Dec. 2018, doi: 10.3389/fenrg.2018.00141.

[73] K. T. X. Tong, I. S. Tan, H. C. Y. Foo, M. K. Lam, S. Lim, and K. T. Lee, "Advancement of biorefinery-derived platform chemicals from macroalgae: a perspective for bioethanol and lactic acid," Biomass Convers Biorefin, Mar. 2022, doi: 10.1007/s13399-022-02561-7.

[74] M. Aamer Mehmood et al., "Advances in developing metabolically engineered microbial platforms to

produce fourth-generation biofuels and high-value biochemicals," BioresourTechnol, vol. 337, p. 125510, Oct. 2021, doi: 10.1016/j.biortech.2021.125510.

[75] F. Cherubini, "The biorefinery concept: Using biomass instead of oil for producing energy and chemicals," Energy Convers Manag, vol. 51, no. 7, pp. 1412–1421, Jul. 2010, doi: 10.1016/j.enconman.2010.01.015.

[76] B. Kamm and M. Kamm, "Principles of biorefineries," Appl MicrobiolBiotechnol, vol. 64, no. 2, pp. 137–145, Apr. 2004, doi: 10.1007/s00253-003-1537-7.

[77] N. MOSIER, "Features of promising technologies for pretreatment of lignocellulosic biomass," BioresourTechnol, vol. 96, no. 6, pp. 673–686, Apr. 2005, doi: 10.1016/j.biortech.2004.06.025.

[78] A. V. Bridgwater, "Review of fast pyrolysis of biomass and product upgrading," Biomass Bioenergy, vol. 38, pp. 68–94, Mar. 2012, doi: 10.1016/j.biombioe.2011.01.048.

[79] A. Singh, D. Pant, N. E. Korres, A.-S. Nizami, S. Prasad, and J. D. Murphy, "Key issues in life cycle assessment of ethanol production from lignocellulosic biomass: Challenges and perspectives.," BioresourTechnol, vol. 101, no. 13, pp. 5003–12, Jul. 2010, doi: 10.1016/j.biortech.2009.11.062.

[80] G. Stephanopoulos, "Metabolic fluxes and metabolic engineering.," Metab Eng, vol. 1, no. 1, pp. 1–11, Jan. 1999, doi: 10.1006/mben.1998.0101.

[81] L. R. Lynd et al., "How biotech can transform biofuels," Nat Biotechnol, vol. 26, no. 2, pp. 169–172, Feb. 2008, doi: 10.1038/nbt0208-169.

[82] J. P. Barnett, A. Millard, A. Z. Ksibe, D. J. Scanlan, R. Schmid, and C. A. Blindauer, "Mining Genomes of Marine Cyanobacteria for Elements of Zinc Homeostasis," Front Microbiol, vol. 3, 2012, doi: 10.3389/fmicb.2012.00142.

[83] S. Atsumi, T. Hanai, and J. C. Liao, "Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels.," Nature, vol. 451, no. 7174, pp. 86–9, Jan. 2008, doi: 10.1038/nature06450.

[84] A. Chowdhury and C. D. Maranas, "Designing overall stoichiometric conversions and intervening metabolic reactions," Sci Rep, vol. 5, no. 1, p. 16009, Nov. 2015, doi: 10.1038/srep16009.

[85] R. W. Kozak, C. N. Durfor, and C. L. Scribner, "Regulatory considerations when developing biological products. Report of the Center for Biologics Evaluation and Research.," Cytotechnology, vol. 9, no. 1–3, pp. 203–10, 1992, doi: 10.1007/BF02521747.

[86] A. Lorenz, M. Raven, and K. Blind, "The role of standardization at the interface of product and process development in biotechnology," J TechnolTransf, vol. 44, no. 4, pp. 1097–1133, Aug. 2019, doi: 10.1007/s10961-017-9644-2.

[87] T. Tomlinson, "A Crispr Future for Gene-Editing Regulation: a Proposal for an Updated Biotechnology Regulatory System in an Era of Human Genomic Editing.," Fordham Law Rev, vol. 87, no. 1, pp. 437–83, Oct. 2018.

[88] A. S. Bawa and K. R. Anilakumar, "Genetically modified foods: safety, risks and public concerns—a review," J Food Sci Technol, vol. 50, no. 6, pp. 1035–1046, Dec. 2013, doi: 10.1007/s13197-012-0899-1.

[89] A. A. Shukla and U. Gottschalk, "Single-use disposable technologies for biopharmaceutical manufacturing.," Trends Biotechnol, vol. 31, no. 3, pp. 147–54, Mar. 2013, doi: 10.1016/j.tibtech.2012.10.004.

[90] S. Mitra and G. S. Murthy, "Bioreactor control systems in the biopharmaceutical industry: a critical perspective," Systems Microbiology and Biomanufacturing, vol. 2, no. 1, pp. 91–112, Jan. 2022, doi: 10.1007/s43393-021-00048-6.

[91] M. A. Heller and R. S. Eisenberg, "Can patents deter innovation? The anticommons in biomedical research.," Science, vol. 280, no. 5364, pp. 698–701, May 1998, doi: 10.1126/science.280.5364.698.

[92] D. S. Siegel, D. A. Waldman, L. E. Atwater, and A. N. Link, "Commercial knowledge transfers from universities to firms: improving the effectiveness of university–industry collaboration," The Journal of High Technology Management Research, vol. 14, no. 1, pp. 111–133, Mar. 2003, doi: 10.1016/S1047-8310(03)00007-5.