



ADVANCEMENTS IN BIOREACTOR TECHNOLOGY: UNVEILING THE PINNACLE OF BIOTECHNOLOGICAL INNOVATION

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ABSTRACT

This review explores the pivotal role played by bioreactors in the field of bioprocessing, serving as essential tools for cultivating diverse biological entities to yield valuable products, spanning pharmaceuticals to biofuels. The significance, applications, and evolving trends of bioreactors are examined, emphasizing their role in providing controlled environments for the thriving of cells, microorganisms, and tissues. This controlled setting facilitates the efficient and scalable production of various bio-products by ensuring precise regulation of critical factors such as temperature, pH, dissolved oxygen, and nutrient availability. The paramount importance of this controlled environment lies in its contribution to consistent and high-quality output. In the realm of bioprocessing, bioreactors find widespread applications across pharmaceuticals, agriculture, biofuels, and beyond. Their utility extends to the production of therapeutic proteins, vaccines, enzymes, and bio-based materials, with profound implications for healthcare, sustainability, and industry. Furthermore, bioprocessing bioreactors play a crucial role in advancing the field of synthetic biology, enabling researchers to engineer biological systems for specific functionalities. This comprehensive examination underscores the multifaceted impact and versatility of bioreactors in shaping the landscape of bioprocessing and its broader implications.

Keywords: Bioreactors, controlled environment, cultivation, scalability, optimal growth, industry

INTRODUCTION

Biotechnology, a dynamic and multidisciplinary field that seamlessly integrates biology, chemistry, and engineering, has emerged as the vanguard of transformative solutions to a myriad of global challenges. At the heart of this revolution lies the exponential growth and ground breaking advancements in biotechnology, shaping diverse sectors ranging from pharmaceuticals to agriculture. Instrumental to this progress is the remarkable evolution of bioreactor technology, the crucible where living systems and organisms converge to yield unprecedented outcomes [1]. The trajectory of bioreactor technology has been nothing short of revolutionary, catalyzing the development of novel bio products and redefining the production of refining

compounds. The scope of its applications is expansive, encompassing the production of life-saving drugs through recombinant DNA technology, as well as the engineering of crops resilient to environmental stressors and enriched in nutritional value [2]. At the epicenter of this biotechnological metamorphosis, the bioreactor vessel emerges as a pivotal component, orchestrating innovation and serving as the nexus for transformative biological processes. Within the confines of the bioreactor vessel, the crucial dance of molecular interactions unfolds. Achieving optimal performance necessitates a nuanced understanding of fluid dynamics, mixing rates, and mixing times, which collectively contribute to maintaining a homogeneous solution [3].

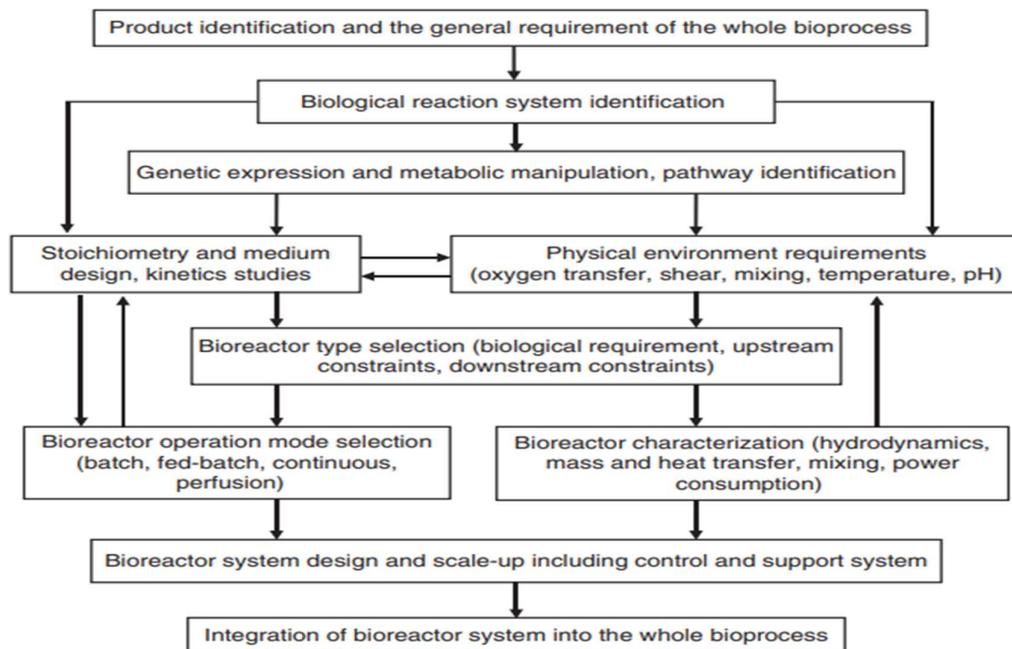


Figure 1: Schematic representation of the process and scope of bioreactor engineering

The meticulous control of environmental parameters within the vessel, such as pH, temperature, dissolved oxygen concentration, and foam dynamics, is paramount to ensuring an ideal milieu for bacterial growth and the production of valuable bioproducts [4]. Unlike their conventional chemical reactor counterparts, bioreactors are designed with a unique mandate – to provide heightened control over process disruptions and fortified defences against contamination by competing microbes [5]. These vessels serve as the lifeblood of biotechnology, fostering the cultivation and growth of a diverse array of biological entities, including cells, microorganisms, and tissues. The applications of bioreactors are far-reaching, encompassing the production of pharmaceuticals, biofuels, enzymes, biopolymers, and an array of other bio products that underscore their indispensability in the modern biotechnological landscape [6].

This review embarks on a comprehensive exploration of the strides made in bioreactor technology, delving into its historical roots, pivotal technological milestones, and its transformative impact across a spectrum of applications. As we navigate the intricate landscape of biotechnological innovation, which aim to unravel the complexities and unveil the pivotal role played by bioreactors in

unlocking the full potential of biological advancements.

Bioreactors play a critical role in supporting the growth and maintenance of stem cells, and their design incorporates several key features. Agitation and mixing, facilitated by stirrers, impellers, and gas spargers, ensure uniform distribution of nutrients and gases essential for cell growth. Temperature control systems, including heating and cooling mechanisms, maintain a stable environment conducive to optimal stem cell proliferation. Automated pH control systems monitor and adjust pH levels, influencing cell metabolism [7]. Dissolved oxygen (DO) control, achieved through DO probes and gas sparging, regulates oxygen availability vital for aerobic cultures. Bioreactors are designed to supply a continuous stream of nutrients necessary for cell growth and metabolism. Modern bioreactors are equipped with monitoring and control systems, utilizing sensors and data logging to track variables such as temperature, pH, DO, and nutrient levels, allowing for real-time adjustments [8].

Fundamental Designing Principles of Bioreactor

In designing and selecting bioreactors, fundamental principles emphasize adequate oxygen transfer, low shear stress, and effective mixing. The consideration of nutrient supply and waste removal is

crucial, along with assessing cell growth kinetics for defining optimal environmental conditions [9]. Transport phenomena, including mixing, shear force, and oxygen transfer, are studied to establish criteria for bioreactor design and scale-up. Operating parameters such as temperature, pH, DO concentration, and substrate concentrations should be easily controllable and monitorable. The bioreactor design aims for simplicity, cost-effectiveness, and freedom from contamination [10]. In the biopharmaceutical industry, compliance with current good manufacturing practice (cGMP) is crucial in bioreactor design and selection. While meeting all requirements may be challenging, a balanced compromise, such as considering the interplay between mixing and mass transfer requirements and the shear sensitivity of cells in large-scale bioreactor systems, becomes essential [11].

Types of bioreactors

In general, biological reaction systems can be broadly categorized into two main groups: suspension systems and immobilization systems. Bioreactors such as stirred tanks, air-lift systems, and bubble column reactors are primarily designed for suspension cultures. On the other hand, membrane reactors, packed bed reactors, and fluidized bed reactors are tailored for cultivating attached cells or supporting immobilized enzymatic reactions. There are

instances where certain bioreactors can be versatile and applied to both suspension and immobilization categories [12]. For instance, by employing suitable carriers, immobilized cells or enzymes on carriers can be suspended in stirred tank bioreactors or air-lift/bubble column bioreactors. While the design and selection of each bioreactor type are unique, some fundamental principles are universally followed. Effective nutrient supply and waste product removal are essential considerations. Assessment of cell growth and product formation kinetics helps define optimal environmental conditions and operational modes. Transport phenomena, encompassing mixing, shear force, and oxygen transfer, are studied to establish criteria for bioreactor design and scale-up [13].

Control and monitoring of operating parameters such as temperature, pH, dissolved oxygen concentration, and substrate concentrations are crucial. Additionally, bioreactors should be designed to be simple, cost-effective, and easily operated while minimizing the risk of contamination with microorganisms [14]. In the biopharmaceutical industry, cGMP compliance is a critical factor in bioreactor design and selection. However, meeting all requirements is often challenging, requiring compromises. For example, finding a balance between mixing

and mass transfer requirements and the shear sensitivity of cells is crucial in the design of large-scale bioreactor systems [15]. The following sections briefly discuss some basic types of bioreactors widely used in industrial fermentation, including those designed for solid-state fermentation and photobioreactors for algal cultures.

1. Stirred-Tank Bioreactors: In the vibrant realm of biotechnology, the pivotal role played by stirred-tank bioreactors has been nothing short of transformative. These vessels, characterized by their dynamic mixing mechanisms, have become the linchpin in the cultivation and production processes that underpin a myriad of biotechnological applications. Stirred-tank bioreactors stand as the stalwart workhorses in the biotechnologist's arsenal, providing a controlled environment for the growth and manipulation of various biological entities. As vessels of innovation, they have enabled the production of diverse bioproducts, ranging from pharmaceuticals to biofuels, through the precise orchestration of environmental conditions and fluid dynamics [16].

The historical journey of stirred-tank bioreactors traces back to their conceptualization and early applications, progressing to the present day where their design and functionality have been refined to meet the demands of modern biotechnological processes. This

manuscript delves into the pivotal technological milestones that have shaped stirred-tank bioreactors into the versatile tools they are today, facilitating advancements in fields as diverse as medicine, agriculture, and environmental sustainability [17]. One of the defining features of stirred-tank bioreactors is their ability to achieve optimal mixing, ensuring a homogeneous distribution of nutrients and fostering an environment conducive to robust biological growth. The review explores the various mixing strategies employed, considering parameters such as fluid flow, mixing rates, and mixing times, which collectively contribute to the efficiency and scalability of biotechnological processes [18].

Stirred-tank bioreactors have emerged as indispensable tools in the biotechnological arsenal, with their efficiency and scalability directly influenced by strategic considerations in fluid dynamics, mixing rates, and mixing times. These parameters collectively shape the performance of stirred-tank bioreactors, impacting the cultivation and production processes across diverse biotechnological applications [19].

Fluid Dynamics: The fluid flow within stirred-tank bioreactors is a critical determinant of mass and heat transfer, nutrient distribution, and overall process efficiency. Various strategies are employed to optimize fluid dynamics, such as the use

of baffles and impellers. Baffles enhance the circulation patterns, preventing the formation of stagnant zones and promoting uniform nutrient distribution. The choice of

impeller design and placement is crucial, influencing the generation of turbulence and ensuring thorough mixing of the culture medium [20].

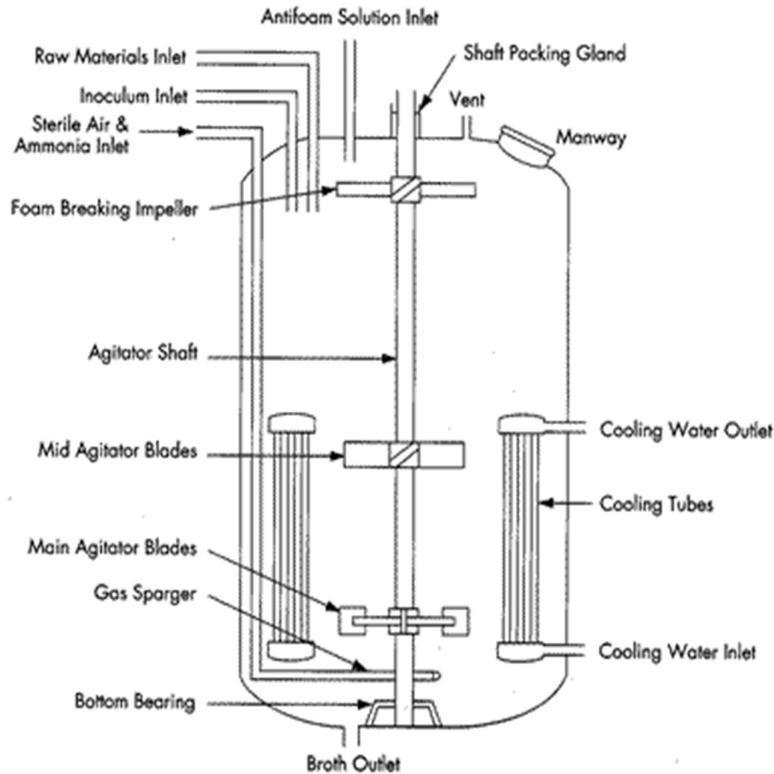


Figure 2: Stirred-tank bioreactors

Mixing Rates: Achieving the optimal mixing rate is paramount for maximizing contact between microorganisms or cells and the growth medium. This involves selecting the appropriate impeller speed and design, considering the viscosity of the culture medium and the specific requirements of the biological entities being cultivated. The right mixing rate prevents the formation of gradients in temperature, nutrient concentration, and dissolved gases, ensuring a homogeneous

environment conducive to robust biological growth [21].

Mixing Times: Mixing times play a pivotal role in determining the duration for which the culture medium is subjected to the optimal mixing conditions. Striking the right balance between rapid mixing for efficient nutrient distribution and extended mixing for uniformity is crucial. The mixing time influences the kinetics of biochemical reactions, the distribution of oxygen, and the removal of metabolic by-

products. Understanding the specific requirements of the bioprocess is essential for tailoring mixing times to achieve desired outcomes [22].

Scale-up Strategies: Scalability is a key consideration in industrial bioprocessing. Strategies for maintaining efficiency during scale-up involve adjusting impeller designs, optimizing agitation strategies, and accounting for changes in fluid dynamics. Computational fluid dynamics (CFD) simulations and empirical studies are often employed to predict and optimize performance at larger scales, ensuring that the benefits observed in small-scale systems are translated successfully to industrial applications.

Adaptive Control Systems: Implementing adaptive control systems allows for real-time monitoring and adjustment of mixing parameters. Sensors for temperature, pH, dissolved oxygen, and other relevant parameters provide feedback to control systems, enabling dynamic adjustments to maintain optimal conditions. This adaptability is crucial for handling variations in microbial growth, changing nutrient demands, and fluctuations in environmental conditions.

Understanding and optimizing these parameters collectively contribute to the efficiency and scalability of stirred-tank bioreactors in biotechnological processes. As these vessels continue to play a pivotal

role in diverse applications, ongoing research and innovations in mixing strategies will further enhance their performance, opening new frontiers in bioprocessing and bioproduct development. Stirred-tank bioreactors offer several advantages, such as precise control over various parameters (temperature, pH, aeration, agitation, etc.), scalability for industrial production, and the ability to maintain consistent and controlled conditions for optimal microbial growth and product formation. However, the design and operation of these bioreactors can vary based on the specific requirements of the bioprocess and the microorganisms being used [23].

2. Airlift Bioreactors:

Airlift bioreactors represent a dynamic and innovative branch of bioprocessing technology, playing a pivotal role in the cultivation of microorganisms, cells, and tissues for diverse applications. The roots of airlift bioreactors can be traced back to the mid-20th century when researchers sought alternatives to traditional bioreactor designs. The airlift concept, characterized by the use of gas-liquid circulation induced by aeration, emerged as a promising solution. Early applications were primarily in wastewater treatment, and it wasn't until the latter half of the century that airlift bioreactors gained traction in broader biotechnological contexts. Airlift

bioreactors distinguish themselves through their unique design, featuring two interconnected zones – a riser and a down comer – where gas-liquid circulation is maintained by the introduction of gas (typically air or oxygen). This design not only provides an efficient means of mass transfer but also eliminates the need for mechanical agitators, reducing shear stress on cells and microorganisms [24].

Airlift Bioreactor Design:

An airlift bioreactor consists of a vertical column or vessel divided into two main zones:

a. Riser Zone: This is the portion where gas and liquid interact to create a buoyant force. Gas is introduced at the bottom of the riser, which causes the liquid to rise along with the gas bubbles. This rising liquid-gas mixture provides the necessary mixing and circulation.

b. Down comer Zone: The down comer is a separate column connected to the riser, and it typically has a larger diameter. In this section, the liquid-gas mixture descends due to gravity, creating a flow of liquid that completes the circulation loop.

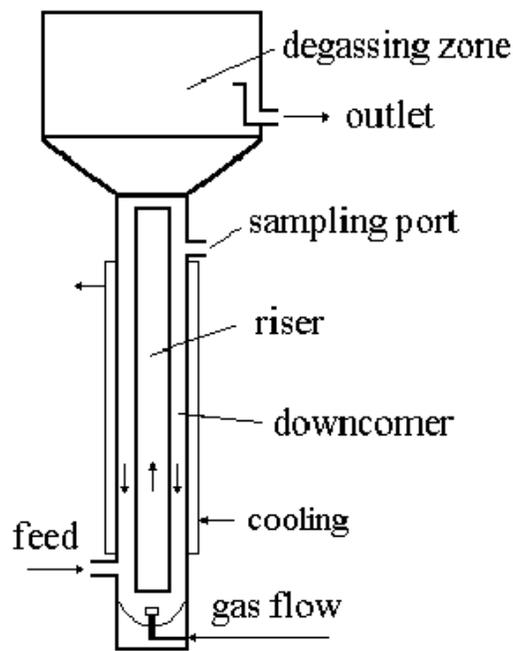


Figure 3: Airlift Bioreactor

Recent Technological Advancements:

Recent advancements in sensor technologies and control systems have heightened the precision with which airlift bioreactors can be managed. Real-time

monitoring of key parameters such as pH, dissolved oxygen, and temperature allows for dynamic adjustments, optimizing conditions for cellular growth and product formation. Scaling up airlift bioreactor

processes has been a focus of recent developments [25]. Computational modeling and simulation tools, such as computational fluid dynamics (CFD), aid in predicting and optimizing performance at larger scales, ensuring seamless transitions from laboratory to industrial production [26].

Integration with Genomic Tools: Airlift bioreactors are increasingly being integrated with genomic tools and synthetic biology approaches. This synergy allows for the cultivation of genetically modified organisms with precision, unlocking new possibilities in the production of bio-based products and therapeutics [27].

Flexible and Modular Designs: Advancements in materials and engineering have led to more flexible and modular designs, facilitating customization based on specific bioprocessing requirements. This adaptability enhances the versatility of airlift bioreactors across a range of applications.

Application in Tissue Engineering: Airlift bioreactors have made significant strides in the field of tissue engineering. Their gentle fluid dynamics and efficient mass transfer properties make them conducive to the cultivation of three-dimensional cell cultures for tissue regeneration and organoid production. While airlift bioreactors have specific advantages, their design and operation also require careful

consideration of parameters such as gas flow rates, liquid circulation rates, and nutrient availability to ensure optimal growth conditions and product yields [28].

3. Membrane Bioreactor

Utilizing specialized membranes to retain cells within reactors, membrane bioreactors aim to achieve in situ separation of cells from the medium, effectively integrating production and separation in a single step. The key benefits of membrane bioreactors include high cell density, elevated volumetric productivity, and minimal shear stress [29]. However, challenges such as poor cell viability, process instability (due to issues like membrane fouling and clogging), product inhomogeneity, and diffusion gradients pose limitations on large-scale applications. Despite these drawbacks, various membrane configurations, including flat sheet and rotating bioreactors, have been explored. Among these, the hollow-fiber configuration stands out as particularly intriguing. Membranes are constructed from diverse materials, including cellulose, acetate, nitrate, polyvinylidene difluoride, polysulfone, polypropylene, polytetrafluoroethylene (PTFE), ceramic, silicone rubber, and ion exchange membranes. Microfiltration and ultrafiltration membranes, with varying pore sizes, are commonly used [30].

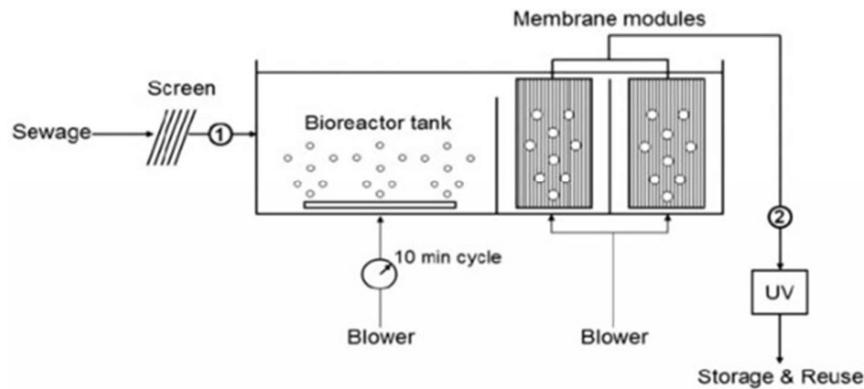


Figure 4: Membrane Bioreactor

Recent Advances in Membrane Bioreactors:

MBRs find extensive applications in biocatalysis, fermentation, cell cultures, and wastewater/waste gas treatments. In biocatalysis, enzymatic membrane reactors (EMRs) integrate membrane separation with enzymatic reactions, offering advantages such as gentle enzyme entrapment, improved stability, and enhanced productivity. MBRs have been employed in wastewater treatment for the removal of phenol and catechol [31].

High Cell Density in Microbial Fermentation: Membrane reactors facilitate high cell density in microbial fermentation, leading to increased productivity. Examples include the rapid fermentation of lactic acid and the production of superoxide dismutase by

Streptococcus lactis, both showing significant improvements over conventional methods [32].

Animal Cell Cultures: In animal cell cultures, MBRs offer benefits such as high cell density and productivity, along with the elimination of shear stress issues encountered in stirred-tank reactors. However, challenges exist in large-scale applications, leading to limitations in their use primarily to small-to-medium scales [33].

Wastewater Treatment and Waste-Gas Treatment: MBRs enable the complete retention of sludge, allowing operation at higher biomass concentrations. This leads to reduced excess sludge production, as demonstrated in the aerobic treatment of municipal wastewater. Membrane fouling, a common challenge, can be mitigated by

appropriate backwashing and air scouring. Consequently, MBRs have found widespread use in large-scale wastewater treatment plants. The technology also holds promise in waste-gas treatment [34].

4. Fluidized Bed Bioreactors:

Fluidized bed bioreactors (FBBs) represent a cutting-edge paradigm in bioprocessing technology, offering unique advantages in terms of mass transfer, mixing efficiency, and scalability. Most of the FBBs developed for biological systems involving cells as biocatalysts are three phase systems. Fluidized bed bioreactors are characterized by the suspension of solid particles (usually support media) in a liquid medium, creating a "fluidized bed" of particles. This design allows for efficient mixing, mass transfer, and enhanced contact between microorganisms and nutrients. These bioreactors involve a bed of solid particles suspended by the upward flow of liquid, creating an environment that facilitates cell attachment and growth [35].

The concept of fluidization, where solid particles are suspended and behave like a fluid when subjected to a gas or liquid flow was first recognized in the early 20th century. Pioneering work in fluidized bed technology began in the 1930s and 1940s, primarily in the field of chemical engineering and catalysis. The application of fluidized beds to bioprocessing emerged in the mid-20th century. Researchers

started investigating the potential of fluidized beds for cultivating microorganisms, particularly in the context of wastewater treatment. The high surface area and efficient mass transfer properties of fluidized beds were recognized as advantageous for promoting microbial activity [34].

In the 1960s and 1970s, there was a notable shift toward utilizing fluidized bed technology for wastewater treatment. Researchers explored the use of fluidized beds to support microbial biofilms that could efficiently degrade organic pollutants. This marked the initial foray of fluidized bed bioreactors into environmental biotechnology. As the understanding of fluidized bed bioreactors grew, the 1980s witnessed an expansion of their applications into bioenergy production. FBBs became instrumental in anaerobic digestion processes, fostering the generation of biogas through the microbial degradation of organic matter. In the late 20th century and early 21st century, advancements in immobilization techniques played a pivotal role in enhancing the effectiveness of fluidized bed bioreactors. Improved methods for attaching microorganisms to fluidized particles were developed, addressing challenges related to stability and maintaining biofilm integrity [36].

The 21st century has seen a diversification of fluidized bed bioreactor applications. Beyond wastewater treatment and bioenergy production, FBBs have been employed in pharmaceutical and fine chemical manufacturing, demonstrating their versatility and adaptability to various bioprocessing needs. In recent years, there has been a trend toward integrating fluidized bed bioreactors with genetic engineering tools. This integration allows for the cultivation of genetically modified microorganisms tailored for specific bioprocesses, including the production of therapeutic proteins and specialty chemicals. On-going research in materials science has led to the development of smart materials and coatings for fluidized particles. These innovations aim to improve microbial attachment, stability, and overall reactor performance. The history of fluidized bed bioreactors reflects a journey of continual exploration, innovation, and diversification. From its origins in fluidized bed technology, FBBs have evolved into versatile bioprocessing tools with applications spanning environmental remediation, bioenergy, and pharmaceutical manufacturing. The ongoing research and technological advancements in this field promise further refinements and expanded applications in the years to come [37].

Fluidized bed bioreactors are particularly advantageous for processes involving immobilized cells or solid-phase reactions. They are commonly used in the production of enzymes and certain plant cells. They have been most successful in large-scale processes that use stable cell lines, including wastewater treatment and bulk anaerobic fermentations (ethanol, lactic acid, etc.). Among aerobic processes they are used for tissue culture where the fragility of the cells makes the low shear a definite advantage, and the low metabolic rate makes slow oxygen diffusion less of a limitation. They are also used for aerobic wastewater treatment where the solubility of oxygen, although low, is only one order of magnitude less than the concentration of organic matter, not the two or three typical of aerobic fermentations designed to maximize production of metabolites [38].

Fluidized Bed Bioreactor Design:

Fluidized bed bioreactors consist of a column or vessel containing solid particles as a support matrix for microbial attachment and growth. The fluid flow introduced from the bottom of the column causes the solid particles to become suspended and form a fluidized bed. This bed of particles behaves like a fluid, providing continuous mixing and excellent mass transfer [39].

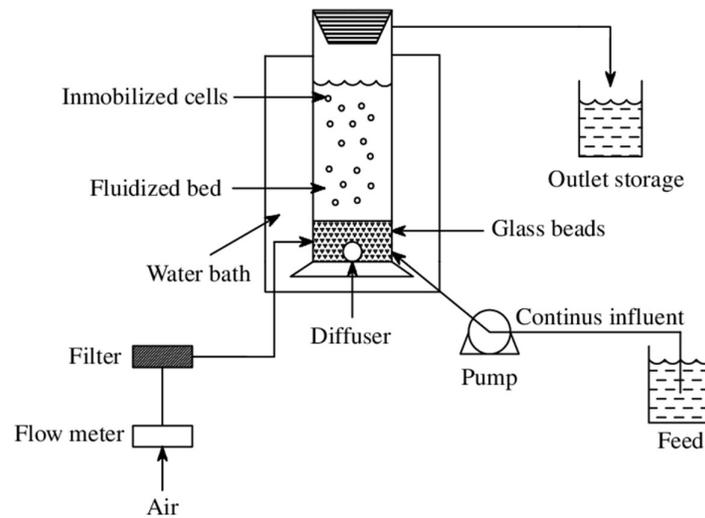


Figure 5: Fluidized bed bioreactor

Recent Advances in Fluidized Bed Bioreactors:

Fluidized bed bioreactors (FBBs) have witnessed a surge in research and development, with recent advancements focusing on refining design principles, enhancing operational efficiency, and expanding applications across various bioprocessing domains [40]. Here, we delve into some of the noteworthy strides in fluidized bed bioreactor technology.

1. Immobilization Techniques:

Advancements in immobilization techniques have improved the attachment of microorganisms to fluidized particles, enhancing stability and facilitating the continuous operation of FBBs.

2. Online Monitoring and Control Systems:

Integration with advanced monitoring and control systems allows real-time adjustment of key parameters,

ensuring optimal conditions for microbial growth and bioprocess efficiency.

3. Genetic Engineering Integration:

FBBs are increasingly integrated with genetic engineering tools, enabling the cultivation of genetically modified microorganisms tailored for specific bioprocesses, such as the production of therapeutic proteins or specialty chemicals.

4. Smart Materials and Coating Technologies:

Innovations in materials science have led to the development of smart materials and coatings for fluidized particles, providing improved support for microbial attachment, stability, and ease of handling.

Despite the notable advantages, challenges in terms of particle attrition, reactor design optimization, and control of microbial biofilms persist. Addressing these

challenges will be critical for further expanding the applications of FBBs and realizing their full potential in bioprocessing. It's important to note that the design and operation of fluidized bed bioreactors require careful consideration of factors such as particle size, flow rates, and the choice of support material to create and maintain an optimal fluidized bed environment for microbial growth and activity [41].

5. Packed Bed Bioreactors:

Packed bed bioreactors (PBBs) stand as stalwart contributors to the realm of bioprocessing, embodying a versatile platform for the cultivation of microorganisms and the production of bio-based compounds. This comprehensive review traces the historical roots, explores advancements, and delves into present-day applications, unraveling the multifaceted contributions of Packed Bed Bioreactors to the landscape of biotechnology. The inception of packed bed bioreactors finds its roots in the mid-20th century, a period marked by an increased focus on developing alternative bioprocessing technologies [42]. Early experiments explored the concept of immobilizing microorganisms on solid supports, and the idea of creating a three-dimensional matrix for microbial attachment gained prominence. As the understanding of biofilm dynamics and mass transfer

processes deepened, packed bed bioreactors emerged as a viable and efficient platform for various biotechnological applications [43].

Packed bed bioreactors contain a fixed bed of solid support materials, such as beads or particles, on which microorganisms or enzymes are immobilized. The design of packed bed bioreactors allows for efficient contact between the immobilized biocatalysts and the liquid flowing through the bed. It must include mass transfer in the bed as well as heat transfer and chemical reactions. Heat transfer, packed catalyst, and pressure drop are essential characteristics for lowering machine operating costs. They are used in wastewater treatment and some bioprocesses [42]. They exhibit great potential in tissue engineering applications, yet their full utilization remains untapped. These bioreactors can effectively sustain diverse cell lines over extended culture durations, owing to the immobilization of cells within macroporous matrices, thus operating under conditions of minimal shear.

Packed Bed Bioreactor Design: Packed bed bioreactors consist of a column or vessel filled with solid support materials that provide a surface for microbial attachment or enzyme immobilization. The liquid containing the substrate or nutrients flows through the packed bed, allowing for

interactions between the immobilized biocatalysts and the target molecules. Among the process factors that must be optimised during process development are carrier selection, medium selection, proper

flow velocity, and long-term performance. All these information are required to evaluate the overall performance, e.g., productivity, and to layout the scale-up strategy [42].

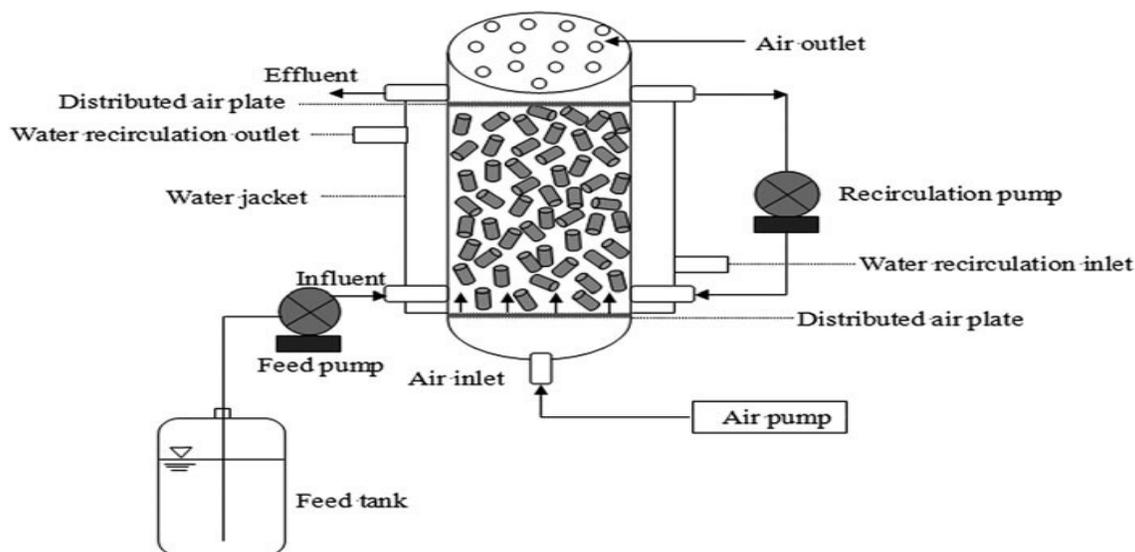


Figure 6: Packed Bed Bioreactor

Recent Advances in Packed Bed Bioreactor:

Recent advancements in materials science have ushered in a new era for PBBs, featuring innovative support materials. These materials, including synthetic polymers and modified natural substances, enhance the stability, durability, and versatility of Packed Bed Bioreactors [42].

Microbial Consortia Engineering:

Contemporary research focuses on engineering microbial consortia within PBBs to achieve tailored and optimized bioprocessing outcomes. Manipulating the composition of biofilms allows for the creation of intricate microbial communities

with enhanced metabolic pathways and increased bioprocess efficiency.

In situ Monitoring and Control:

Integration with sophisticated in situ monitoring and control systems represents a significant leap forward. Real-time data acquisition for parameters such as pH, dissolved oxygen, and temperature enables precise control over the bioprocess conditions, optimizing microbial growth and productivity.

The success of packed bed bioreactors depends on factors such as the choice of solid support material, appropriate immobilization techniques, and optimal flow rates to ensure efficient contact between the immobilized biocatalysts and

the liquid phase. Proper design and operation are essential to achieve the desired outcomes in biotechnological processes using packed bed bioreactors. The trajectory of packed bed bioreactors points toward a future marked by continual innovation. Ongoing efforts in smart materials, automation, and the integration of PBBs with emerging technologies such as synthetic biology hold promise for expanding the applications and impact of Packed Bed Bioreactors in the ever-evolving landscape of bioprocessing. As a cornerstone in biotechnological advancements, PBBs are poised to shape the future of sustainable and efficient bio manufacturing processes [44].

6. Perfusion Bioreactors:

Perfusion bioreactors are specialized types of bioreactors used in biotechnology for continuous cell culture and production of valuable bioproducts. Unlike traditional batch or fed-batch bioreactors where cells are cultured for a specific period and then harvested, perfusion bioreactors allow for continuous growth and maintenance of cells in a controlled environment [45]. In these systems, fresh media is continuously added to the bioreactor, while waste products are simultaneously removed. This enables longer cell cultivation periods and higher cell densities. This continuous process offers several advantages for certain applications, particularly those

involving sensitive or high-value cell cultures [46]. Here's an overview of perfusion bioreactors and their applications:

Perfusion Bioreactor Design:

Perfusion bioreactors are one of the upcoming reactor technologies based on continuous bioprocessing that offers the ease of continuous culturing of cells without nuisance of filter clogging or low throughput. In addition, there are less possibilities of waste accumulation and, hence, minimized chances of any product inhibition, especially while dealing with proteins prone to instability. Perfusion bioreactors are designed to maintain a constant flow of fresh nutrient-rich media into the bioreactor while simultaneously removing spent media and waste products. This creates a steady-state environment where cells are constantly exposed to optimal conditions for growth and productivity [47]. There are two main types of perfusion bioreactors:

- i. **Tangential Flow Filtration (TFF):** In TFF perfusion bioreactors, cells are retained within the bioreactor while the media is circulated through a filtration system that removes waste products and replenishes nutrients.
- ii. **Continuous Media Exchange:** In this approach, media is continuously added to the bioreactor at a controlled rate while spent

media is simultaneously removed, nutrients.
creating a continuous flow of fresh

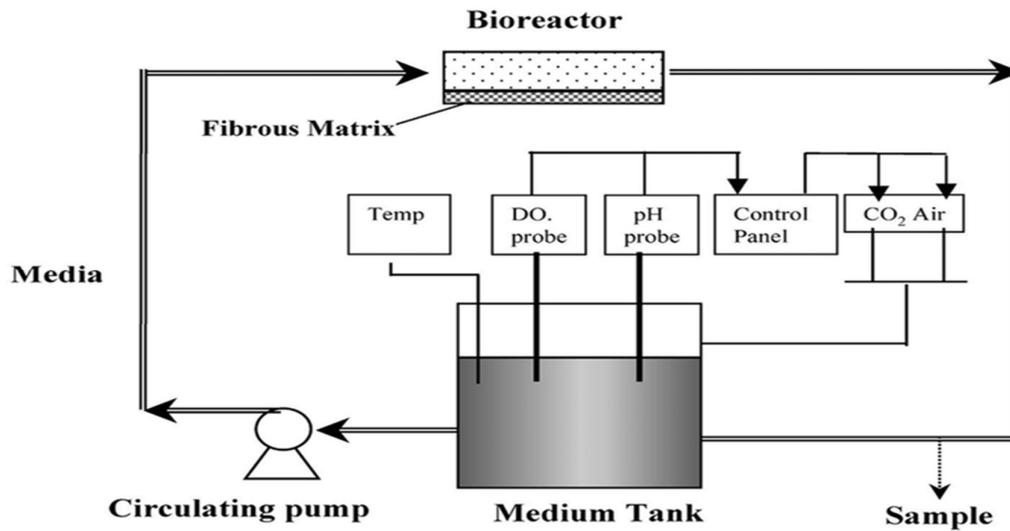


Figure 7: Perfusion Bioreactor

Recent Technological Advancements:

Advancements in microfluidics technology are being integrated into perfusion bioreactors, allowing for precise control over fluid dynamics, nutrient gradients, and cell microenvironments.

Online Monitoring and Control Systems:

Perfusion systems are incorporating advanced monitoring and control systems, enabling real-time analysis of key parameters such as cell density, viability, and metabolite concentrations. This ensures dynamic adjustments to optimize the bioprocess.

Single-Use Systems: The adoption of single-use technologies in perfusion bioreactors reduces the risk of contamination, enhances flexibility, and simplifies the overall bioprocessing workflow.

Challenges in terms of system complexity, scalability, and cost-effectiveness remain areas of active research. The future of perfusion bioreactors holds promise for addressing these challenges through continued technological innovation, process optimization, and broader industry adoption [47]. Proper design, operation, and management are critical to achieving successful and efficient cell culture in perfusion bioreactors.

7. Stem Cell Bioreactors:

Stem cell bioreactors stand as pivotal tools in the realm of regenerative medicine, offering controlled environments for the expansion, differentiation, and cultivation of stem cells. These sophisticated systems play a crucial role in unlocking the therapeutic potential of stem cells for applications ranging from tissue

engineering to cell-based therapies. Stem cell bioreactors have revolutionized the field of regenerative medicine and tissue engineering by providing a more controlled and reproducible environment for cultivating stem cells. They have enabled advancements in understanding stem cell biology, optimizing differentiation protocols, and generating functional tissues for transplantation [48]. The development of stem cell bioreactors represents a significant milestone in the field of regenerative medicine, offering a transformative approach to the cultivation and manipulation of stem cells for therapeutic applications. This historical review traces the evolution of stem cell bioreactors, shedding light on key milestones, technological advancements, and the pivotal role they have played in

shaping the landscape of regenerative medicine [49].

The conceptual foundations of stem cell bioreactors were laid in the mid-20th century, coinciding with the recognition of the regenerative potential of stem cells. Early experiments involved static cultures, where stem cells were cultivated in simple laboratory vessels. These foundational studies paved the way for the exploration of more sophisticated bioprocessing methods. The 1970s and 1980s witnessed a notable shift with the introduction of stirred-tank bioreactors for stem cell culture. This marked a departure from static cultures, offering a more controlled and scalable environment. The incorporation of mechanical agitation and continuous media exchange laid the groundwork for improved nutrient supply and waste removal, enhancing cell proliferation [50].

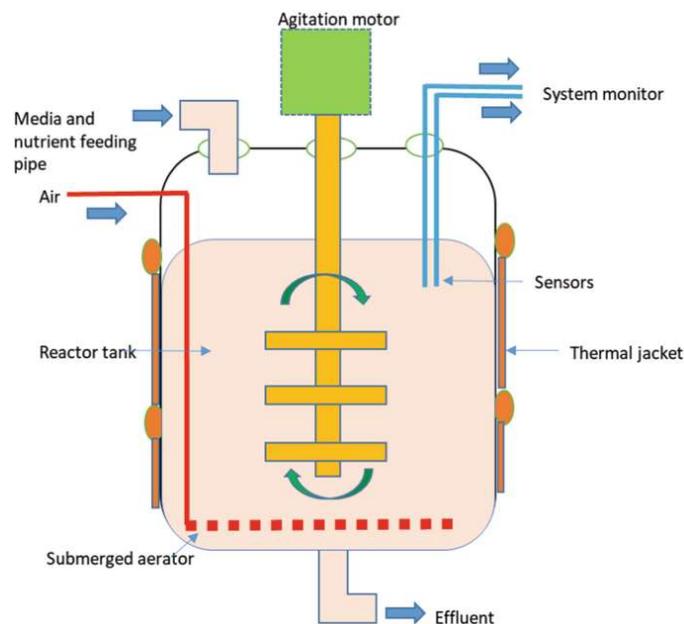


Figure 8: Stem cell bioreactor

The integration of microcarrier technology in the 1980s represented a breakthrough, enabling the cultivation of stem cells in suspension cultures. Microcarriers, often coated with biocompatible materials, provided a larger surface area for cell attachment and supported the expansion of stem cell populations. This innovation facilitated scale-up efforts for clinical applications. The 1990s and early 2000s witnessed a paradigm shift with the adoption of perfusion bioreactors. These systems introduced continuous media exchange, enabling sustained nutrient supply while removing waste products. The continuous nature of perfusion bioreactors enhanced cell viability, prolonged culture duration, and laid the groundwork for large-scale stem cell expansion. In the 21st century, a focus on mimicking the *in vivo* microenvironment led to the integration of 3D culture systems and scaffold technologies within stem cell bioreactors [50]. These advancements aimed to replicate the physiological conditions for stem cell growth and differentiation more accurately. 3D bioprinting technologies further expanded the possibilities of creating complex tissue structures within bioreactors. The last decade has seen the integration of microfluidic systems and organ-on-a-chip platforms into stem cell bioreactors. These technologies provide precise control over microenvironments,

allowing researchers to create highly specialized and biomimetic conditions for stem cell culture. The marriage of stem cell biology with microengineering offers unprecedented insights into cell behaviour and tissue development.

Challenges in stem cell bioreactor technology include optimizing differentiation protocols, achieving standardized processes, and addressing the complexities of heterogeneous stem cell populations. Future perspectives involve refining culture conditions, incorporating advanced monitoring techniques, and expanding the range of stem cell applications in personalized medicine. The evolution of stem cell bioreactors mirrors the dynamic progress in regenerative medicine, from rudimentary static cultures to sophisticated perfusion and 3D culture systems. As technology continues to advance, stem cell bioreactors are poised to unlock new possibilities, bringing the promise of regenerative medicine closer to widespread clinical applications. This historical journey underscores the interdisciplinary efforts that have propelled stem cell bioreactors from conceptualization to their current pivotal role in shaping the future of healthcare [51].

Additionally, bioreactor systems have potential applications in drug testing, disease modelling, and personalized

medicine. The rapidly evolving domain of stem cell bioreactor cultivation holds promise for shaping future systems capable of producing clinical or pharmacological-grade cellular products derived from stem cells several joint research programmes between industry and universities are focusing on the development of stem cell bioreactor systems. Applikon Biotechnology has participated in several of these projects and has developed a number of successful designs.

Stem Cell Bioreactors Design & features:

Stem cell bioreactors play a pivotal role in the controlled cultivation and expansion of stem cells for various biomedical applications, including regenerative medicine and cell-based therapies. The design of an effective stem cell bioreactor involves several key features to ensure optimal cell growth and functionality. Firstly, bioreactors typically have a controlled environment with precise regulation of temperature, pH, and oxygen levels to mimic the physiological conditions necessary for stem cell proliferation. Additionally, these systems often incorporate advanced monitoring and feedback mechanisms to continuously assess and adjust the culture parameters in real-time, ensuring a stable and conducive environment for cell growth. Bioreactors may also feature scalable configurations to accommodate varying cell quantities,

facilitating the production of large quantities of stem cells for clinical applications. Furthermore, the incorporation of specialized scaffolds and three-dimensional culture systems within bioreactors helps mimic the in vivo microenvironment, promoting differentiation and tissue-specific functionality of stem cells. The design of stem cell bioreactors is an interdisciplinary endeavor, combining principles of engineering, biology, and materials science to create efficient and reproducible platforms for the scalable production of high-quality stem cell populations. The design of a stem cell bioreactor can vary significantly based on the specific requirements of the stem cell type being cultured, the intended application, and the available technology. Collaboration between biologists, engineers, and bioprocess experts is essential to develop an effective and efficient stem cell bioreactor system [52].

Recent Advances in Stem cell bioreactors

Stem cell bioreactors have undergone remarkable advancements in recent years, contributing significantly to the field of regenerative medicine. These innovations aim to improve scalability, reproducibility, and the ability to precisely control the microenvironment of stem cells. Here, we explore the cutting-edge developments that

are reshaping the landscape of stem cell bioreactors.

Integration of Microfluidics and Organ-on-a-Chip Technologies: Recent years have seen the integration of microfluidics and organ-on-a-chip technologies into stem cell bioreactors. Microfluidic systems offer precise control over fluid flow, nutrient gradients, and oxygen levels, creating microenvironments that closely mimic physiological conditions. This integration enhances the ability to study complex interactions within tissues and organs, providing more accurate in vitro models for drug testing and disease modeling.

3D Bioprinting for Complex Tissue Engineering: 3D bioprinting has emerged as a game-changer in stem cell bioreactors, allowing for the precise deposition of stem cells and biomaterials to create complex tissue structures. This technology enables the engineering of tissues with intricate architectures, closely resembling native tissues. The ability to print multiple cell types and extracellular matrix components within a controlled environment holds great promise for tissue regeneration and transplantation.

Smart Materials and Scaffolds: Advancements in biomaterials have led to the development of smart materials and scaffolds within stem cell bioreactors. These materials can dynamically respond to environmental cues, providing mechanical

support and biochemical signals that influence stem cell behavior. Engineered scaffolds play a crucial role in creating biomimetic microenvironments, guiding stem cell differentiation and tissue development.

Artificial Intelligence and Data Analytics: The incorporation of artificial intelligence (AI) and data analytics has revolutionized stem cell bioreactors by enabling real-time monitoring and control. AI algorithms analyze vast datasets generated during stem cell culture, allowing for predictive modeling and optimization of culture conditions. This data-driven approach enhances reproducibility and accelerates the development of robust protocols for stem cell expansion and differentiation.

Single-Use Bioreactor Systems: The adoption of single-use bioreactor systems is gaining prominence in stem cell bioprocessing. These systems eliminate the need for traditional stainless-steel bioreactors, reducing the risk of contamination and enabling more flexible and cost-effective manufacturing processes. Single-use bioreactors are particularly advantageous for clinical-scale production of stem cells for cell therapies.

CRISPR-Based Genome Editing in Bioreactor Environments: The integration of CRISPR-based genome editing tools into stem cell bioreactors allows for the precise

modification of stem cell genomes during culture. This technology enables the creation of genetically modified stem cell lines tailored for specific therapeutic applications. The ability to perform genome editing within the bioreactor environment streamlines the process and enhances efficiency [52].

Closed-Loop Systems for Automated Control: Closed-loop systems with automated control mechanisms are becoming integral to stem cell bioreactors. These systems continuously monitor critical parameters such as pH, temperature, and oxygen levels, making real-time adjustments to optimize culture conditions. Automated control enhances the reproducibility of stem cell cultures and minimizes the need for manual intervention.

Expansion of Personalized Medicine Applications: Recent advancements in stem cell bioreactors are facilitating the expansion of personalized medicine applications. Patient-specific stem cells can be cultured and differentiated within bioreactors to generate cells for autologous transplantation, reducing the risk of immune rejection and broadening the scope of individualized regenerative therapies. These recent advancements collectively underscore the rapid evolution of stem cell bioreactors, positioning them as instrumental tools for advancing

regenerative medicine and translating stem cell therapies from the laboratory to clinical practice. As research continues to unfold, the synergistic integration of these technologies holds great promise for unlocking the full therapeutic potential of stem cells.

Trends in Bioreactor Engineering

Bioreactor engineering science has undergone significant advancements, and recent years have seen a surge in interest in microbioreactors. This heightened interest is fueled by remarkable progress in functional genomics, metabolic engineering, and systems biology. These developments open up immense potential for leveraging a single cell as a super bioreactor, capable of intricate and highly controlled bio-manufacturing processes. The integration of cutting-edge technologies allows researchers to manipulate cellular functions at a molecular level, optimizing production pathways and enhancing overall bioprocess efficiency [53].

An exciting trend is the exploration of plants and animals as integrated bioreactor systems. This approach involves harnessing the inherent biological capabilities of living organisms to produce valuable compounds. Plants and animals can be genetically modified or engineered to act as living factories, expressing and secreting specific proteins, enzymes, or pharmaceuticals. This

integrated bioreactor concept offers a novel and sustainable approach to bioproduction. The synergy between bioreactor engineering and advancements in genomics and systems biology has paved the way for a deeper understanding of cellular processes [54]. This knowledge is crucial in designing and optimizing bioreactors to meet the demands of various industries, including pharmaceuticals, biotechnology, and bio-manufacturing. As a result, the field is moving toward more precise and tailored approaches, with the potential to revolutionize the production of bio-based products and therapeutic agents. The ongoing progress in bioreactor engineering holds promise for continued innovation, pushing the boundaries of what is achievable in the realm of biological manufacturing [55].

1. Microbioreactor

Low-cost microbioreactors or miniature bioreactors have been specifically engineered for high-throughput bioprocessing applications, spanning media development, strain enhancement, and process optimization. An optical sensing system was employed for continuous monitoring of pH, dissolved oxygen (DO), and optical density (OD) in a microbioreactor featuring a 2-ml working volume. In *E. coli* fermentation experiments, this microbioreactor exhibited pH, DO, and OD profiles analogous to

those observed in a conventional 1-liter bioreactor [56]. This research lays the foundation for the development of a multiple-bioreactor system tailored for high-throughput bioprocess optimization. This includes the integration of a low-cost non-invasive optical CO₂ sensing system for fermentation and cell culture, a 24-well plate miniature bioreactor for microbial cultivation assessments, and its utility as a scale-down model for cell culture process development. A gas-permeable PDMS membrane facilitated aeration, while integrated sensors allowed online measurement of OD, DO, and pH using optical methods. Bacterial fermentations conducted in the microbioreactor under well-defined conditions demonstrated comparable outcomes to those observed in a 500-ml bench-scale bioreactor [57].

The microbioreactor system exhibited sensitivity and reproducibility, enabling the detection of statistically significant differences in the time evolution of OD, DO, and pH, distinguishing between different physiological states. Additionally, two types of PDMS microbioreactors were developed to enhance primary adult rat hepatocyte cultures, featuring membranes as scaffolds for cell attachment. These membrane-based PDMS microbioreactors, closely mimicking in vivo liver architecture, showcased promise for applications in drug screening and liver

tissue engineering. In the pursuit of a microbio reactor device for animal cell culture processing, a microfluidic array for continuous perfusion culture was recently designed. The compact device, measuring 2 X 2 cm, comprised a circular microfluidic chamber, narrow perfusion channels surrounding the main chamber, and four ports for fluidic access. Successful continuous perfusion culture of human carcinoma (HeLa) cells was verified over 16 days, demonstrating the device's functionality for repeated cell growth/passage cycles, reagent introduction, and real-time optical analysis [56].

2. Cell as a Super Bioreactor

Metabolic engineering, employing rational design and mathematical tools, is crucial for creating novel cell factories. Lactic acid bacteria, modified through this approach, produce essential compounds like diacetyl and alanine (Lee *et al.*, 2021). Functional genomics, aided by complete genomic sequences, focuses on assigning functions to open reading frames, providing a macroscopic view of cell function. Systems biology aims to quantitatively describe cellular interactions, facilitating studies in microbial physiology and metabolic engineering [57].

In plant cell factories, engineering for secondary metabolite production is vital due to the diverse applications of these

compounds. Overcoming challenges in synthesizing pharmaceutical metabolites involves strategies such as addressing rate-limiting steps and minimizing catabolism. Advances in plant genomics and metabolite profiling offer opportunities to explore plant biochemical capacity. State-of-the-art genomics tools enable enhancing known metabolite production or synthesizing novel compounds in cultivated plant cells. While commercially produced recombinant proteins from plant cell cultures are not widespread, ongoing investigations suggest their potential feasibility. Overall, the integration of metabolic engineering, functional genomics, and systems biology holds promise for optimizing valuable compound production in both microbial and plant cell factories [58].

3. Protein-Producing Bioreactors

The biopharmaceutical industry is exploring new protein expression systems due to limited bioreactor capacity. Whole plants are gaining attention as natural bioreactors for recombinant protein production, offering economic, scalable, and safe advantages. Effective transgene expression in plant cells relies on optimized expression systems using promoters. A strategy involving chloroplast transformation, GUS fusions, and affinity-tag-based chromatography successfully overexpressed and purified human interferon gamma (IFN- γ) in tobacco

plants, achieving promising results with high yields [58].

Transgenic animals, particularly in milk and potentially egg white, are emerging as industrial bioreactors for pharmaceuticals. Transgenic cattle produced gram-per-liter concentrations of recombinant human lactoferrin in bovine milk. Similarly, transgenic goats expressed recombinant human α -fetoprotein (rhAFP) at an industrial scale in their milk [59]. Transgenic rabbits produced recombinant human erythropoietin (rhEPO) in their mammary glands, demonstrating viability, fertility, and successful gene transmission to offspring. These advancements pave the way for further studies on glycosylation and function, contributing to transgenic methods in the biopharmaceutical industry [60].

CONCLUSION

Bioreactors play a crucial role in modern biotechnology, serving as controlled environments for the cultivation and manipulation of various biological entities, such as microorganisms, cells, and enzymes. These devices enable researchers and industrialists to efficiently produce a wide range of products, from biofuels and pharmaceuticals to food additives and biomaterials. Bioreactors provide precise control over environmental parameters such as temperature, pH, dissolved oxygen, and nutrient availability. This control allows for

optimization of growth conditions, enhancing the yield and productivity of the desired biological processes. Bioreactors are used both in laboratory-scale research and industrial-scale production. Scaling up involves maintaining process parameters while increasing volume, while scaling down refers to reproducing laboratory conditions at a smaller scale. Achieving consistency and reproducibility across scales is a significant challenge. Various types of bioreactors exist, including stirred-tank bioreactors, airlift bioreactors, packed bed bioreactors, and immobilized cell bioreactors. Each type offers distinct advantages and disadvantages, depending on the specific process requirements. Bioreactors are integral to cell culture and tissue engineering applications. They provide the necessary environment for the growth and differentiation of cells, which is essential for the production of therapeutic proteins, vaccines, and even organs for transplantation. Advanced bioreactors are equipped with sensors and monitoring systems that continuously gather data on various parameters. This data is used to adjust process conditions in real-time, optimizing productivity and ensuring product quality. Despite their advantages, bioreactors also face challenges. Maintaining sterility, preventing contamination, and managing shear stress on cells are critical concerns. Additionally,

designing and operating bioreactors for novel processes or sensitive organisms can be complex and costly. The field of bioreactors is continually evolving. Advances in automation, artificial intelligence, and synthetic biology are likely to lead to more efficient and adaptive bioreactor systems. Additionally, efforts to incorporate renewable feedstocks and improve waste utilization will contribute to the sustainability of bioprocesses. Continued research and innovation in bioreactor design, operation, and integration with other technologies will further drive the advancement of bioprocessing and its contributions to various industries.

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