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Review

Bioreactors: Applications and Innovations for a Sustainable and Healthy Future—A Critical Review

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Abstract: Biotechnological processes are essential for developing economies that aim to stand out in future markets. The use of bioreactors is one of the most important unit operations of biotechnological processes, and real-time monitoring of bioreactors is essential to ensure precise bioprocess control. This review presents different types of bioreactors, sensors, and applications in other sectors. Bioreactors, controlled systems for cultivating microorganisms and cells, are essential tools in various fields, from scientific research to industrial production. The use of a variety of sensors is critical for accurate, real-time monitoring, early problem detection, reproducibility, cost reduction, and increased efficiency. These benefits are being realized in numerous applications, including biofuel production, bioremediation and leaching processes, tissue engineering, and drug manufacturing. Innovations in bioreactor technology are expanding opportunities for a more sustainable and healthier future. By developing new types of bioreactors, integrating advanced sensors, and exploring promising applications, bioreactors are playing a key role in addressing global challenges and sustainably advancing science and technology.

Keywords: bioreactors; innovations; sensors; applications

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1. Introduction

The first bioreactors in history were probably ceramic vessels that ancient people, such as Egyptians, Mesopotamians, Romans, and Greeks, used to ferment foods such as bread, wine, beer, and mead. At that time, there was no idea about the microbial world and fermentation process technology. In the 19th century, with the discovery of microorganisms by Louis Pasteur, attention turned to experimentation. He produced microbial butanol with a mixed bacterial culture that included at least one strain of *Clostridium*. After, in the 20th century, during the First World War, Chaim Weizmann also used a strain of *Clostridium* to produce acetone for use in the production of artillery projectiles at the time.

Later, with the discovery of penicillin by Alexander Fleming and the need for global production of these antibiotics, bioreactors began to be designed for larger scales. In 1945, industrial bioreactors could produce 7 trillion units of penicillin. In the second half of the 20th century, bioreactor technology was revolutionized, and improvements and innovations in the process only grew, including sterilization methods, agitation and

aeration systems, multivessel systems to produce multiple products in parallel, and the manufacture of equipment with increasingly larger volumetric capacity. Starting in the 1980s, the world of bioreactors moved beyond microorganisms to cultivating animal and plant cells. Due to this, new types and configurations of bioreactors were built to meet the interests of the industry [1]. In the 21st century, technologies such as automation, artificial intelligence, and 3D printing also became part of new bioreactors, making them automated, versatile, and highly efficient equipment. In addition to these technologies, different bioreactor projects and designs have been developed over the years to ensure the safe and cost-effective manufacturing of biotechnology products. Bioreactors are the cornerstone of the bioprocessing industry, although each bioreactor design has its advantages and disadvantages. Between 2003 and 2023, studies on bioreactor technology and the design of these devices increased by approximately 1200% (Figure 1) in an effort to develop versatile and sophisticated equipment that meets the needs of the current market in the various industrial sectors in which fermentation processes are used.

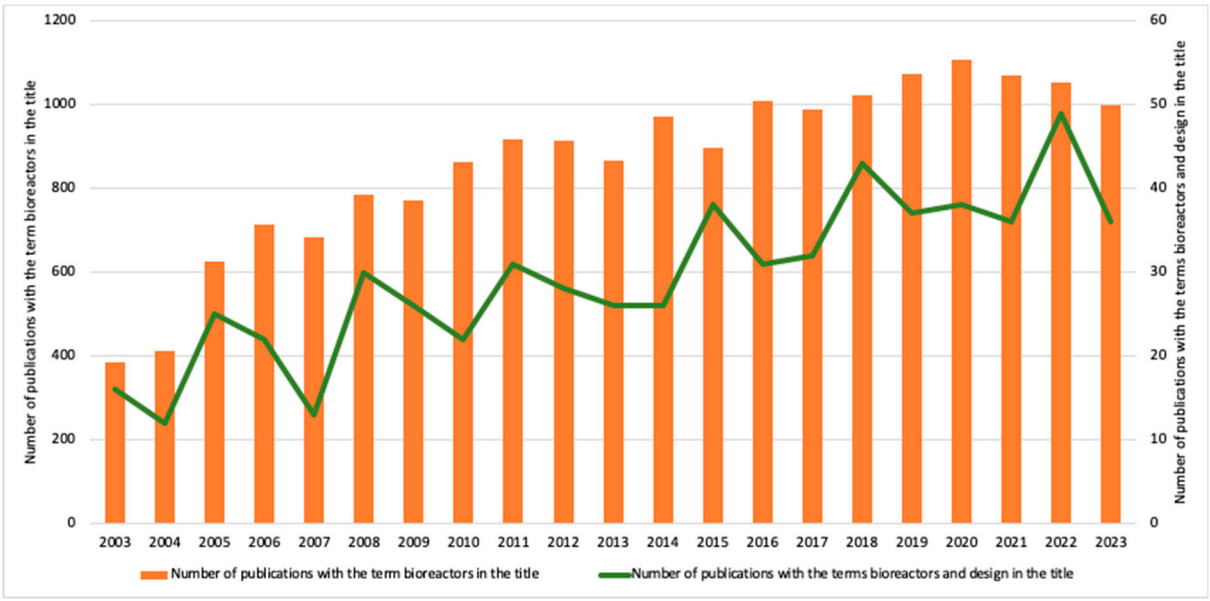

Figure 1. Evolution of the number of publications involving the keywords "bioreactors" and "bioreactor design" between 2003 and 2023. Source: Scopus (2024).

Currently, bioreactors play a fundamental role in the advancement of biotechnology, they are essential equipment in the interaction between small-scale bioprocesses outlined in laboratory studies with large-scale bioprocesses in the industrial scope. Bioreactors can be defined as equipment used to cultivate animal, plant, or microbial cells on a small or large scale. Generally, bioreactors have diverse systems for controlling operational variables such as agitation, aeration, temperature, pH, nutrient supply, and product removal, among others. Bioreactors ensure an ideal environment for cell growth and the synthesis of desired bioproducts, enabling the optimization of bioprocesses and reducing costs and production time [2]. Table 1 presents the types, advantages, and disadvantages of bioreactors. Choosing the appropriate bioreactor is essential for the process to be carried out efficiently.

Bioreactors are indispensable tools in modern biotechnology, enabling the production of a wide range of high-value-added products. This manuscript presents a comprehensive review of fundamental concepts and types as well as the challenges associated with monitoring bioprocesses in bioreactors. Then, we delve into the most recent advancements in optical and electrical sensor technologies, which have significantly improved the precision and efficiency of bioprocess control. Finally, we discuss the latest trends in bioreactor applications, highlighting their potential to revolutionize various industrial sectors.

Table 1. Types, advantages, and disadvantages of bioreactors.

Type of Bioreactor	Advantages	Disadvantages	Reference
Stirred Tank Reactor (STR)	✓ Simple design ✓ Versatility and scalability ✓ Greater control of cultivation conditions ✓ Reduced risk of contamination	✓ High shear stress, not suitable for shear-sensitive cells ✓ Complex mechanical agitation system	[1,3,4]
Modified STR	✓ Suitable for animal cells—Lower height/diameter ratio ✓ Reduced shear stress ✓ Homogeneous mixture	✓ Specific design modifications required ✓ Potentially higher costs	[4]
Airlift Bioreactor	✓ High mass transfer ✓ Good mixing with low-shear stress ✓ Low energy consumption ✓ Easy operation in sterile conditions	✓ Difficult to scale up ✓ Limited use compared to STR in industrial settings	[3,5]
Bubble Column Bioreactor	✓ High mass transfer–Low shear stress ✓ Low energy consumption ✓ Simple design	✓ Difficult to scale up ✓ Limited use compared to STR	[3,6]
Wave Bioreactor	✓ No need for sterilization (disposable) ✓ Suitable for low oxygen demand cells ✓ Simple configuration	✓ Limited scale expansion (up to 500 L) ✓ Not suitable for high oxygen-demanding crops	[7,8]
Disposable Bioreactor	✓ Eliminates the need for cleaning and sterilization ✓ Reduces contamination risk ✓ Flexible and efficient ✓ Suitable for various scales	✓ Environmental concerns (plastic waste) ✓ Potentially higher long-term costs	[9,10]
Membrane Bioreactor (MB)	✓ Efficient separation ✓ Continuous operation ✓ Contamination control ✓ Space saving	✓ High costs ✓ Complex maintenance ✓ Flow limitations	[11,12]
Solid-State Fermentation	✓ Suitable for low water activity media ✓ Can use various substrates—Less energy intensive	✓ Damage to filamentous fungi ✓ Uneven biomass growth ✓ Issues with aeration/heat transfer—inoculation and sterilization challenges	[13]
3D-Printed Bioreactor	✓ Customizable design ✓ Efficient process optimization ✓ Application in various bioprocesses	✓ High initial setup cost, complexity in design and manufacturing	[14–16]

2. Fundamental Concept Types of Bioreactors

Bioreactors for submerged fermentations (SmFs) and solid-state fermentations (SSFs) have been highlighted in recent years due to the intensification of the use of industrial biotechnological processes, mainly with the popularization of concepts such as biorefineries and bioeconomy, due to the global appeal for processes and sustainable products as well as growth in the production of biopharmaceuticals and vaccines. According to the concepts of Biochemical Engineering, biotechnological processes can occur in a liquid medium (SmF), in a solid medium, with low water activity (SSF), and in the presence or absence of agitation (static and agitated) and aeration (aerated and non-aerated) [3,17]. For submerged fermentations (SmFs) with microbial cells, three types of bioreactors are used, already well known in the literature: the stirred tank (STR), the airlift, and the bubble column bioreactor [3]. According to projections by Procedence Research (2023), the global bioreactors market size was evaluated at 9.3 billion dollars in 2022, and it is expected that over the next 10 years, a growth rate (CAGR) of 12.58% will result in a market size of 30.42 billion dollars by 2032. The main companies producing and selling bioreactors for bench and industrial scales are GE Healthcare (Chicago, IL, USA), Merck KGaA (Darmstadt, Germany), Eppendorf

AG (Hamburg, Germany), Sartorius AG (Gottingen, Germany), Thermo Fisher Scientific Inc. (Waltham, MA, USA), Bioengineering AG (Wald, Switzerland), Danaher Corporation (Washington, DC, USA), Infors HT (Bottmingen, Switzerland), Solaris Biotech Solutions (Sant'Antonio, Italy), and Lonza (Basel, Switzerland).

The aerated STR bioreactor is the most used in the biotechnology industry due to its advantages, such as simple design, versatility (operational flexibility and adaptability to different types of microorganisms), scalability, greater control of cultivation conditions, and reduced risk of contamination. Generally, this type of bioreactor is used in the cultivation of bacterial or yeast microbial cells, as they are less sensitive to shear stress. It is known that one of the main elements of STR bioreactors is the mechanical agitation system, which uses agitators such as blades, propellers, or turbines, according to the needs and particularities of the bioprocess, to promote efficient mixing of the cultivation medium. This agitation is crucial to ensure the homogenization of nutrients, uniform distribution of oxygen, and maintenance of ideal conditions throughout the reactor volume [1]. Among the agitation systems most used in bioprocesses are the Rushton turbine and the blades on pitched-blade impellers. The Rushton turbine is the most common impeller for microbial cultures of bacteria and yeasts that are more resistant to shear stress. Pitched-blade impellers are used for low-shear cultivation so that no physical damage occurs to the cells. They are ideal for the cultivation of sensitive cells such as mammalian and insect cells growing in suspension or on microcarriers, or for the cultivation of viscous microbial cells such as some filamentous fungi. Depending on the application, a combination of impeller types is also possible to increase mixing characteristics and reduce shear force [18].

With the advancement of SmF from animal cells, used in the production of some vaccines, biopharmaceuticals, and even in some cases of tissue engineering, modified STR bioreactors have stood out. According to [4], due to the greater sensitivity of animal cells to shear stress, the main modifications that STR bioreactors present that aim at adapting them to the production of biopharmaceuticals are as follows:

- ✓ A height/diameter ratio is less than two;
- ✓ For crops with a greater need for aeration, perforated-type sprinklers are used with a gas spray speed and bubble size monitored to generate lower shear stress, avoid foam formation, and ensure a homogeneous mixture;
- ✓ Marine-type impeller.

In addition to STR bioreactors, airlift and bubble column types, also known as pneumatic bioreactors, have also gained prominence in recent years. Despite being less used than STRs in the industrial environment, they are necessary equipment for aerated processes, but intense agitation is a critical parameter, as the cells used as fermenting agents are sensitive to shear stress. Airlift bioreactors are based on the draft tube principle, consisting of a cylindrical vessel connected to an aeration system. The interior of the cylindrical vessel is divided into two distinct parts, being an ascending tube or riser, in which the gas is injected and released in the upper part, and a descending tube or degassed downcomer [3,5]. This airlift bioreactor configuration allows air movement and aeration of the system. The bubble column bioreactor, like the airlift, consists of a cylindrical vessel with an aeration system implanted in the lower part. Pneumatic bioreactors have the following main advantages: (i) high mass transfer, (ii) good mixing with low shear stress, (iii) low energy consumption, (iv) easy operation in sterile conditions, and (v) simple design. However, these bioreactors have the disadvantage of being difficult to scale up to larger volumes [3,6]. With the intensification of studies and the use of microalgae in various industrial sectors, vertical tubular airlift and bubble column photobioreactors have been used on laboratory scales of up to 20 L [19].

An alternative to the STR bioreactor in submerged cultivation of animal and plant cells, especially those most sensitive to shear, are wave bioreactors [7]. This type of bioreactor was developed in the late 1990s for crops with low oxygen demand, which do not require submerged gasification, and for cells with anchorage-dependent growth [20]. The equipment has a simple configuration, as it consists of a Cellbag (plastic bag of varying

volume and sterile) with a hydrophobic filter for gas exchange coupled to a rocking table responsible for oscillatory movements and temperature control. Despite advantages such as no need for sterilization, as the Cellbag is sterile and disposable, this type of bioreactor has the disadvantage of limited scale expansion up to a capacity of 500 L of effective volume. This type of bioreactor is currently used in the pharmaceutical industry for the production of vaccines and other biopharmaceuticals, as it is suitable for cultivating animal cells and also unstable products, such as bioconjugates [8].

Another recent innovation is disposable or single-use bioreactors, also widely used in the pharmaceutical industry for the growth of animal cells in SmF. They are equipment manufactured with high-quality plastic materials, such as low-density polyethylene, polypropylene, or polycarbonate, which eliminates the need for cleaning and sterilization between cultivation batches. This reduces the risk of cross-contamination and simplifies validation and regulatory compliance processes. When compared to glass or stainless-steel bioreactors, they are flexible and efficient. These bioreactors can come in various sizes and configurations, from bench to industrial scale with a volume of 2000 L. This type of equipment is adaptable to different types of crops, allowing parallel experiments to be carried out or production to be scaled as necessary [9]. Although made of plastic, many disposable bioreactors are designed for energy-efficient recycling or incineration after use. Furthermore, its energy efficiency during use can offset the environmental impact associated with manufacturing and disposal. This represents an advantage in terms of sustainability compared to conventional stainless steel or glass bioreactors, which consume more resources and energy throughout their life cycle [10]. Among the main advances in the development of disposable bioreactors, the possibility of customizing the design according to the application stands out, offering different types of vessel and impeller geometry for mixing and mass transfer, as well as the development of non-invasive sensors and the integration of process analytical technologies (PAT) with the principles of quality by design (QbD). These advances will allow the development of more versatile bioreactors, for the cultivation of a variety of cells under physiologically favorable conditions and in the development of robust and repeatable processes [21].

Membrane bioreactors (MB) have also gained prominence in recent years, especially when they are applied in the industrial treatment of effluents. MB is equipment designed with semi-permeable membranes that allow the selective passage of substances of varying sizes while retaining larger cells or particles inside the reactor. These membranes can be microfiltering, ultrafiltering, or nanofiltering, depending on the size of the particles that you want to separate from the cultivation medium. The main advantages of this equipment are (i) efficient separation, (ii) the possibility of continuous operation, (iii) contamination control, and (iv) space savings. However, their disadvantages are (i) cost, (ii) complex maintenance, and (iii) flow limitations [11]. In addition to application in effluent treatment, these bioreactors can also be used in the intensification and consolidation of bioprocesses to obtain value-added products (polyols, biosurfactants, organic acids, and bacterial polyesters) in which the upstream and downstream steps can occur concomitantly. The membrane system acts to purify the biomolecules of interest. This strategy can reduce processing steps, directly affecting process time and costs, and can make the process economically viable [12].

Static or agitated bioreactors (occasional, continuous, or only with rotation) can also be used in SSF. The main types of bioreactors used for bench and industrial-scale SSF are (i) trays, (ii) fixed beds, and (iii) stirred drums. Despite their widespread use across various industries, there remains a continual need to evaluate and improve these systems due to several challenges [13]:

- ➢ Most SSF processes involve filamentous fungi as fermentation agents, and their delicate hyphae are prone to damage by mechanical agitation systems;
- ➢ The solid medium can agglomerate during fermentation, leading to uneven microbial biomass growth and complications with aeration and heat transfer;
- ➢ The large-scale inoculation, control, and sterilization of media volumes are often difficult.

Improvements to bioreactors for SSF often require customizations based on the specificities of the bioprocess, as they depend on the microorganism used, the type of substrate, and the operational conditions [13]. On an industrial scale, key challenges include heat transfer and humidity control due to the larger volumes of substrate involved. As a result, research has focused on developing bioreactors with innovative designs and automation systems to mitigate these issues and boost productivity [22].

One promising area of development is the application of 3D printing technology, which has gained popularity in recent years. This technology enables the development of micro- and macrobioreactors for bench-scale cell or enzyme cultivation. Additionally, 3D printing can be employed to manufacture micromixers and matrices for the immobilization of cells and enzymes [15,16,23]. Three-dimensional printing offers a solution for optimizing both SmF and SSF processes by facilitating the design of bioreactors and accessories that enable easy performance optimization. This technology can be applied to various aspects of the process, including the bioreactor itself, support structures for biocatalyst confinement, and peripheral accessories, allowing for highly controlled bioprocess [16]. In pharmaceutical processes, micro bioreactors are primarily used for drug testing, cellular response optimization, and disease modeling, while macrobioreactors support the cultivation of functional tissues for implantation in tissue engineering studies [14–16,23]. Operational safety includes preventing contamination and managing waste, with materials like PDMS (polydimethylsiloxane) playing a crucial role. PDMS is a versatile silicon-based organic polymer known for its flexibility, transparency, and gas permeability. These properties make it ideal for use in reaction microdevices, where it helps ensure the integrity and control of bioprocesses [24,25].

In recent years, energy consumption and process productivity have been improved using artificial intelligence (AI), with machine learning algorithms adjusting parameters in real time, improving efficiency and reducing waste. These algorithms predict energy needs and implement predictive control strategies, which are particularly useful in continuous processes [24,26]. Challenges in industrial-scale bioreactors involve engineering, safety and energy efficiency. Maintaining culture homogeneity and operational safety are essential. The choice of impellers and the integration of AI improve bioreactor efficiency. Automation, modeling, and the use of advanced materials ensure safer and more efficient operations, optimizing biotechnological processes. Frontistis et al. [27] reported the use of neural networks in MB control systems for wastewater treatment. The integration of neural networks with advanced algorithms and the implementation of Internet of Things (IoT) devices and new-generation sensors have the potential to transform the advanced wastewater treatment scenario towards the development of intelligent and self-adaptive systems.

Table 2 compares multiple types of bioreactors, including traditional systems such as Stirred Tank Reactors (STRs), innovative options like 3D-printed bioreactors, and single-use/disposable models, across six critical parameters:

- ➢ Mass Transfer: The ability of the bioreactor to facilitate the transfer of nutrients, gases, and substrates between phases, which is essential for maintaining cell viability and product yield.
- ➢ Heat Transfer: The efficiency of the bioreactor in maintaining optimal temperature conditions, which can significantly influence reaction rates and organism performance.
- ➢ Shear Stress: The mechanical forces exerted on cells or organisms in the bioreactor, where high shear stress can damage sensitive cultures.
- ➢ Medium Homogeneity: The uniform distribution of nutrients, gases, and other components throughout the culture medium, ensuring consistent conditions for all organisms.
- ➢ Scalability: The ability to increase the reactor size or replicate its conditions for larger production scales without losing performance.
- ➢ Oxygen Transfer: A vital aspect of aerobic processes, measuring how effectively oxygen is delivered to the culture.

Table 2. Hydrodynamics and scalability of different bioreactor types.

Reactor Type	Mass Transfer	Heat Transfer	Shear Stress	Medium Homogeneity	Scalability	Oxygen Transfer	References
Stirred Tank Reactor (STR)	High, mechanical agitation facilitates efficient transfer	High, controlled with external heat jackets	High, due to mechanical agitation	High, mechanical mixing ensures uniform conditions	Excellent, most scalable type of bioreactor	High, efficient oxygen transfer with proper agitation	[1,28]
Modified Stirred Tank Reactor (STR)	High, mechanical stirring provides excellent mass transfer	High, effective due to stirring and jacketed vessels	Moderate, controlled with proper agitation	High, stirring ensures even distribution	Good, highly scalable, common for large-scale production	High, impeller design can enhance oxygen transfer	[4]
Airlift Bioreactor	High, due to circulation and organized gas–liquid flow	High, due to efficient fluid circulation	Low to moderate, more controlled shear	High, consistent circulation ensures good mixing	Good, maintains performance at larger scales	High, good oxygen transfer via gas-lift mechanism	[5,29]
Bubble Column Bioreactor	Moderate, depends on gas flow and bubble size	Moderate, relies on gas–liquid interactions	Low, ideal for shear-sensitive organisms	Moderate, can be less uniform in larger volumes	Easy, but efficiency decreases with larger volumes	Moderate, depends on bubble size and gas flow rate	[3,6,30]
Wave Bioreactor	Moderate, depends on wave motion for oxygen transfer	Moderate, good for small volumes	Low, gentle wave motion	High, wave motion provides good fluid mixing	Easy to scale, widely used for cell culture applications	Moderate, limited by wave amplitude and frequency	[7,8]
Disposable Bioreactor	Moderate, dependent on bag design and aeration	Limited, generally relies on external temperature control	Low, typically used for shear-sensitive cultures	Moderate to high, depending on design and agitation	Easy, designed for modular scale-up	Moderate, typically relies on sparging or external aeration	[9,10]
Membrane Bioreactor (MB)	High, depends on membrane type and configuration	High, membranes can provide efficient heat exchange	Low, no agitation involved	High, consistent flow across the membrane	Moderate, membrane surface area limits scalability	Low, oxygen transfer is limited by membrane diffusion	[11,12]
Solid-State Fermentation	Limited, relies on diffusion in solid medium	Challenging due to solid substrates	Low, typically no agitation	Low, difficult to maintain uniform conditions	Low, scalability is complex due to heat and mass limitations	Low, oxygen transfer is poor in solid media	[13,31]
3D-Printed Bioreactor	Moderate, highly customizable mass transfer	Moderate, depending on design	Low, can be optimized for shear-sensitive processes	High, design flexibility allows for uniformity	Good, but depends on printing technology and design	Moderate, can vary depending on the design and material	[14–16]
3D-Printed Microbioreactor	Moderate, highly customizable with good surface-to-volume ratio	Good, depends on material properties and design	Low, can be tailored for shear-sensitive cultures	High, uniform conditions due to small size	Moderate, scalability is design-dependent	Moderate, can vary depending on the design and material	[32–35]

●: high efficiency, ●: medium efficiency, ●: low efficiency

Each bioreactor type is evaluated and color-coded based on its performance in these categories. This comparison serves as a guide to selecting the most suitable bioreactor for different biotechnological applications, ranging from lab-scale research to full-scale industrial production.

3. Challenges in Monitoring Bioprocesses in Bioreactors

Sensors and operational control systems in a bioreactor are vital for monitoring and maintaining controlled conditions, thereby enhancing the efficiency of chemical, biochemi-

cal, and biological processes. Despite their importance, significant challenges remain in monitoring and controlling bioprocesses due to the diversity of bioreactor models and biological models. As far as reactors are concerned, there are batch reactors, fed-batch reactors, continuous bioreactors, magnetic bioreactors, perfusion bioreactors, wave bioreactors, pneumatic bioreactors, rotating wall bioreactors, stirred bioreactors, and more recently, microfluidic reactors [36,37]. The engineering differences in these bioreactors, both in physical structure and operational mechanisms, lead to varying sensor applications [37]. Regardless of the specific type of bioreactor, several basic operational parameters must be measured. These include pH, temperature, pressure, feed flow rate, output flow rate, agitation, oxygen level, CO_2 level, and N_2 level. Additionally, there are parameters specific to each bioprocess, such as cell growth, enzyme release, nutrient metabolite levels, and secondary metabolite concentration. These parameters can vary based on the type of cell or microorganism used, the metabolic pathway employed (aerobic or anaerobic), the product of interest (which could be the cell itself or an extra- or intracellular product), and cell viability [38–40].

Sensors are also crucial for monitoring substances harmful to bioprocesses, such as endotoxins, contaminant bacteria, and cell culture stress. A significant current challenge is developing in-line or at-line sensors, including optical and electrical sensors, to measure cell growth, cell density, cell viability, enzyme release, nutrient metabolites, and secondary metabolite concentration. Many of these analyses are performed using spectroscopic techniques, which, although well-established, require specialized equipment and are often not integrated into bioreactors and are thus classified as off-line monitoring [41,42].

The aim of new sensor technologies is to create monitoring methods that can replace spectroscopic techniques, enabling continuous, integrated monitoring that can be performed remotely. This shift to automatic measurements reduces human operational errors and the risk of contamination, which can render a bioprocess unfeasible. An additional advantage of an online monitoring model is the constant flow of information for mathematical adjustment models, allowing for more robust, precise control and automated conditioning of bioreactor parameters. Figure 2 shows a diagram of an online and offline monitoring system [36,43,44].

Artificial intelligence (AI) has become a pivotal tool for controlling and monitoring bioreactor processes. With advancements in computing and programming, it is now possible to create sophisticated analysis algorithms that leverage extensive databases of pre-existing information or real-time data gathered during process monitoring [27,45]. AI's learning capabilities, combined with its ability to rapidly respond to critical variables, enable precise adjustments, ensuring that processes operate at optimal or near-optimal conditions. Beyond operational control, AI is extensively used to develop protocols, optimize workflows, and correlate large data sets. This allows researchers and engineers to identify the conditions under which processes achieve peak performance, enhancing overall efficiency and productivity [27,45,46]. In the bioprocessing sector, many researchers are integrating artificial intelligence (AI) into membrane bioreactors for a range of applications, including biohydrogen production, wastewater treatment process control, identification of critical factors in membrane fouling, and filtration process analysis. Various AI models can establish correlations between key bioprocess parameters such as temperature, time, agitation, pH, pressure, nitrogen concentration, CO_2 levels, biological and chemical oxygen demand, dissolved oxygen, suspended solids, volatile suspended solids, total dissolved solids, total organic carbon, and volatile organic compounds. This capability allows researchers to gain preliminary insights into potential optimized scenarios without requiring significant time or resource investment [45,47].

There are numerous AI models available for such tasks, each with unique operating mechanisms and outcomes. These models include Artificial Neural Network (ANN), Bayesian Network (BN), Elman Neural Network (ENN), Feed Forward Neural Network (FFNN), Fuzzy Inference System (FIS), Model Tree (MT), Multilayer Perceptron (MP), Radial Basis Function (RBF), Wavelet Neural Network (WNN), Long Short-Term Memory

(LSTM), Self-Organizing Map (SOM), Support Vector Machine (SVM), Adaptive Neuro-Fuzzy Inference System (ANFIS), Backpropagation (BP), Genetic Algorithm (GA), Random Forest (RF), Artificial Bee Colony Optimization (ABC), and Recurrent Neural Network (RNN) [48,49]. Given the distinct results produced by each model, they are often studied individually or in combination. Among the most widely applied models in bioprocesses are Artificial Neural Networks (ANNs), Support Vector Machines (SVMs), Long Short-Term Memory (LSTM), and Adaptive Neuro-Fuzzy Inference Systems (ANFISs) [45,47] (Table 3).

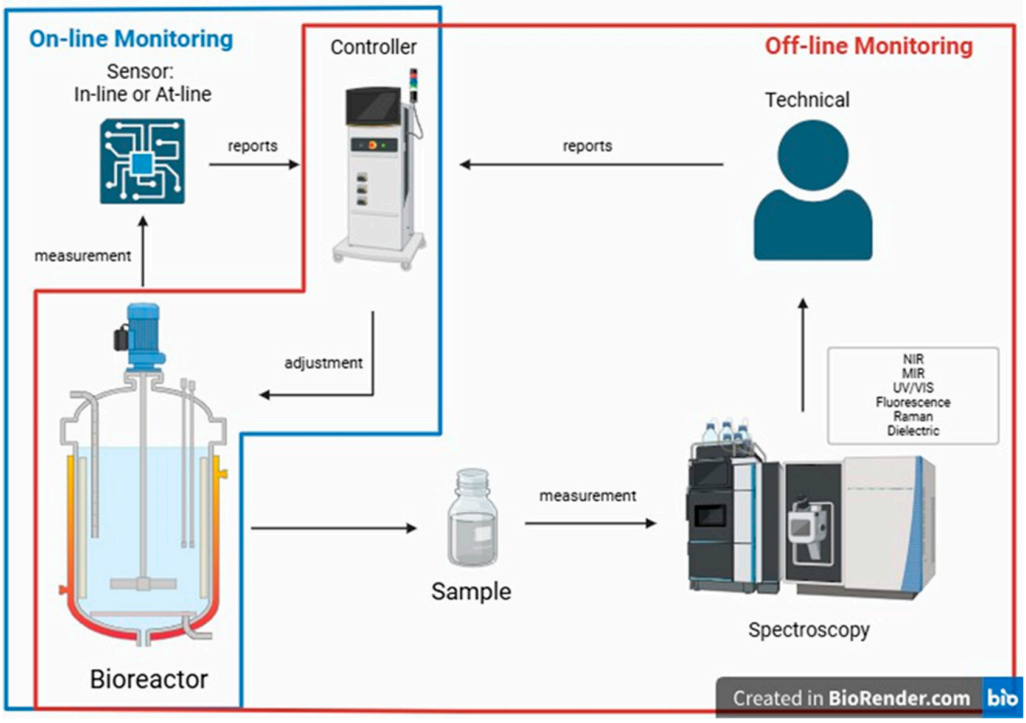

Figure 2. Schematic of an on-line and off-line monitoring system.

Table 3. Artificial intelligence models and their applications in bioreactors.

Models	Description	Applications	References
Artificial Neural Network (ANN)	The Artificial Neural Network (ANN) is a computational model inspired by the functioning of biological neural networks, structured using algorithms across multiple layers of neurons and an output layer. Each node in the network receives input data, processes it through an activation function weighted by coefficients (weights), and transmits the results to the subsequent layer	Study of the effects of hydrogen bonding in the gel fouling phenomenon; prediction of membrane fouling from long-term anoxic-aerobic MBR operational data, To study the membrane fouling behaviors in aerated MBR, it predicts transmembrane pressure in the anaerobic membrane bioreactor-sequencing batch reactor during biohydrogen production, identifying critical fouling factors and predicting fouling behavior in anaerobic membrane bioreactors.	[45,49–51]
Long Short-Term Memory (LSTM)	The Long Short-Term Memory (LSTM) is a specific architecture of recurrent neural networks (RNNs). Its distinction lies in its specialization for addressing the problem of long-term dependencies in sequential data. The model's structure includes memory cells, which retain information for extended periods. The control mechanism, known as "gates" (input, forget, and output gates), regulates the flow of information within each cell	Predictive Control with Rationality Verification for Bioreactors in Wastewater Treatment, predicting biogas production from large-scale anaerobic digesters to predict the nutrient removal efficiency in sewage treatment.	[45,52,53]

Table 3. *Cont.*

Models	Description	Applications	References
Support Vector Machine (SVM)	The Support Vector Machine (SVM) is a classification algorithm that identifies a hyperplane separating data sets into different classes, aiming to find the maximum margin of distance between the classes.	Optimization of Membrane Permeability of a Membrane Rotating Biological Contactor for Wastewater Treatment, including optimization of microbial lipid fermentation from cellulosic ethanol wastewater by *Rhodotorula glutinis.*	[48,54,55]

4. Advances in Optical Sensors in Bioreactors

Optical chemosensors, also known as optodes, are devices that operate through the interaction between an analyte and a chemical indicator incorporated into a matrix immobilized at the tip of the sensor. The operation of these sensors is based on illuminating the indicator with a light-emitting diode (LED) via an optical fiber. Changes in the optical properties of the indicator, such as photoluminescence intensity, absorption, or reflection, are detected by a photodiode. These changes are directly correlated with the concentration of the analyte of interest, enabling precise and reliable measurements [44,56]. The versatility of optodes allows for their in situ use in stainless steel bioreactors, utilizing standard ports that facilitate integration with existing systems. In small-scale contexts, such as deep-well plates and shake flasks, optical sensors can be applied via adhesive patches. This application is particularly valuable in low-volume systems where in situ sensors may not be feasible or could interfere with the system's hydrodynamics. These sensors are widely used to monitor critical parameters such as dissolved oxygen, carbon dioxide, and pH, with few innovations in their operating mechanisms. The ability to be sterilized by gamma radiation before use ensures that they meet the stringent sterility standards required in bioprocesses [37,43]. Significant innovations include reducing the size of spectrometry sensors to a miniaturized form, capable of performing microscopic scans and optical density measurements. Additionally, the development of optical biosensors involves immobilizing enzymes, substrates, or bacteria in a stationary phase, which can be a resin or some nanostructure. Figure 3 shows the main technologies being studied in the field of optical sensors [38,43,56].

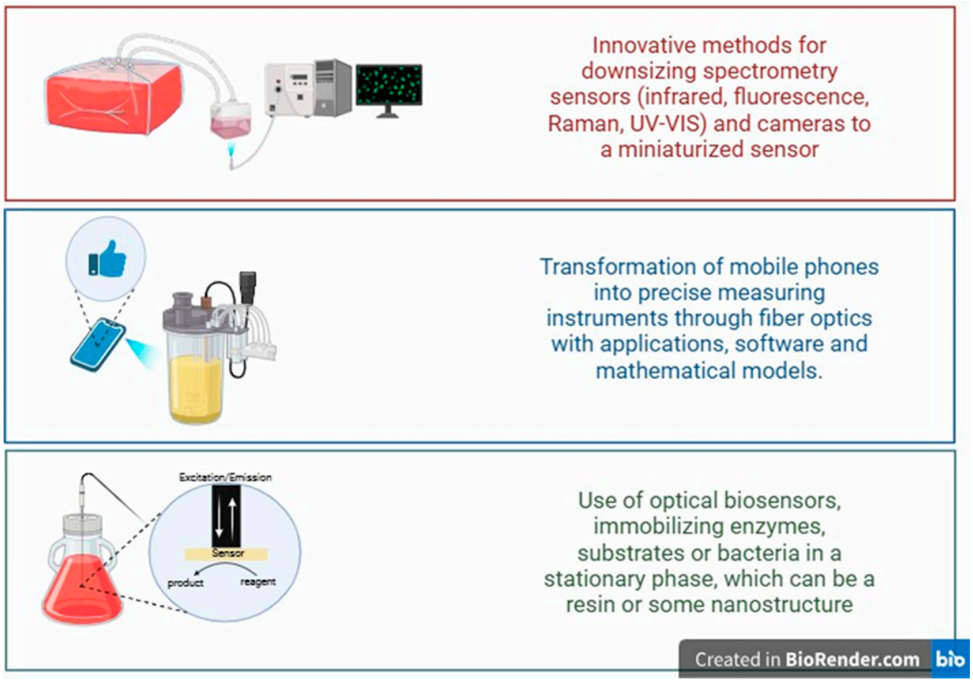

Figure 3. Key technologies being studied in the field of optical sensors.

The ability to integrate with remote monitoring systems and industrial automation is another strong point of optical chemosensors. With the growing adoption of Industry 4.0 technologies, the ability to collect and analyze data in real time is crucial. Optodes can be easily connected to sensor networks and control systems, allowing continuous monitoring and optimization of biotechnological processes. New projects are studying the transformation of smartphones into optical sensors, turning an everyday device into a precise measurement instrument [42,43,56,57]. Table 4 below lists some new optical sensors being developed and their applications.

Table 4. Optical sensor models under development and their applications.

Application	Mechanism	References
Cell proliferation	Measurement of optical density in the cell culture environment	[56]
Algae proliferation and reduction of QA (quinone acceptor in photosystem II)	Measurement using dual modulation LED kinetic fluorescence	[58]
Cellular imaging	Digital microscopic scanning in cell cultures using a miniaturized automatic microscope device	[37,42]
Surface affinity of molecules or cells	Using surface plasmon resonance to measure wavelength shifts	[42,44]
OD measurement by Infrared spectroscopy	Miniaturized probe-type infrared spectrometry sensor	[37]
Fermentation process analysis	Smartphone-based optical fiber sensor with colorimetric analysis of images captured by the camera	[44]

Conventional optical microscopy is constrained by the diffraction limit of light, which prevents the resolution of objects smaller than approximately 200 nanometers. Super-resolution microscopy overcomes this limitation by employing strategies that precisely pinpoint the location of individual fluorophores, enabling imaging at resolutions far beyond the diffraction barrier. This technique has revolutionized bioimaging, allowing for the visualization of cellular structures with extraordinary detail. In super-resolution microscopy, specific cellular structures are labeled with fluorescent molecules, and these fluorophores are activated one at a time, enabling the precise localization of each molecule and resulting in a much higher-resolution image. Another approach to improving resolution involves the modulation of fluorophore emission, where stimulated emission depletion (STED) microscopy uses a laser to suppress fluorescence in specific regions, further enhancing image clarity [59].

Super-resolution microscopy has significant potential in biotechnology, particularly within bioreactors. It enables the detection and analysis of individual cells, revealing previously inaccessible details about their internal organization, molecular interactions, and dynamics. This technology could facilitate real-time monitoring of critical biological processes, such as cell proliferation, biofilm formation, and metabolite production, directly within bioreactors. Additionally, it could optimize cultivation conditions, such as temperature, pH, and nutrient concentration, to maximize the yield of biomolecules of interest.

Moreover, super-resolution microscopy can monitor the formation and organization of engineered tissues, supporting the development of more realistic models of human organs and tissues. While super-resolution techniques are traditionally applied to intracellular environments, recent research has focused on using fluorescence microscopy to investigate nanoscale physicochemical variations in the extracellular matrix—a key biophysical environment that undergoes dynamic changes during various physiological processes. This technology offers novel insights into molecular-level processes that are difficult to measure with current spectroscopic methods [59,60].

5. Advances in Electrical Sensors in Bioreactors

Electrochemical sensors play a crucial role in monitoring bioprocesses, classified into several categories such as potentiometric, conductometric, voltammetric, and amperometric. Each type serves unique functions: potentiometric sensors detect changes in electrical

potential, conductometric sensors measure variations in conductivity, voltammetric sensors track alterations in charge transport under variable potential, and amperometric sensors measure charge transport while maintaining a constant potential. Similar to optical sensors, they are highly valued for their rapid response, wide measurement range, cost-effectiveness, and widespread adoption across various industries. Current applications include monitoring pH levels, dissolved oxygen (DO), and quantifying concentrations of glucose and glutamate [44,61,62].

Advancements in electronics and semiconductor technologies have facilitated the development of compact, portable electrochemical sensors that operate efficiently with minimal energy consumption. Innovations in chemical detection methods, biomodification techniques, and manufacturing processes have significantly enhanced the sensitivity, selectivity, and compatibility of these sensors with biological systems. Emerging models of electrical sensors are exploring sophisticated applications, including real-time analysis of cell growth dynamics in culture plates and assessment of cell adhesion behaviors on diverse surfaces [36,39,63]. These assessments utilize changes in electrical conductivity in the growth medium, microfluidic dynamics between interconnected microplates, and fluctuations in electrical potentials indicative of biochemical reactions. Figure 4 shows an example of an electrical sensor developed for monitoring cell growth [63–65].

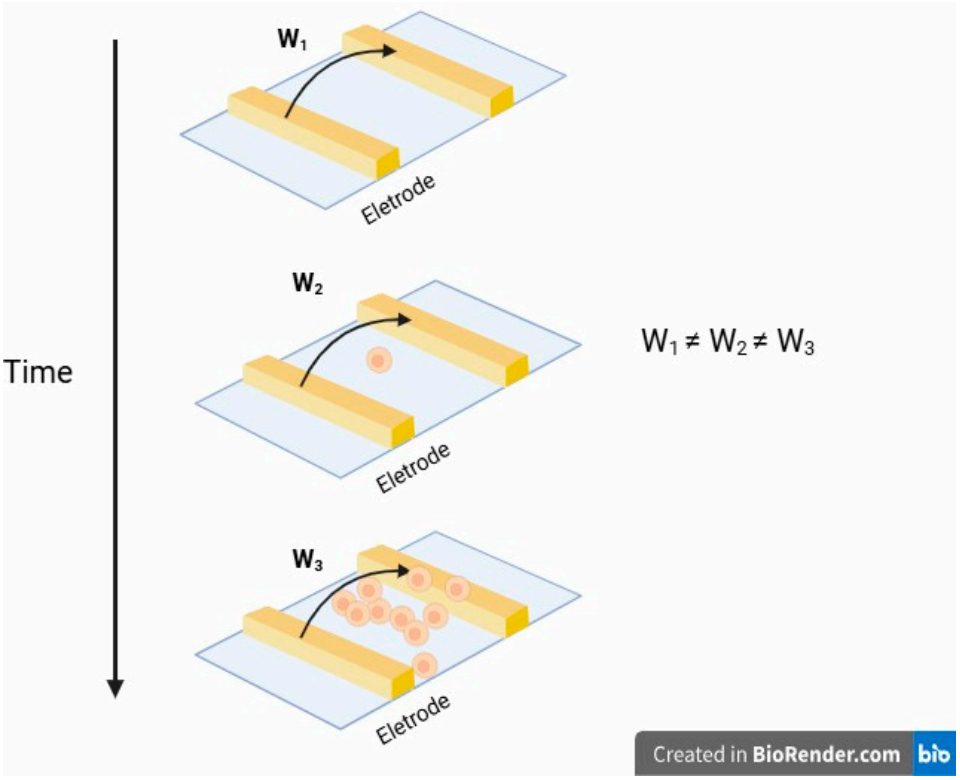

Figure 4. Schematic of an online and offline monitoring system.

The integration of electrochemical sensors into bioreactors and laboratory setups has simplified bioprocess monitoring by offering immediate and accurate measurements. For example, sensors designed to monitor cell growth use enzymatic oxidation processes to transfer electrons from specific substrates to an electrode interface [38,41,42]. This capability ensures precise monitoring of cellular metabolic activities in real time, crucial for optimizing biotechnological processes. Electrochemical sensors for measuring glucose and glutamate represent significant advancements in bioprocess monitoring, enabling real-time and precise monitoring of cellular metabolism. These sensors utilize enzymatic oxidation processes to transfer electrons from the measured substrate to an electrode and are designed

for immediate use, easily integrated into shake flasks or disposable bioreactors. Figure 5 shows an example of an electrical sensor developed for monitoring cell growth [38,65,66].

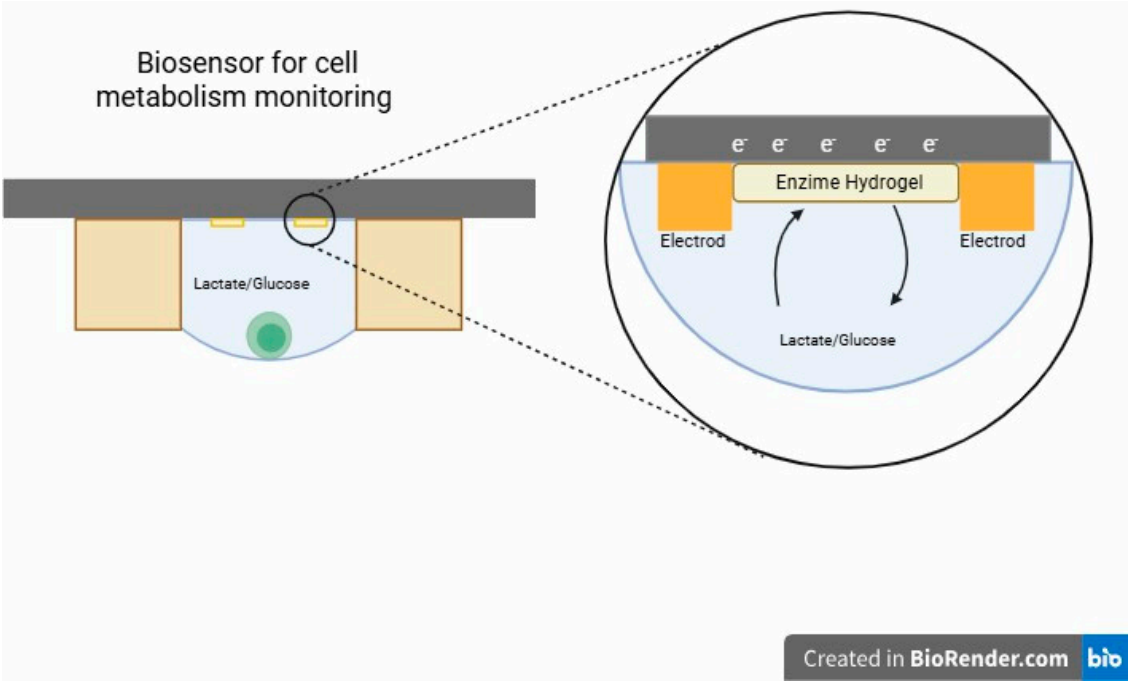

Figure 5. Electrical sensor developed for monitoring cellular metabolism.

6. Recent Advances in Bioreactor Applications

6.1. Production of Biopharmaceuticals

Given the high variability and complexity of biological processes, the necessity to maintain a sterile environment in some cases and the availability of real-time direct measurements for controlling bioreactors present distinct challenges. These challenges have led to the development of innovative solutions and highlighted areas needing further research and development [67]. Single-use technology is being used to address these demands [68]. Single-use bioreactors may also be used in pilot-scale continuous processes, reaching 4.6 times greater productivity than a batch process, with a 15% cost reduction [69]. Regarding cost reduction, developing bioreactors and the associated equipment demands substantial investment. CFD (Computational Fluid Dynamics) may be applied to model and simulate bioreactor operations, avoiding the exclusive need for empirical tests and reducing operational costs [70]. Single-use sensors have also been widely used to avoid contamination [71]. The sensors arrive to the user already sterilized with gamma radiation and can be set up for immediate use [37].

Corbin et al. [72] evaluated the use of a techno-economic model in SuperPro Designer. This model reduced costs by 4–11% compared to traditional batch operation for large-scale butyrylcholinesterase (BChE) production in a two-stage semi-continuous bioreactor, representing a valuable simulation tool for the plant-based pharmaceutical sector and a computational system adaptable to other biotechnological processes. Ruiz-Molina et al. [73] used Physcomitrella moss as a host to produce the complement regulator MFHR1 in 5 L photobioreactors. Recombinant protein production doubled with fed-batch or batch compared to semi-continuous operation, and specific productivity increased by 35%. They also studied an unstructured kinetic model to predict protein production, nitrate uptake, and biomass growth, allowing process control and optimization. They observed that the addition of auxin increased the specific productivity of MFHR1 by up to 470% in shake flasks and 260% in bioreactors, demonstrating the potential of Physcomitrella moss as a biopharmaceutical production platform that can be applied to other plant-based expression systems.

6.2. Production of Biofuels

Biofuel production has gained significant attention as a sustainable alternative to fossil fuels. Microalgae cultivation is been widely studied as third-generation biofuel production, mainly for biodiesel [74]. The cultivation in open raceway ponds is cheaper to build and operate but lacks contamination control and is susceptible to environmental influence [74,75]. Photobioreactors provide higher process control, leading to higher product yield and purity, but they present higher operation and build costs [76]. Many cost-reduction alternatives are being developed, such as the use of aquaculture water for microalgae cultivation in open ponds [77], as well as the coproduction of high-value-added products in photobioreactors, such as astaxanthin, antioxidants, hormones, pigments, etc. [75,78]. Biojet fuels may also be produced on bioreactors using genetically modified microorganisms [79]. The use of lignocellulosic biomass as a carbon source for the fermentation process requires pretreatments and sequential saccharification [80], increasing the operational cost and hindering the scale-up process [81]. Consolidated bioprocessing is a promising alternative, creating a single system in which cellulase production, as well as saccharification and fermentation, may occur simultaneously [79,82].

6.3. Bioremediation

Bioreactors may also be used for bioremediation, providing a controlled environment for the breakdown of pollutants by microorganisms [33]. Pollutants are converted into less toxic or environmentally friendly compounds by microorganisms. Microbial remediation can be applied in ex situ or in situ conditions. However, in situ bioremediation is a slow process that is difficult to optimize and control. Bioreactors provide optimum conditions for the growth of microorganisms and microbial biodegradation mechanisms. [83]. Several wastes may be treated, such as dye pollutants [33], aquaponics waste [84], and even petroleum hydrocarbons [85]. Usually, bioreactors are used in bioremediation, such as the fluidized-bed bioreactor [86], fixed-bed bioreactor [87], stirred-tank bioreactor [88], airlift bioreactor [89] and packed-bed bioreactor [85]. Promising alternatives are being studied, such as microbioreactors to treat wastewater from several processes, reducing operational costs and carbon footprint, enhancing process control, and allowing high-throughput screening [32,33]. Microbioreactors may involve complex structures, such as a lab-on-a-chip device, containing microchannels that allow a controlled flow of liquids [34], or be as simple as polyurethane foam impregnated with oil-eating bacteria [35]. The treatment of open spaces, such as petroleum leaks on seawater, is usually performed by recovering the oil, transporting it to a place onshore, and processing that oil with physical and chemical processes [85,90]. An alternative suggested by [85] is to use floating oil spill containment booms as a bioreactor basin, allowing the bioremediation to be performed on site, reducing costs associated with transportation.

6.4. Cell Culture and Tissue Engineering

Tissue engineering integrates biology, engineering, and material science to innovate in repairing and enhancing damaged tissues, showing potential to transform regenerative medicine by addressing challenging diseases and injuries [91,92]. Bioreactors are crucial for advancing tissue engineering by enabling scalability, making the technology more accessible and cost-effective, and providing controlled environments for the in vitro growth of functional tissues [93]. However, mammalian cells must maintain strict culture conditions, including temperature (approximately 37 °C), pH (7.2–7.4), and oxygen levels; environmental control is critical for maintaining cell viability and functionality, so smooth circulation and homogeneity of nutrients and metabolites must be ensured to avoid damage to shear-sensitive cells, which lack cell walls and are voluminous [94].

The customization of bioreactors for different tissue types is a promising area. Each tissue type requires specific growth conditions, and the ability to adjust bioreactors to meet these needs represents a significant advancement. Multidisciplinary collaborations among engineers, biologists, medical professionals, and material scientists are driving innovations

and fostering the development of more sophisticated and effective bioreactors [95]. For this purpose, in silico studies have brought advancements in optimizing the mechanisms incorporated into bioreactor equipment [96,97]. Prospective manufacturers interested in exploiting animal cells face challenges in selecting from numerous cell preparation methods and reactor designs: microcarriers, microcapsules, suspended cells, the Mass Culturing Technique (MCT), hollow fibers, spiral tubes, compartmented ceramic cylinders, air-lift reactors, gently stirred tanks, disposable rocking (wave) crossflow reactors, compartmented plates, and stacked plate units [94].

Eukaryotic bioreactors are like miniaturized laboratories, providing a controlled and optimized environment for the cultivation of eukaryotic cells, such as animal and plant cells. They are essential tools for a variety of fields, from scientific research to the industrial production of medicines and bioproducts. However, the handling of these bioreactors presents several challenges that can impact their efficiency and effectiveness [98–100]. Among these challenges we can mention the following:

- Scale-Up: Scaling up from laboratory to industrial scale can be complex. Maintaining optimal conditions such as aeration, agitation, and nutrient supply is crucial but challenging [101,102].
- Shear Stress: Eukaryotic cells are often more sensitive to shear stress caused by agitation and aeration, which can lead to cell damage or death, affecting overall productivity [97,103].
- Contamination: Eukaryotic cultures are more prone to contamination by bacteria, fungi, or viruses, which can compromise the entire bioprocess [104,105].
- Nutrient Supply and Waste Removal: Ensuring a consistent supply of nutrients and efficient removal of waste products is critical. Imbalances can lead to reduced cell growth and productivity [106].
- Oxygen Transfer: Eukaryotic cells, especially mammalian cells, have high oxygen demands. Efficient oxygen transfer is essential but can be difficult to achieve in large-scale bioreactors [96,107].
- Cost: The cost of maintaining eukaryotic bioreactors, including media, supplements, and equipment, can be significantly higher compared to prokaryotic systems [108].

These challenges require careful consideration and innovative solutions to optimize bioreactor performance for eukaryotic cell cultures. Initially, bioreactors for eukaryotic cells were used to produce therapeutic proteins, monoclonal antibodies, and cell biology studies. However, with the advent of tissue engineering, the challenges have intensified, necessitating the cultivation of cells in three dimensions for small to medium-sized tissue constructs [109,110] and even entire organs [111]. Cell therapy has inspired studies on the medical application of bioreactor lymphocytes and macrophages derived from human induced pluripotent stem cells (iPSCs), which have the potential to enable the development of cell-based therapies for numerous disease conditions. However, despite the great potential, the limitation that needs to be overcome is the necessity of ex vivo expansion because of the insufficient number of hMSCs presented within adult organs and the high doses required for transplantation. Thus, it is possible to find different studies in the literature that explore bioreactors for the clinical uses of mesenchymal cells [112].

Ackermann et al. [113] outline a detailed protocol for mass producing macrophages derived from human induced pluripotent stem cells (iPSC-Mac) in scalable suspension cultures using an orbital shaker or stirred-tank bioreactors (STBRs). The approach is simple and robust, involving the differentiation of primed iPSC aggregates into "myeloid-cell-forming-complex" intermediates with the use of a minimal cytokine cocktail.

Abdin et al. [114], attempted to scale up the production of human macrophages from human induced pluripotent stem cells (hiPSCs) with chimeric antigen receptors (CARs), referred to as CAR-iMacs. This approach is promising due to the challenges posed by the low yields of cancer patients' monocytes, inefficient ex vivo expansion, and the limited efficiency of genetic engineering in primary monocytes. While CAR-macrophage production has been previously demonstrated in 2D systems, the upscaled 3D

production of genetically modified macrophages is a completely new development. The team employed an automated CERO 3D bioreactor® to differentiate CAR-iPSCs into CAR-iMacs using a continuous suspension differentiation protocol. The results demonstrated that iMac differentiation protocols can be successfully adapted to upscaling platforms while preserving the functionality and phenotype of the modified cells. However, the scaling up of bioreactors for mammalian cells presents significant challenges that still need to be overcome [115]. The complexity of tissues remains one of the greatest challenges due to each tissue type's unique cellular composition, structure, and function, making artificial replication highly intricate. While the challenges associated with eukaryotic bioreactors are significant, they also present unique opportunities for innovation and advancement in tissue engineering. Recent advances in tissue engineering demonstrating notable progress and innovation include organoids [91], bioprinting [116,117], and the use of the natural extracellular matrix (ECM) structure to bioengineer an organ [118]. Ho et al. [119] were able to scale up cell culture/produce 1 billion cells in 5-day cultures at a 250 mL scale and 4 billion cells in 4-day cultures at a 1 L scale, showing the feasibility of printing tissues of approximately 1 cm × 1 cm × 0.1 cm; however, to be able to print a solid organ, they point out that, according to other studies, billions or even trillions of cells will be needed.

Another alternative being studied is perfusion bioreactors. A special type of bioreactor is used to continuously grow cells in a controlled environment. In cell culture, a perfusion system generally refers to a bioreactor coupled to a CRD, which separates and retains the majority of the cells in the bioreactor while the product-containing harvest is collected [120]. It differs from traditional bioreactors in that it constantly adds fresh nutrients to the culture while removing dead cells and products. Most perfusion bioreactors apply non-uniform flow and shear stress to the biological material due to the geometry of the sample chamber and/or flow channel. Acute expansions or non-rotationally symmetrical geometries tend to generate irregular flow speed areas in the periphery of the sample chamber; disposable rocking bioreactors (wave) are widely used in developing and scaling up in vitro bioprocesses once they overcome these problems. Recent publications suggest a likely direction for widening the applicability of this in supporting regenerative medicine and strategies for continuous bioprocessing and developing systems for the metabolic responses of non-natively stress-induced cells maintained in vitro [121,122].

Villiger et al. [102] investigated scaling mammalian cell culture processes over different stirred and aerated bioreactors ranging from 15 mL to 15,000 L using computational and experimental methods. Key parameters (maximum hydrodynamic stress, mixing time, and oxygen mass transfer coefficients) were determined experimentally for all scales. Computational fluid dynamics simulations integrating time-averaged equations of motion for fluid flow and considering bubble size population balance equations pointed to local and average hydrodynamic stresses and mass transfer coefficients. This integrated approach supports Quality-by-Design strategies, facilitating the transfer of mammalian cell cultures across reactors of different geometries. For controlling cell behavior, the integration of nano-vibrational stimulation mechanisms (initially designed via computer simulations and verified using laser interferometry) offers an interesting alternative [123].

It is important to point out that gamma-sterilized single-use plastic equipment plays a crucial role in reducing manufacturing costs, increasing plant flexibility, and shortening turnaround times while ensuring a safe and regulatory-compliant product. These characteristics support the needs of the biopharmaceutical industry, and the issue of leachables has been addressed for decades [124]. For example, 3,5-Dinitro-bisphenol A, found in extracts from polycarbonate flasks, can arrest the cell cycle in CHO-S cells, while bis(2,4-di-tert-butylphenyl) phosphate (bDtBPP), a prominent leachable, has growth-inhibiting effects on cell cultures [125]. Two of the most common leachables are plasticizers and antioxidants, which are typically used in the production of polymeric materials. These substances can be released during the sterilization process, through exposure to solvents, mechanical stress, or even under normal culture conditions, posing risks to cell viability and bioproduction consistency [126]. Therefore, it is essential to monitor the levels of leachables and their

effects, especially in single-use devices, which are often used with plastic bags. Regarding plant cells, the distinct types of bioreactors currently used for quality biomolecule production are discussed, with a focus on some species used to obtain important metabolites, by [127], with an insight into the type of bioreactor and production protocols.

To support bioreactor design, computational simulations are a powerful tool to avoid trial-and-error approaches. The discussion includes general and stimulus-specific requirements (e.g., perfusion, mechanical, and electrical) that must be considered during the design phase based on the tissue target. Computational models support designing bioreactors based on the provided stimulus, with a special focus on additive manufacturing techniques [128,129].

The ultimate trend in tissue engineering is cultured meat. Reviewing the literature highlights several studies indicating that some bioreactor systems are best suited for producing a particular sort of meat product in vitro and might not work well for producing meat in other forms or sizes. Therefore, there is still progress to be made in this area [130,131].

6.5. Food Production

SSF is widely used in the production of foods fermented by filamentous fungi in oriental cultures. In Japan, Koji, a specific fungal culture produced from steamed cereals, is used to obtain miso, shoyu, sake, and other products. After World War II, with the modernization and industrialization of Japan, the processes for obtaining Koji began to be developed in bioreactors on a larger scale, made of stainless steel or plastic and with aeration of the types (I) internal ventilation, (II) surface ventilation, and (III) non-ventilated (Table 5). The market for *Koji* fermentation equipment is dominated by internal ventilation bioreactors with various configurations [22]; https://controlledmold.com/industrial-koji-fermentation-equipment/ Accessed on 1 September 2024).

In addition to traditional fermentation processes for obtaining food, studies on cultured meat (also known as artificial meat or in vitro meat) are currently being highlighted. Artificial meat is produced through the process of in vitro myogenesis. When compared to the conventional meat that today's society consumes, laboratory-grown meat has some advantages, such as (i) the reduction of greenhouse gas emissions into the atmosphere, as it is not necessary to maintain large herds, and (ii) the reduction of the huge number of animals [130,131].

Aiming to achieve increasing industrial production rates, studies to obtain cultured meat using bioreactors with different configurations are of fundamental importance. Currently, studies can be found that report the production of meat on a bench and pilot scale using STR reactors in batch, fed-batch, and continuous modes. According to Lindskog [132], the use of STR bioreactors in continuous mode when compared to batch mode has as its main advantage more optimized cell growth rates, due to the maintenance of nutrient levels. In addition, a subtype of continuous batch reactors, known as perfusion reactors, is based on changing the culture medium without disturbing the cellular content of the reactors, which allows for achieving maximum recycling of the medium and the volume of the container. However, this bioreactor type depends on cell retention devices, ensuring uninterrupted cell proliferation and differentiation during medium change [133]. Culture systems based on recirculation of the cell culture supernatant (Alternating Tangential Flow, ATF; Tangential Flow Filtration, TFF), which differ from normal flow filtration, are also used in the context of perfusion reactors. They are designed to achieve cell retention and increase cell density [131,134].

In addition to mechanically agitated STR bioreactors, studies have also been carried out with pneumatic bioreactors, such as hollow fiber and airlift. Although pneumatic reactors ensure lower shear stress on cells and allow a relatively easy exchange of nutrients and gases between the culture medium and cells, they have the disadvantages of limited scalability, propensity for clogging, limited oxygenation, and relatively high cost [135].

Finally, when designing a bioreactor for the production of cultured meat, several issues must be considered: (i) the bioreactor may need to have a surface for cells to adhere to or

be able to support growing cells attached to scaffolds; (ii) the setup should be simple and make use of safe and inexpensive materials, aiming to reduce manufacturing costs; (iii) the setup should allow for easy scalability, aiming at the development of equipment for larger volumes [131].

Table 5. Some types of industrial bioreactors used in the Koji process and their main characteristics.

Bioreactor	Capacity	Characteristics
Static flat table type (also known as tent type, ventilated box type, and "Castan" type)	100–1500 kg	Interior ventilation/Forced aeration; Tray-type bioreactor; Substrate layer thickness (Koji bed depth): 5–7 cm; This type of bioreactor provides better substrate aeration and heat transference/regulation throughout the thick substrate mass; Larger capacity configurations feature an air-handling unit coupled to the blower, facilitating temperature and humidity control; Larger capacity configurations feature a rail-mounted mixer that runs horizontally across the Koji bed, facilitating substrate spreading and layer homogenization; Application: Shochu, Shoyu, Miso, and Sake.
Multistage conveyor type	1000–3000 kg	Interior ventilation (stage 1) and surface ventilation (stage 2)/Forced aeration; Tray-type bioreactor with multistage system; Substrate layer thickness (Koji bed depth): 15 cm (stage 1), 3–6 cm (stage 2); Due to the multistage system, this bioreactor can perform the fermentation process in a static bed in the first 24 h, because the growth of the inoculum does not require high aeration; There is no excessive heat emission during the process; after the growth of the inoculum on the substrate, it is distributed in a thin layer through a conveyor system that operates intermittently to mix and redistribute it; Currently, few industries use this system due to the greater possibility of contamination and its more sophisticated configuration that increases costs; Application: Sake and Ginjo/Daiginjo.
Vapor exchange non-ventilated type	100–500 kg	Non-ventilated; Tray-type bioreactor; Substrate layer thickness (Koji bed depth): 3–6 cm; In this type of bioreactor, there is a homogeneous distribution of the heat generated in the system during the fermentation process; Easy-to-clean bioreactor; Application: Sake and Ginjo/Daiginjo.
Drum type (also known as Tomuzetto)	500–3000 kg	Interior ventilation/Forced aeration; Drum-type bioreactor; The configuration allows for on-site substrate sterilization; temperature regulation for substrate cooking; substrate agitation during the fermentation process, improving aeration and homogenization; This type of bioreactor has been less used in recent years; Application: Miso.
Rotary disc type	1000–50,000 kg	Interior ventilation/Forced aeration; Substrate layer thickness (Koji bed depth): 15–60 cm; Facilitates loading and distribution of Koji; Distribution of Koji in a uniform layer; Features its own air treatment unit; Bioreactor for the Koji process most commonly used on an industrial scale; Application: Shochu, Shoyu, Miso, and Sake.

Adapted from https://controlledmold.com/industrial-koji-fermentation-equipment/. Accessed on 1 September 2024).

7. Future Perspectives: Trends for the Future of Bioreactor Systems and the Impact of Advances in Bioreactors on Society and Industry

Looking ahead, the evolution of bioreactor systems holds profound implications for both industry and society. Anticipated trends point towards the increasingly sophisticated integration of sensors and automation, ushering in an era of real-time monitoring and

control. These advancements promise heightened precision and efficiency, not only in the production of biopharmaceuticals and vaccines, bolstering global healthcare capabilities, but also for other biotechnological products, such as biofuels and food.

Moreover, sustainability is poised to drive innovation in bioreactor design, with a growing emphasis on eco-friendly materials and energy-efficient processes. The advent of disposable bioreactors, designed for minimal environmental impact and recyclability, underscores a shift towards greener biotechnological practices. This aligns with broader societal demands for sustainable solutions across industrial sectors.

Looking beyond industry impacts, the democratization of biotechnology is foreseen as barriers to entry lower, facilitating broader participation from startups and small enterprises. This democratization fosters a diverse ecosystem of innovation, potentially unlocking novel applications and solutions that cater to a wide array of societal needs.

In essence, the future trajectory of bioreactor systems envisions a transformative role in global biotechnology, characterized by sustainability, precision, and accessibility. These advancements not only promise to elevate industrial capabilities but also to address pressing societal challenges through innovative biotechnological solutions.

8. Conclusions

Bioreactors have emerged as transformative tools in the biotechnology landscape, enabling the cultivation of microorganisms and cells for the production of a wide spectrum of products. However, the transition from small-scale laboratory settings to large-scale industrial production poses significant challenges that must be addressed to fully realize the potential of this technology. Overcoming these challenges requires a multifaceted approach that integrates automation, computational modeling, and advanced materials. Automation systems enable precise control of multiple bioreactors, facilitating real-time monitoring and adjustments. Computational modeling tools can simulate bioreactor conditions, predicting performance and identifying potential bottlenecks. Advanced materials like PDMS play a crucial role in enhancing bioreactor safety and efficiency. By bridging the gap between small-scale research and industrial production, bioreactors are poised to revolutionize various industries. Machine learning algorithms and innovative impeller designs optimize energy consumption and ensure process homogeneity. Additionally, materials like PDMS enhance safety and bioprocess control. As bioreactor technology continues to evolve, we can expect even greater efficiency, sustainability, and personalization in the field of biotechnology, ultimately leading to breakthroughs in human health, novel therapies, and a brighter future. In general, bioreactors are essential equipment for the development of many new high-value products, being essential for both upstream and downstream stages. The future of bioreactors is promising and full of opportunities. Constant innovation in this field allows us to imagine a future where biotechnology is more efficient, sustainable, and personalized, benefiting human health and driving the development of new therapies and treatments.

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