APPLICATION OF BIOREACTORS IN PRODUCTION OF ALTERNATIVE PROTEINS - REVIEW

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Abstract. Interest in the development of technologies aimed at producing alternative proteins is growing significantly, driven by the need to identify sustainable production and consumption methods to meet the demands of the global population. This field involves the cultivation of microorganisms and animal cells in controlled environments, ensuring both their viability and efficient growth. Cultivation takes place in a diverse array of bioreactors, spanning from traditional models to cutting-edge designs, each offering unique features and benefits. The most widely utilized bioreactors include stirred tank reactors, which rely on mechanical agitation, airlift or bubble column bioreactors, which employ pneumatic agitation, fluidized-bed reactors and other bioreactor models, which will be discussed in detail in this review. The deployment of a diverse array of sensors is vital for ensuring accurate real-time monitoring, early detection of issues, reproducibility, cost efficiency, and enhanced overall performance. These applications of bioreactors are not limited to the production of alternative proteins but are also essential across various sectors, from scientific research to industrial production. This review highlights and describes the different types of bioreactors currently employed in the production of combined proteins, the use and integration of advanced sensors, exploration of promising applications, and the technological challenges that need to be addressed.

Keywords: biotechnology, alternative protein, sensors, bioreactors.

Introduction

The Sustainable Development Goals outlined by the United Nations prioritize the elimination of hunger, the preservation of biodiversity both in aquatic and terrestrial ecosystems and the mitigation of climate change. Nonetheless, the rapid growth of the global population, coupled with persistent malnutrition and limited access to high-quality protein, poses significant challenges to public health. Traditional agricultural practices, which heavily depend on fertile land and freshwater resources, are increasingly viewed as unsustainable due to their inefficiency and high resource consumption, such as water, energy, feedstock, fertilizers, pesticides, and veterinary medicine. For example, traditional meat production generates 37 gigatonnes of CO_2 per year and accounts for 15% of global anthropogenic emissions [1]. These vulnerabilities are expected to be exacerbated by the impacts of climate change, pollution, population growth and environmental stress [2].

Projections indicate that the global population will surpass 10 billion by 2050, leading to a dramatic rise in the demand for food [3]. This surge is further driven by increasing incomes in industrializing nations, where dietary preferences are shifting towards protein-intensive, meat-based diets. And in addition to environmental concerns, excessive reliance on animal-based protein has been linked to heightened health risks, including cardiovascular diseases and certain types of cancer [4].

Addressing the interconnected challenges of rising nutritional demands, environmental degradation and health risks necessitates urgent adoption of sustainable consumption and production practices. Transforming global food systems through innovative solutions is vital to ensuring long-term food security and ecological sustainability. Among these solutions, the development of alternative protein technologies approaches to reimagining protein production and consumption for a growing global population [1].

This emerging technology focuses on cultivating microorganisms and animal cells under carefully controlled conditions to optimize their growth and viability. The production process is facilitated by the use of specialized bioreactors, with range from traditional models to cutting-edge designs tailored to enhance efficiency and scalability [5].

Bioreactors are integral to the progress of biotechnology, serving as critical tools that bridge the gap between small-scale bioprocesses developed in laboratory settings and large-scale industrial applications. They can be described as devices designed to cultivate animal, plant, or microbial cells at both small and large scales. Typically, bioreactors are equipped with various systems for regulating

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operational parameters such as agitation, aeration, temperature, pH, nutrient delivery, and product extraction, among other factors [6].

The design and operational mode of a fermenter are primarily determined by the characteristics of the production organism, the optimal conditions required for synthesizing the target product, the value of the product, and the scale of production. Additionally, economic considerations such as capital investment and operational costs play a critical role. For instance, large-scale production of low-value products like plant-based protein concentrates requires relatively simple fermenters and does not necessitate aseptic conditions. In contrast, the production of high-value, low-volume products such as cultured meat or specialized protein isolates demands more sophisticated systems with stringent aseptic requirements. Similarly, the design and configuration of bioreactors are tailored to meet the specific needs of the cells, enzymes, or microorganisms involved, depending on the nature of the bioprocess. For large-scale alternative protein production, conventional bioreactor models can be utilized effectively, provided they are adapted to the unique requirements of the process [7].

For instance, large-scale production of cultivated meat in bioreactors involves two essential stages – cell proliferation and differentiation – where optimizing bioprocess parameters, such as dissolved oxygen, pH, and temperature, is critical for ensuring the proper development of muscle and fat cell types. To achieve this, various bioreactor designs are employed, with the most commonly used being stirred-tank reactors (STRs), wave bioreactors, and fluidized-bed bioreactors. A practical example is demonstrated in a recent study that achieved a cell density of 5.053×10^5 bovine stem cells per millilitre in a 1 L STR bioreactor using SoloHill microcarriers [8]. In another case, the formation of mycelial architectures in bioreactors is influenced by spore inoculum properties and cultivation conditions, including medium composition, hydrodynamics, and oxygen availability, with innovations such as advanced stirrer designs helping reduce shear stress [9].

The design and configuration of bioreactors are pivotal in determining the success of biotechnological processes, involving careful selection of materials, control systems, and strategies to optimize cellular growth and metabolic production. In this regard, a deep understanding of process kinetics, necessary agitation, energy and mass balance, along with heat and mass transfer, is crucial for enhancing the process efficiency.

The integration of specialized sensors, which resulted in only marginal improvements in process performance, highlights the challenges associated with optimizing bioreactor conditions for alternative protein production [10]. Despite their potential to monitor key variables such as cell viability and growth, the high cost of these sensors may limit their practical application. Thus, while bioreactor systems are essential for enhancing the efficiency and scalability of alternative protein production, the need to address issues such as energy consumption, resource management, and economic feasibility remains crucial to ensuring their long-term sustainability and contribution to food security [5].

A key focus of this review is to explore bioreactor models used in alternative protein production, focusing on the importance of integrating sensors to optimize performance and ensure stable biotechnological processes.

Bioreactor types for cultivation of various alternative proteins: characteristics and applications

Alternative proteins, sourced from plants and microorganisms (fungi, yeast, bacteria, and insects), are increasingly used as substitutes for animal protein in dairy-free and meat-free products. Meeting this demand will require significant investment in bioreactor capacity, with an estimated $30 billion needed for expansion [11]. The selection of a bioreactor for alternative protein production will be determined by its particular characteristics (Fig 1).

Mechanical agitation bioreactors are the most commonly used type of bioreactors and are currently constructed at scales up to 20.000 litres. These bioreactors consist of a cylindrical vessel with a central shaft driven by a motor, which supports one or more agitators or impellers. In large-scale production, fed-batch or continuous operations are preferred as they allow for higher yields. Although a potential drawback of using this type of bioreactor is the increased shear stress on cells due to the moving internal parts and the agitation speed, this issue is less concerning for cultivated meat production. In fact, nitric oxide signalling may have a protective effect on cells exposed to shear stress in mechanical agitation bioreactors [5]. Cultivated meat is produced through the ex-situ culture of animal cells, which are obtained via biopsy and then cultured under controlled conditions with appropriate nutrients. This process results in the formation of structures similar to muscle tissue, which are subsequently shaped, seasoned, and coloured to produce a final product resembling conventional meat [4]. Additionally, several companies in Europe and North America – such as Clara Foods, Remilk, and Those Vegan Cowboys – are leveraging microbial fermentation to produce animal-free dairy proteins, including casein, whey, and egg proteins. This approach aligns with the growing demand for sustainable and alternative protein sources. STR are predominantly used for microbial protein synthesis due to their high scalability and precise control [12].

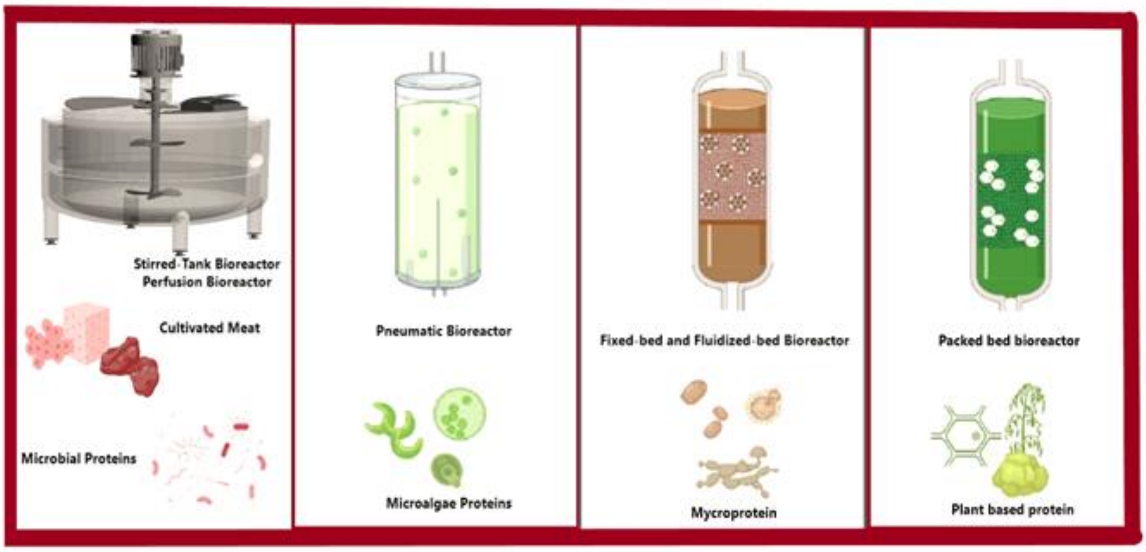

Fig 1. **Bioreactors used in production of alternative protein [5]**

Airlift bioreactors and bubble column bioreactors facilitate efficient gas-liquid dispersion, with their gentle mixing method making them particularly suitable for cultivating sensitive cells, such as plant and animal cells. These bioreactors are also highly effective for the growth of microalgae, including *Chlorella vulgaris* and *Arthrospira sp*., which are widely used in the food industry due to their high protein content [13]. Furthermore, these reactor systems play a significant role in recombinant protein production. One notable example is miraculin, a protein capable of altering sour tastes into sweet sensations, successfully synthesized in carrot callus cultures [14]. Additionally, recombinant milk proteins, such as caseins and whey proteins, are being explored as potential alternatives for dairy production [15].

Fixed-bed bioreactors consist of a solid matrix in which the biocatalyst is either immobilized on the surface or embedded within the structure. These systems provide a high conversion rate and are relatively simple to operate. Their application in the cultivation of animal or plant cells for cultured meat is widely reported, as most cell lines can be grown in these bioreactors without requiring additional adaptation. Similarly, fluidized-bed bioreactors operate on a comparable principle; however, instead of a fixed support matrix, the culture medium flows upward through a suspended bed of support material. This dynamic environment is particularly advantageous for cultured meat production, as it promotes efficient cell adhesion and proliferation on microcarriers, ensuring enhanced cell viability within a homogeneous system [8].

From a cost perspective, bubble columns are the most economical, followed by STRs, while fluidized and fixed bed bioreactors require significant investment. Each offers distinct advantages: STRs for easy scale-up, bubble columns for cell protection, fluidized beds for adherent cells, and fixed beds for advanced tissue engineering.

Initial process conditions are established at laboratory scale. Scaling these optimized conditions to industrial levels often proves challenging due to the precise performance replication required, as these parameters critically influence cellular growth and biomolecule biosynthesis. Mammalian cells require attachment surfaces within bioreactors for proper growth and proliferation. Consequently, cellular aggregates (spheroids) or microcarriers are employed to overcome this limitation. A key innovation involves edible microcarriers composed of plant-derived collagen and modular systems, which could reduce processing costs by 15% while significantly streamlining production [16].

Microcarrier properties (rigidity, surface texture) can significantly influence cellular differentiation. All biological tissues undergo a maturation phase involving mechanical stimulation (compression, tension, bending, torsion) to develop physical characteristics like elasticity and strength. Successful induction of contractile functions can be achieved through integrated mechanical or electrical bioreactor devices. For instance, Dursun et al. [17] describe a piston-based bioreactor for cartilage tissue compression, while Hairong et al. [18] developed a chamber with clamps and rods for stretching cultured muscle, achieving conventional meat-like texture.

During mammalian cell culture scale-up, shear stress generated by agitation or bubble bursting may compromise cell viability. While cellular tolerance thresholds remain debated, potential solutions include specialized impellers (e.g. marine or pitched-blade designs) and packed-bed bioreactors that shield cells from hydrodynamic forces. Such implementations may reduce cellular damage by 20% at agitation rates exceeding 200 rpm [5]. These technological approaches hold transferable applications in both regenerative medicine and pharmaceutical manufacturing, significantly broadening their potential for commercial scalability.

Sensors and monitoring technologies in bioprocessing

The production processes of alternative proteins are highly complex systems that require optimal operating conditions to prevent potential losses and declines in productivity. Deviations from these conditions can significantly impact product quality, as alternative proteins are highly sensitive to environmental changes. Therefore, continuous monitoring of key parameters, such as temperature, pH, carbon source concentration, metabolites, agitation speed, using high-quality sensors is essential for ensuring product integrity and maintaining high production standards [19].

Ideally, sensors should be non-invasive, versatile, highly accurate, sensitive, and stable, while also being cost-effective and capable of providing rapid feedback to the control system**.** Sensors can be classified as in-line, where they are in direct contact with the culture medium, enabling real-time monitoring, or at-line, where samples are extracted and analysed externally, providing results within minutes [20]. For example, critical dissolved process variables such as dissolved oxygen partial pressure (pO_2), dissolved carbon dioxide partial pressure (pCO_2), and pH values are typically monitored using electrochemical sensors, categorized into conductometric, potentiometric, and amperometric types [21]. Rapid analytical systems, such as High-Performance Liquid Chromatography (HPLC) and ultraviolet-visible (UV-VIS) spectroscopy, serve as effective tools for at-line analysis. These techniques enable accurate and timely monitoring of alternative protein production, facilitating the quantification of protein concentration and purity, as well as the identification of relevant metabolic intermediates and impurities [22]. The type of data collected in bioprocessing depends on the organism used (bacteria, yeast, mammalian cells, etc.) and the production techniques applied.

A new class of sensors, based on digitalization and IoT, estimates variables through mathematical models, enabling real-time monitoring and anomaly detection. These sensors facilitate remote control of bioprocesses, reducing the need for sample handling. Software sensors enable the estimation of variables that are otherwise challenging to measure directly, such as substrate concentration or specific growth rates in bacterial fermentation processes [24]. In such scenarios, Kalman filters or extended Kalman filters are commonly employed for estimating the system variables [25]. In the production of alternative proteins (derived from microbial fermentation, algae, or mammalian cell cultures), Kalman filters play a crucial role in estimating nutrient concentrations (e.g. glucose, amino acids) to optimize feed strategies, minimize substrate waste, and detect real-time process deviations such as contamination or cellular stress. The Kalman filtering approach integrates sensor data with mechanistic or data-driven mathematical models, enhancing the process control accuracy. A notable example is demonstrated in a study on plant-based haemoglobin production using Pichia pastoris, where an Extended Kalman Filter (EKF) was employed to provide real-time estimations of recombinant protein concentration and methanol consumption. This enabled automated feed-rate adjustments [26].

For instance, Sundström et al. utilized four software sensors based on simplified mathematical models and online data to monitor biomass concentration, specific growth rate, oxygen transfer capacity, and the oxygen-to-energy substrate consumption ratio in fed-batch fermentations using E. coli [27]. In this context, E. coli functions effectively as a cell factory for alternative proteins, synthesizing recombinant milk proteins [5]. One of the earliest applications was demonstrated in the study by Warth et al., which monitored the expression of a fluorescent protein (e.g. GFP – Green Fluorescent Protein) in E. coli cultivated in a fed-batch bioreactor. The fluorescence of this protein was measured in real time using an optical probe, and the data were fed into a mathematical algorithm that estimated the culture growth rate and glucose consumption rate. Compared to traditional methods, this approach demonstrated an accuracy of up to 95%, reducing the need for manual sampling and providing results at intervals of 2-5 minutes [28].

Despite their efficiency, these sensors have not yet been widely adopted due to their complexity, stringent regulatory requirements, and high costs. Nevertheless, technological advancements and the ongoing trend toward digitalization suggest that they will likely be implemented on an industrial scale within the next decade [28].

Another study has demonstrated that the bio-calorimetric method enables real-time estimation of microbial growth rate, even in small bioreactors or at low biomass concentrations, by applying digital filtering to reduce noise and enhance accuracy. While capacitance spectrometry provides a rapid and precise alternative, bio-calorimetry leverages existing equipment, minimizing costs and maintenance requirements. The calorimetric model exhibited stability throughout the cultivation process, and integration of the oxygen uptake rate (OUR) sensors could further improve estimation accuracy. The findings suggest that this method, when combined with digital filtering, is comparable to or superior to conventional hardware sensors, reinforcing its potential as a PAT-compatible tool for monitoring alternative protein production processes [3].

While the discussed applications highlight the transformative potential of soft sensors, it is critical to emphasize that their adoption extends far beyond alternative protein production. A key challenge lies in bridging the gap between laboratory-scale validation and industrial deployment. Despite their demonstrated efficacy, widespread implementation is hindered by high integration costs and regulatory barriers, particularly compliance with Good Manufacturing Practice (GMP) standards. Many soft sensor solutions remain confined to academic research due to unresolved concerns regarding data validation and regulatory acceptance.

Nevertheless, the trajectory is clear: digitalization is inevitable, and soft sensors are evolving from passive data collectors into active participants in bioprocess optimization. Advanced computational approaches, including Kalman filtering techniques that facilitate real-time process adjustments, alongside with non-invasive optical monitoring systems that supersede conventional analytical methods, demonstrate measurable improvements in the process efficiency, cost containment, and quality assurance metrics.

Conclusions

The implementation of bioreactors for the production of alternative proteins represents a significant advancement in the field of biotechnology, providing a sustainable solution to contemporary agricultural challenges. This approach contributes to reducing greenhouse gas emissions, requires less water and land usage, and minimizes waste generation. Recent innovations in the bioreactor design, along with the integration of soft sensors, have the potential to significantly enhance the production of microbial proteins and cultivated meat. These advancements could facilitate large-scale industrial and commercial manufacturing while ensuring high standards of quality and consistency.

As biomanufacturers increasingly prioritize Industry 4.0 frameworks, hybrid solutions integrating hardware sensors with artificial intelligence-based models are poised to become standard practice. Early adopters - particularly in precision fermentation and cellular agriculture sectors - are already leveraging these tools to achieve accelerated scale-up and enhanced product consistency.

This study underscores the urgent need for standardization of the Process Analytical Technologies (PAT) and the development of hybrid models combining mechanistic approaches with artificial intelligence. Soft sensors demonstrate remarkable accuracy in estimating critical parameters, particularly in alternative protein production where precise estimation is paramount. However, key opportunities must be identified to reduce implementation costs and overcome regulatory barriers. Despite current challenges, advancements in bioreactor technology and intelligent monitoring systems are paving promising pathways toward more efficient, sustainable, and scalable protein production. The implementation of these solutions will require close collaboration among researchers, food industry stakeholders, and regulatory authorities to ensure a harmonious transition toward viable long-term food systems.

The findings highlight that while technical solutions exist, their successful industrial deployment necessitates addressing both technological and systemic barriers through coordinated multi-stakeholder efforts. This transition represents not merely an operational upgrade, but a fundamental paradigm shift in biomanufacturing methodologies.

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