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Review

Is It Possible to Produce Meat Without Animals? The Potential of Microorganisms as Protein Sources

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Abstract: Climate change and environmental impacts from greenhouse gas emissions have spurred on efforts to reduce these emissions. Meat production, especially from cattle, is a significant contributor, releasing methane—a greenhouse gas far more potent than CO_2—and driving deforestation for pastureland. As a sustainable alternative, Single-Cell Protein (SCP), derived from microorganisms like bacteria, yeast, and algae, offers high nutritional value with a lower environmental impact. SCP production has advanced through process optimization, the use of eco-friendly substrates such as agro-industrial and food waste, and the cultivation of safe microorganisms classified as Generally Regarded as Safe (GRAS). Innovations in flavor and texture, including the use of myoglobin and natural polymers to mimic meat properties, have further improved SCP's appeal. Despite these advances, challenges remain in optimizing production parameters, enhancing sensory acceptance, and ensuring regulatory compliance for market introduction. This review explores the potential of SCP to serve as a sustainable protein source, addressing both environmental concerns and nutritional demands. It highlights recent advancements in production techniques and sensory improvements while discussing their role in environmentally friendly and health-conscious food systems. SCP stands out as a promising solution for reducing greenhouse gas emissions, offering an efficient and sustainable alternative to conventional protein sources.

Keywords: Single-Cell Protein; microbial protein; cultivated meat; mycoprotein

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1. Introduction

Climate change, driven by greenhouse gas emissions, has prompted significant transformations across various sectors, particularly in the food industry. Among the most substantial contributors to these emissions is meat production, especially from cattle, which releases methane—a greenhouse gas approximately 25 times more potent than carbon dioxide in trapping atmospheric heat. Beyond emissions, livestock farming has profound environmental impacts, including deforestation, biodiversity loss, and the degradation of natural ecosystems to create pastures and produce animal feed. In this context, the search for alternative protein sources has gained momentum, offering a dual benefit: mitigating environmental harm and fostering economic diversification while reshaping the dynamics of the food supply chain [1].

Alternative protein sources have been proposed, such as insects [2], algae [3], fungi [4], and bacteria [5]. These foods usually present a high protein content, lower production cost, and higher yields than traditional animal-based meat. On the other hand, Single-Cell

Protein (SCP) production offers several significant advantages. First, these microorganisms can be cultivated in conditions that utilize more sustainable substrates, such as agro-industrial waste and discarded food, making the process efficient and an opportunity for circular economy practices [6]. Moreover, using microorganisms with a "Generally Recognized as Safe" (GRAS) status has facilitated the development of safe and high-quality food products. However, consumer acceptance remains a significant challenge, as alternative protein products' flavor, texture, and appearance still need to be improved to match the sensory experience of traditional meat [7].

Recent advancements include the development of compounds that mimic the flavor and aroma of meat, such as the introduction of myoglobin, to replicate the texture of muscle fibers [8]. These innovations have enhanced the appeal of SCP-based products and other alternative proteins, making them more attractive to consumers seeking to reduce meat consumption without sacrificing the sensory qualities of traditional meat products [9].

This article discusses advances in the use of microorganisms for the production of Single-Cell Proteins (SCP), emphasizing their potential as a sustainable alternative to conventional protein sources. It explores industrial methods for SCP production, innovations to simulate the taste and texture of meat, and strategies to overcome regulatory and consumer acceptance challenges. This review provides a comprehensive perspective on the role of SCP in creating a more sustainable and environmentally responsible food system, addressing contemporary demands for less impactful and more diverse dietary practices.

2. Single-Cell Protein

Single-Cell Protein (SCP) production is gaining attention as a sustainable alternative to conventional meat production, addressing the growing demand for non-animal-derived proteins [10,11]. SCP refers to proteins derived from microorganisms such as bacteria, fungi, yeast, and microalgae, which contain high protein concentrations and can be cultivated on a large scale using diverse substrates. This approach is environmentally friendly due to its reduced land and water requirements and lower greenhouse gas emissions [12,13].

The primary microorganisms used in SCP production include *Spirulina* (microalgae), *Saccharomyces cerevisiae* (yeast), and fungi such as *Fusarium venenatum*, which produce mycoprotein products like Quorn™. Each type of microorganism offers unique nutritional characteristics; for example, *Arthrospira* is rich in essential amino acids, B vitamins, and iron [14], while *Saccharomyces cerevisiae* provides proteins and dietary fibers [15].

SCP production can utilize a range of substrates, from simple sugars like glucose to agricultural and industrial residues such as molasses and sugarcane bagasse. This versatility enables the recycling of waste materials, supporting a circular economy [16,17]. The fermentation process may be aerobic or anaerobic, depending on the microorganism type and cultivation conditions. Aerobic fermentation is typical for yeasts and fungi, promoting faster cell growth, whereas anaerobic fermentation can be employed with certain bacteria [18–20].

Table 1 presents the average chemical composition of various microorganism sources used for SCP production, including bacteria, yeasts, filamentous fungi, and microalgae. These microorganisms exhibit significant differences in their nutritional profiles, particularly regarding protein content, which is important for SCP applications.

Table 1. SCP production using different microorganism sources.

Microorganisms	Examples	Substrates	Production Methods	Protein Content (%)	Examples of Industrial Products	Reference
Bacteria	*Methylophilus methylotrophus*, *Rhodopseudomonas palustris*, and Hydrogen-oxydizing bacteria	Methane, wastewater, and agroindustrial waste	U-loop bioreactors and CSTR bioreactors	70–76%	Solein® and Air Protein®	[18,21]
Yeast	*Saccharomyces cerevisiae*, *Candida utilis*, *Yarrowia lipolytica*	Agroindustrial, food wastes, and lignocellulosic hydrolysates	Bioreactors and Bubble column reactor	24–54%	Yeast extract	[20,22,23]
Filamentous fungi	*Fusarium venenatum*, *Pleurotus ostreatus*	Food waste, lignocellulosic hydrolisates	Air-lift bioreactor, solid-state bioreactor	15–45%	Quorn®, Promyc® and Perfect Day®	[24–26]
Microalgae	*Arthrospira platensis*, *Chlorella vulgaris*	CO_2, luz solar, água (potável e salina), resíduos de água	Photobioreactor	45–64%	Spirulina food supplementation	[27–29]

Bacteria, such as *Methylophilus methylotrophus*, *Rhodopseudomonas palustris*, and hydrogen-oxidizing bacteria, have high protein content, ranging from 50% to 80% of their dry weight. This high concentration is attributed to their rapid growth rates and efficient substrate-to-biomass conversion. They utilize substrates such as methane, wastewater, and agroindustrial waste, and are commonly cultivated using U-loop bioreactors or continuous stirred-tank reactors (CSTR). Industrial products like Solein® and Air Protein® exemplify the successful application of these bacteria [18,21].

Yeasts, including *Saccharomyces cerevisiae*, *Candida utilis*, and *Yarrowia lipolytica*, exhibit a protein content between 24% and 54% (Figure 1), lower than bacteria. Still, their separation tends to be simpler than when using bacteria [30]. Despite their lower protein concentration compared to bacteria, yeasts are notable for their higher growth rates and easier cell separation during processing, which enhances their industrial feasibility. Saccharomyces cerevisiae, in particular, is widely used in the food industry due to its established safety profile [31]. Yeasts can utilize diverse substrates, such as agroindustrial residues, food waste, and lignocellulosic hydrolysates, and their production is typically conducted in bioreactors or bubble-column reactors. However, yeasts present particular challenges, including a high nucleic acid content that may contribute to undesirable flavors and a cell wall with low digestibility [32]. Despite these limitations, industrial applications of yeast, such as yeast extract, remain prominent in the food and supplement industries [20,23].

Filamentous fungi, such as *Fusarium venenatum* and *Pleurotus ostreatus*, have a protein content ranging from 15% to 45% (Figure 1), which is slightly lower than that of other microorganism groups. However, they exhibit several advantages, including lower nucleic acid levels (2–8%) compared to bacteria and yeasts, which minimizes undesirable flavors associated with higher nucleic acid content. The structural complexity of their cell walls, rich in polysaccharides such as chitin and glucans, also provides functional benefits as dietary fiber [31].

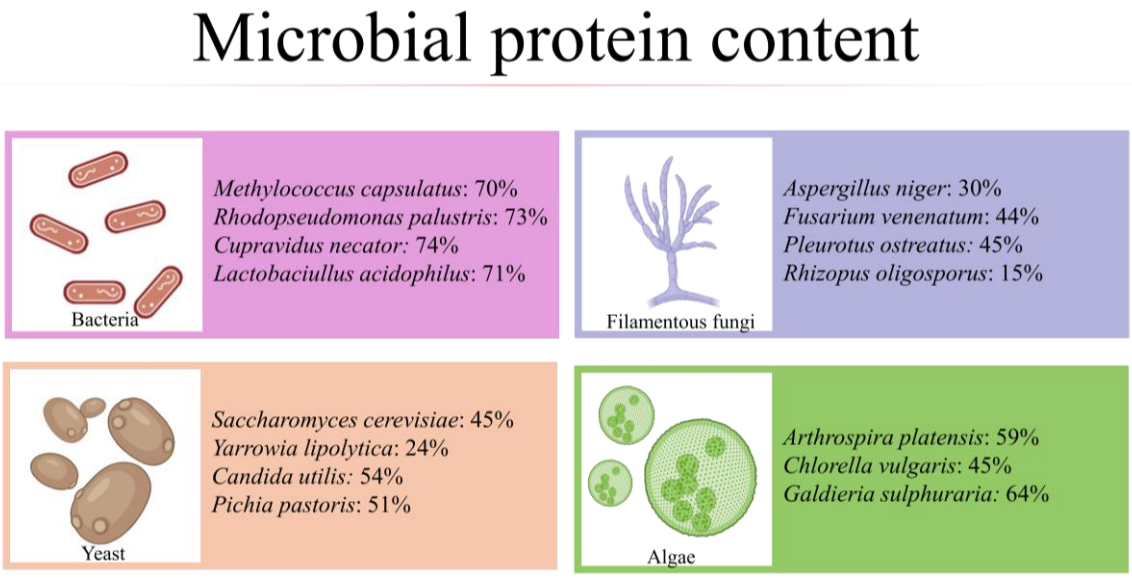

Figure 1. Protein content of different microbial sources.

Bacteria, such as *Methylophilus methylotrophus*, *Rhodopseudomonas palustris*, and hydrogen-oxidizing bacteria exhibit the highest protein content, ranging from 50% to 80% of their dry weight. This high concentration is attributed to their rapid growth rates and efficient substrate-to-biomass conversion. They utilize substrates such as methane, wastewater, and agroindustrial waste and are commonly cultivated using U-loop bioreactors or continuous stirred-tank reactors (CSTR). Industrial products like Solein® and Air Protein® exemplify the successful application of these bacteria [18,21].

Yeasts, including *Saccharomyces cerevisiae*, *Candida utilis*, and *Yarrowia lipolytica*, exhibit a moderate protein content between 24% and 54% (Figure 1). Despite their lower protein concentration compared to bacteria, yeasts are notable for their higher growth rates and easier cell separation during processing, which enhances their industrial feasibility. Saccharomyces cerevisiae, in particular, is widely used in the food industry due to its established safety profile [31]. Yeasts can utilize diverse substrates, such as agroindustrial residues, food waste, and lignocellulosic hydrolysates, and their production is typically conducted in bioreactors or bubble-column reactors. However, yeasts present certain challenges, including a high nucleic acid content that may contribute to undesirable flavors and a cell wall with low digestibility [32]. Despite these limitations, industrial applications of yeast, such as yeast extract, remain prominent in the food and supplement industries [20,23].

Commercially, *Fusarium venenatum* is widely used in mycoprotein-based products, such as Quorn®, which contain only ~1% nucleic acid content. These fungi are highly valued for their ability to grow on food waste and lignocellulosic hydrolysates, offering an effective solution for protein production through waste utilization [25]. Their meat-like texture and the natural umami flavor of mushrooms make them particularly important in developing alternative meat products, enhancing consumer acceptance of such innovations [33]. Industrial cultivation of filamentous fungi is typically carried out in air-lift or solid-state bioreactors, with products like Promyc® and Perfect Day® further exemplifying their potential in the alternative protein market [23,24,26].

Microalgae, such as *Arthrospira platensis* (*Spirulina*) and *Chlorella vulgaris*, exhibit a protein content similar to that of bacteria, ranging from 45% to 64% (Figure 1). These species are widely cultivated for their high protein content, bioactive compounds, and significant lipid levels, which make them particularly valuable in the production of food supplements and functional foods [9]. Microalgae thrive on substrates such as CO_2, sunlight, potable water, or wastewater, making them an environmentally friendly protein source.

Their cultivation is typically carried out in photobioreactors, allowing efficient biomass production while minimizing resource inputs. Products derived from these species, such as spirulina-based supplements, are well-accepted for their nutritional benefits and are increasingly recognized as sustainable alternatives to conventional protein sources [28].

A bibliometric analysis of studies published between 2014 and 2024 in the Scopus database provides valuable insights into research trends on Single-Cell Protein (SCP). From an initial set of 222 documents identified using the keywords "Single-Cell Protein" and "food", a total of 115 papers were selected based on their relevance and thematic alignment. These studies were categorized according to the microbial protein sources investigated: bacteria (29.6%), yeasts (35.7%), filamentous fungi (21.7%), and algae (13.0%). Figure 2 illustrates the distribution and relative emphasis of research across these groups, detailing the specific microorganisms studied within each category.

Yeasts emerged as the most extensively studied SCP source, reflecting their established role in the food industry and their adaptability to protein production. Among these, Saccharomyces cerevisiae accounted for 15.7% of the analyzed studies, underscoring its prominence as a model organism for SCP research. Its widespread use is attributed to its robust safety profile, rapid growth rates, and ability to metabolize various substrates, including food waste and agroindustrial residues. Other yeasts, such as *Yarrowia lipolytica* and *Candida utilis*, have also received significant attention due to their high substrate versatility and industrial relevance. However, challenges such as the high nucleic acid content of yeast biomass—which can impart undesirable flavors—and the relatively low digestibility of yeast cell walls highlight areas where further optimization is required to maximize their potential as protein sources.

Bacteria, accounting for nearly a third of the reviewed studies, have demonstrated significant potential for SCP production, mainly using unconventional and sustainable substrates. Purple non-sulfur bacteria, including *Rhodopseudomonas* and *Rhodobacter*, and methanotrophic bacteria, such as *Methylococcus* and *Methylobacillus*, have been key research focal points. These microorganisms are particularly noteworthy for their ability to grow on methane, wastewater, and organic residues, enabling SCP production that simultaneously addresses waste management and greenhouse gas reduction. Their versatility and high protein yields—often exceeding 70% of biomass dry weight—position bacteria as promising candidates for large-scale SCP production. However, limitations such as the need for precise growth conditions and the optimization of production costs remain challenges to their broader application.

Filamentous fungi represented 21.7% of the studies and were primarily investigated for their unique structural and functional properties, which make them suitable for specific applications, particularly in alternative meat products. *Fusarium venenatum*, for example, is widely used in producing mycoproteins, a key ingredient in commercially available products such as Quorn®. The natural umami flavor and meat-like texture of fungal biomass make it particularly appealing for developing alternative protein products. Additionally, filamentous fungi offer the advantage of ease of separation from growth media and low nucleic acid content (2–8%), which enhances their suitability for human consumption. However, their slower growth rates and lower protein yields compared to other microorganisms—typically between 15% and 45%—may explain why fungi receive less research emphasis. Despite these limitations, the dietary fiber provided by fungal polysaccharides, such as chitin and glucans, add significant nutritional value, reinforcing their potential role in SCP production.

Algae, while representing the smallest share of research (13.0%), hold significant promise due to their exceptional sustainability and nutritional potential. Species such as *Spirulina* (*Arthrospira platensis*) and *Chlorella vulgaris* have been extensively studied

for their high protein content, ranging from 45% to 64%, and their ability to utilize CO_2 through photosynthesis [28,34]. This unique characteristic not only contributes to their environmental appeal but also highlights their potential as a tool for carbon capture. Additionally, their bioactive compounds and lipid content make them valuable for producing functional foods and dietary supplements. However, challenges such as algal biomass's poor digestibility and distinct, sometimes undesirable, flavor profiles have hindered their direct application in traditional food systems. Nevertheless, their successful use in protein supplementation products demonstrates their viability in niche markets, with potential for further innovation.

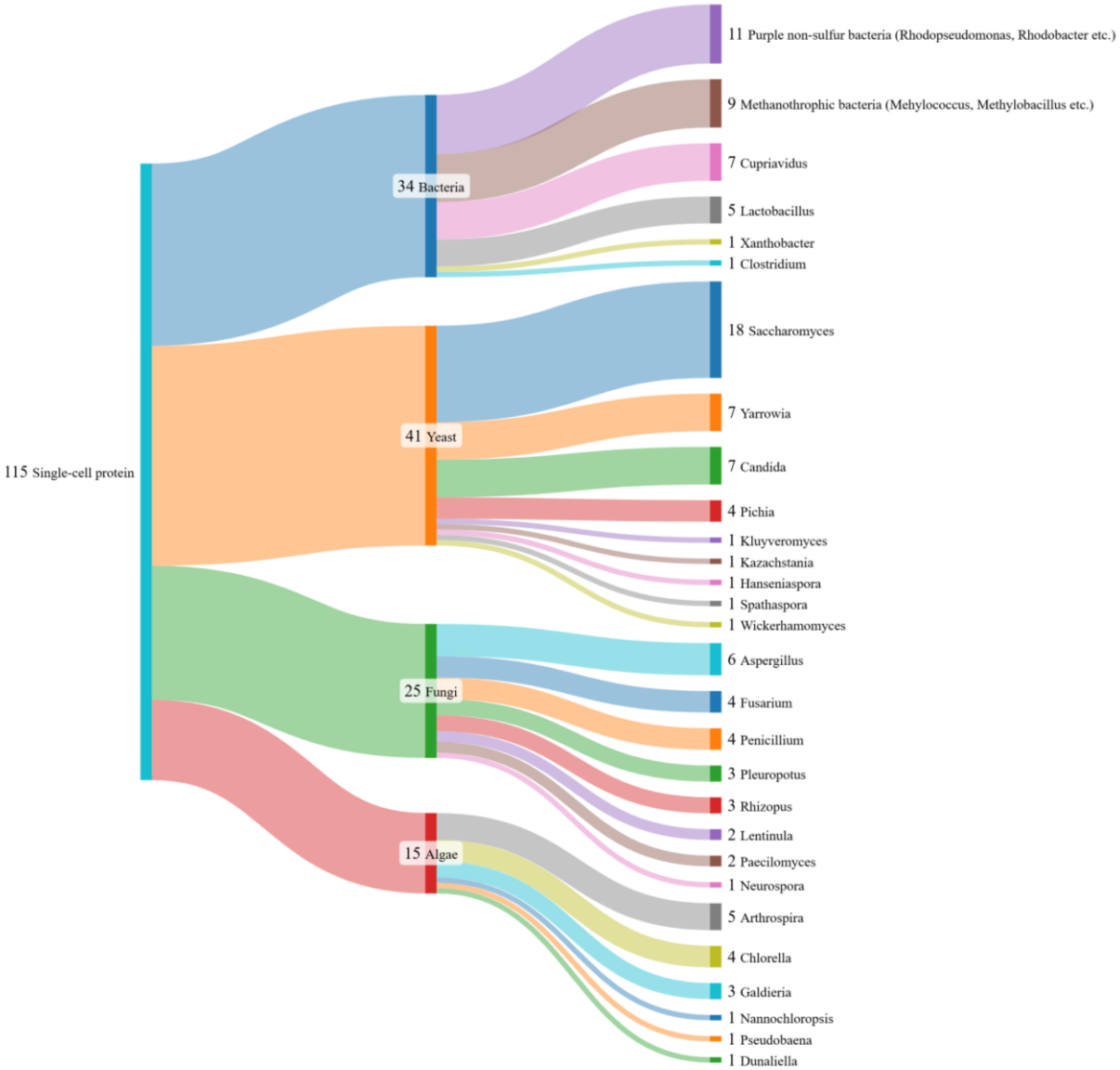

Figure 2. Sankey diagram representing the bibliometric analysis of the recently produced papers (2014–2024) about production of microbial protein.

2.1. Bacteria

Bacteria offer several advantages, including a high growth rate, broad versatility regarding substrate utilization, and a high protein concentration in their biomass. Various bacterial species have been explored for this application, with examples including metilothropic ones [35] which utilize methanol as a carbon source and exhibit high efficiency in biomass conversion; *Lactobacillus* [36], widely recognized for its food safety and frequent use in food fermentations; and *Corynebacterium glutamicum* [37], which, in

addition to its application in amino acid production, possesses a protein profile suitable for SCP production.

Bacterial fermentation for SCP may occur in different substrates, such as methane [5], sticky water [36], and food waste [38]. These low-cost substrates are often discarded, providing an economically viable and environmentally friendly alternative to more traditional carbon sources. However, SCP production using bacteria faces several challenges. Firstly, the high nucleic acid content in bacterial cells poses a drawback, as large-scale consumption of nucleic acids can lead to health issues, such as gout. To enable the use of bacterial SCP in human and animal diets, processes to reduce these compounds must be applied, which can increase production costs [39]. Furthermore, bacterial biomass often has flavors and aromas that may not appeal to consumers. Additional research may be performed to evaluate the collateral effects of bacterial SCP, as it may result in intestinal problems in some animals, as fish fed with bacterial SCP meal have presented necrosis in its guts [40].

Another drawback relates to the complexity of maintaining optimal growth conditions on a large scale. While bacteria such as *Methylococcus* are promising due to their efficiency in converting substrates into protein, maintaining ideal conditions for growth and fermentation at an industrial scale can be both costly and technically challenging [5,21]. Nevertheless, research on bacterial SCP continues to advance, focusing on optimizing microbial strains and fermentation processes to improve efficiency and reduce costs. These advancements could make bacterial SCP a viable solution for global food security and an economically accessible protein source for both human and animal consumption.

2.2. Yeast

The production of SCP from yeasts is a promising approach to meet the growing demand for alternative and sustainable protein sources. Yeasts, such as *Saccharomyces cerevisiae* [41], *Candida intermedia* [42], and *Yarrowia lipolytica* [41], are widely utilized in SCP production due to their high growth rates and ability to metabolize various substrates such as papaya waste [43], sugar cane bagasse [44], molasses [16] and lignocellulosic hydrolysates [42]. The nutritional potential of those microorganisms is important for their potential applications; for example, Saccharomyces cerevisiae is widely applied in the food industry for fermentative purposes, but its extract is also edible and rich in proteins, B vitamins, and fibers, being a safe and nutritious alternative for protein diets [45].

Despite these advantages, using yeasts for SCP production presents some challenges. One of the main drawbacks is the high level of nucleic acids present in yeast cells, similar to bacterial SCP, which can limit direct human and animal consumption due to potential health risks, such as gout [46]. Also, undesirable flavors may reduce consumer acceptance, requiring additional purification processes or flavor enhancers to improve the final product's taste [47]. Furthermore, large-scale SCP production from yeasts may need economic challenges. Although the cost is lower than traditional animal protein sources, it is still higher than other microorganisms, such as bacteria [32].

Current research into yeast SCP aims to address these challenges, including selecting and improving strains with lower nucleic acid content, optimizing fermentation processes, and developing new methods to enhance the flavor and texture of the final product [31,47]. Emerging technologies, such as genetic engineering, are also being explored to improve yeasts' amino acid and vitamin profiles, making them more nutritionally appealing. These yeast SCP are also rich in bioactive amino acids such as arginine, asparagine, and histidine, which are important healthy cofactors [48]. However, challenges like nucleic acid removal [31] and sensory improvements must be addressed to make large-scale use feasible and increase consumer acceptance.

2.3. Filamentous Fungi

The production of SCP from filamentous fungi, or mycoprotein, offers a sustainable protein source, leveraging their high protein content, rich amino acid profiles, and ability to grow on diverse, low-cost substrates. Filamentous fungi, such as *Fusarium venenatum* and *Aspergillus oryzae*, can be used in SCP production. Due to its meat-like texture and substantial protein concentration, *F. venenatum* is remarkably used to manufacture mycoprotein products like Quorn™, a meat substitute popular in several countries [49]. These fungi are known for their efficient biomass production and high protein quality. They contain essential amino acids beneficial for human and animal nutrition, such as arginine, histidine cysteine, and other essential amino acids [32,50].

One of the main advantages of using filamentous fungi for SCP production is their ability to grow lignocellulosic biomass by the production of cellulolytic enzymes [26,51], including agricultural and industrial by-products, such as food waste [52], stickwater [36], and lignocellulosic byproducts [26]. This substrate versatility aligns with sustainable practices, as these processes can help recycle organic waste materials by bioconversion in edible protein [13,51]. The fungal growth process also tends to be robust, with filamentous fungi capable of withstanding various cultivation conditions, allowing for relatively high biomass yields even under suboptimal conditions.

The production of SCP from filamentous fungi typically occurs through submerged or solid-state fermentation processes, with parameters such as temperature, pH, and oxygen levels that optimize fungal growth and protein production, for example, in 25 °C, 150 rpm and using glucose as carbon source, the RNA content in *Fusarium oxysporum* mycoprotein may be reduced [53]. Fungi's unique filamentous structure allows them to produce fibrous biomass with a texture resembling meat, making them particularly suitable for developing plant-based meat analogs. For example, *Fusarium venenatum* biomass, when analyzed under a microscope, has a texture and appearance similar to meat products, enhancing its acceptance as a meat substitute in consumer markets [49].

However, using filamentous fungi in SCP production also presents particular challenges. One primary concern is the potential presence of mycotoxins, toxic secondary metabolites that some fungal species can produce [32], for example, *F. venenatum* may produce fumonisins in low amounts (8.6 μg/kg), which may be enhanced by the moisture content of the production media medium [54,55]. Also, citrinin, a carcinogenic mycotoxin, may be produced by *Aspergillus* and *Penicillium* that contaminate some foods [56]. Additionally, the taste and color of fungal biomass can be off-putting to some consumers, requiring further processing to improve sensory characteristics [57].

2.4. Algae

Microalgae, such as *Spirulina platensis* and *Chlorella vulgaris*, present high-protein content (>50%) [27,58], and the presence of non-essential amino acids such as aspartic acid, glutamic acid, and cysteine [14], and abundant essential nutrients, including vitamins, minerals, and polyunsaturated fatty acids [27,58]. Algae-based SCP offers unique advantages due to the high productivity of microalgae, which can be grown in various environments, including fresh and saltwater, and which have the potential to fix carbon dioxide, making the process environmentally beneficial [27], and also resulting in protein-rich foods which present high potential as substitutes for seafood [59].

Microalgae can achieve high growth rates (1.48 day^{-1}) and present a final biomass per substrate yield of 0.45 ($Y_{x/s}$) [29], producing substantial biomass yields within short periods. One primary issue is the high initial infrastructure cost, especially for photobioreactor systems, which require significant investment and energy input to maintain optimal light exposure and nutrient conditions. However, the major costs are related to the CO_2

supply under industrial levels [60]. Moreover, some microalgae, like *Arthrospira* (*Spirulina*), *Galdieria*, and Chlorella, may present undesirable flavors, colors, and odors due to high chlorophyll, aldehydes, ketones, and geosmin content [29,61,62]. Additional processing is often required to make biomass more acceptable for consumption, which can increase production costs.

Advances in strain selection, genetic engineering, and bioprocess optimization aim to increase protein yield, improve nutrient profiles, and reduce production costs. With its high nutrient content and minimal environmental impact, algae-based SCP holds great potential as a sustainable protein source, particularly as a functional ingredient in human and animal diets [11].

3. SCP Production

Many microorganisms, such as fungi and algae, are rich in essential amino acids such as histidine and cysteine [14,60]. They can be cultivated to achieve a protein content of over 50%, making them comparable to traditional animal proteins. Studies indicate that microalgal proteins can meet essential amino acid requirements while providing bioactive compounds that promote health benefits like antioxidative and anti-inflammatory effects [24].

Through SCP production, microbial protein offers a high-protein alternative to conventional protein sources, such as livestock, which contributes significantly to greenhouse gas emissions, land use, and water consumption. In contrast, microorganisms like fungi, bacteria, and algae can be cultivated on various substrates. The unique ability of microorganisms to grow on unconventional substrates, such as lignocellulosic biomass and organic waste, further supports circular economy initiatives by converting waste into valuable protein, thus reducing both waste generation and the ecological footprint associated with traditional protein sources [63].

Through controlled fermentation, microorganisms convert carbon and nitrogen sources into high-protein biomass. The study highlights the importance of optimizing key variables such as temperature, pH, nutrient availability, oxygen levels, and agitation to maximize protein yield and quality [64]. Statistical methods like Response Surface Methodology and factorial designs have proven effective in fine-tuning these conditions, improving production efficiency and cost-effectiveness of protein extraction [27].

The applied methdologies for SCP production vary from strain to strain. For example, the growth of *M. capsulatus* occurs in anaerobic reactors with a temperature under 42 °C and with a slightly acid pH (6.3) [65]. Fungi, such as yeast and filamentous fungi, usually grow under lower temperatures ($\leq$30°C) [42,66] and in a more acidic pH (<6.0) [54,67]. For algae SCP production, photobioreactors are usually used [68].

Sankar et al. [24] discuss the role of bacteria in sustainable SCP production, utilizing microorganisms such as *Methylococcus capsulatus* and *Bacillus subtilis* to convert low-cost substrates, including methane and agricultural byproducts, into high-quality protein. This method mitigates environmental impacts associated with conventional meat production by reducing greenhouse gases and effectively using waste products. Bacterial SCP serves as a nutrient-dense protein source, often containing over 50% protein by dry weight, and can be cultivated on various substrates, allowing adaptability for applications in human and animal nutrition. The production process incorporates purification steps to remove nucleic acids, enhancing SCP's safety and digestibility, which supports its viability for commercial applications.

Rashid et al. [69] examined the role of purple non-sulfur bacteria in sustainable SCP production, emphasizing the unique metabolic features that enable these bacteria to grow under diverse conditions, utilizing waste carbon sources efficiently. Those non-sulfur

bacteria, such as *Rhodopseudomonas palustris* and *Rhodobacter sphaeroides*, grow under photoheterotrophic conditions, offering an environmentally friendly method for transforming organic waste into edible protein. PNSB's capacity to utilize infrared light for photosynthesis allows them to thrive in mixed microbial cultures, facilitating cost-effective biomass production. Their metabolic versatility and ability to improve water quality make them a promising SCP source, especially for aquaculture feed, while aiding environmental sustainability by reducing waste and recycling nutrients. Also, Ojima et al. [70] also investigated *Rhodopseudomonas* and *Rhodobacter* for their benefits in protein production and pollutant removal. Their photoheterotrophic growth enables them to thrive in high-strength wastewater, allowing effective resource recovery with minimal need for dilution or pre-treatment. The protein content of PNSB can reach up to 70%, and they also contain beneficial biomolecules like carotenoids and coenzyme Q10, enhancing their nutritional value for aquafeed applications. The study also highlights that PNSB-based SCP can improve growth and immunity in aquaculture species without the toxicity concerns associated with other bacteria.

Saccharomyces cerevisiae, *Candida utilis*, and *Kluyveromyces marxianus* are prominent microorganisms for Single-Cell Protein (SCP) production due to their adaptability to various substrates, high protein content, and amino acid profiles comparable to traditional proteins. This versatility makes yeast-based SCP an economically viable and nutritionally valuable source of protein, particularly in regions with limited conventional protein supplies. Industrial processes for yeast achieves high yields and protein content, with additional steps for purification and drying to enhance storage stability and product quality [24].

Liu et al. [41] conducted a comprehensive study evaluating the SCP production potential of different yeast strains, presenting findings highly relevant to food engineering applications. Key strains evaluated included *Saccharomyces cerevisiae* as a control, alongside *Yarrowia lipolytica* and various *Pichia* species, known for their robustness and nutritional value. Experimental data revealed that *Pichia* spp. strains, particularly *P. jadinii*, achieved high protein content, with cellular protein levels peaking at 57.17% during the log phase. This protein concentration surpasses that typically achieved by *S. cerevisiae*, whose log-phase protein content reached approximately 52.69%. The amino acid profiles uncovered a high methionine content in *Y. lipolytica* strains, roughly four times that in *S. cerevisiae* and *Pichia* spp. strains. This finding highlights *Y. lipolytica* as a potential SCP source with enhanced essential amino acid (EAA) content, which is important for applications where nutritional quality is paramount. Furthermore, *P. jadinii* demonstrated resilience under adverse growth conditions (37 °C and pH 4.0), maintaining a cellular protein content of around 50.18–52.66%, underscoring its feasibility for SCP production under less controlled industrial settings. Such resilience reduces costs associated with temperature and pH regulation, presenting a sustainable and economically advantageous SCP source for food engineering. The study's computational analyses, involving metabolic efficiency metrics and amino acid biosynthesis pathways, further support the strategic selection of non-conventional yeasts, like *Y. lipolytica* and *Pichia* spp., for optimized SCP production in industrial applications.

Canedo et al. [71] conducted a study on the protein enrichment of brewery spent grain (BSG) using *Rhizopus oligosporus* through solid-state fermentation (SSF), demonstrating its potential as a valuable protein source in animal feed. To optimize the protein yield, they explored various nitrogen sources—ammonium sulfate, urea, and sodium nitrate—and initial moisture levels (50%, 60%, and 70% *w*/*w*). Notably, the crude protein content of BSG increased from 17.96% to up to 32.90% with ammonium sulfate at 70% moisture, representing nearly a two-fold enrichment compared to unfermented BSG. The study also reported an approximately four-fold increase in soluble protein content. SEM analysis

showed successful fungal colonization on BSG surfaces, which is essential for effective protein conversion. Overall, the results validate the use of *R. oligosporus* in SSF as an efficient method to enhance BSG's nutritional profile, supporting its application in sustainable animal feed production.

Hezarjaribi et al. [72] studied the optimization of SCP production using *Saccharomyces cerevisiae* PTCC5269 in a submerged batch bioprocess, identifying a culture medium composition that maximized cell biomass and protein content. They employed a fractional factorial design and signal-to-noise (S/N) ratio analysis to determine optimal levels of key nutrients. The highest cell count achieved was 8.84 log CFU/mL, using a medium with 0.3 g/L ammonium sulfate, 0.15 g/L iron sulfate, 1 g/L glycine, and 50 g/L glucose at 300 rpm and 35 °C. Glycine and glucose concentrations were the most influential, contributing 39.32% and 36.15%, respectively, to biomass production. Interaction analysis revealed the highest interaction between ammonium and iron sulfate (50.71% severity). At optimal conditions, dried biomass contained 44.6% protein, supporting the commercial viability of *S. cerevisiae* SCP as a nutrient-rich food source.

Bertasini et al. [48] investigated *Saccharomyces cerevisiae* using candy production effluents (CPE) and agricultural digestate as substrates. They performed batch and continuous tests, revealing that aerobic conditions significantly improved SCP yield. In aerobic batch trials, cell counts reached 3.90×10^7 cells, with an SCP concentration of 1.95 g/L and a protein content of 18.63%. Continuous aerobic testing optimized biomass productivity at a dilution rate (D) of 0.50 d^{-1} (2-day hydraulic retention time, HRT), achieving 28% w/w. Amino acid analysis showed suitability for fish and monogastric animal productivity of 0.25 g/L per day and protein content feed. Still, it indicated deficiencies for pet feed applications, demonstrating SCP's promise as an animal feed supplement within agricultural biorefineries.

Khan et al. [73] explored the production of SCP from agricultural peel waste—specifically pea, potato, and banana peels—using *Aspergillus flavus* NRRL 21882. Their solid-state fermentation experiments demonstrated a high protein yield, with pea peels producing the most SCP at 60.67% crude protein content. Amino acid analysis revealed significant levels of aspartic and glutamic acids in SCP derived from pea peels, making this protein source nutritionally valuable. Additionally, when this SCP was incorporated into poultry diets alongside soybean meal, it improved antibody response to the Newcastle disease vaccine without affecting liver enzymes, indicating the SCP's safety and efficacy as a protein supplement for poultry feed.

Babazadeh et al. [74] explored SCP production using *Claveromycice frajilice* and *Fusarium oxysporum* in Kilka fish meal stick water as a growth medium. They conducted experiments with treatments at 50% and 100% stick water concentrations, observing that *C. frajilice* yielded a protein content of 55.35% in 50% stick water and 57.47% in 100% stick water, while *F. oxysporum* produced 53.17% and 54.39% protein, respectively. These results exceeded the protein levels in control groups, suggesting that stick water effectively supports SCP production. Amino acid profiles of the SCP matched the essential amino acid requirements set by FAO/WHO, highlighting its potential as a protein source in animal feed, especially in aquaculture applications.

Upcraft et al. [13] explored mycoprotein production using *Fusarium venenatum* grown on glucose derived from rice straw, demonstrating its potential as a sustainable protein source. They employed food-grade ionic liquids for glucose extraction, achieving a glucose yield of 42.4% with food-grade ionic liquids ([Ch][HSO_4]), compared to 92.8% with non-food-grade [TEA][HSO_4]. Their techno-economic analysis revealed a production cost of $5.04 per kg of crude mycoprotein paste. Life cycle assessment (LCA) showed substantial environmental benefits, with greenhouse gas emissions at less than 14% of those for

beef protein. This approach highlights mycoprotein's viability as a low-carbon protein alternative, particularly advantageous for minimal land usage compared to traditional animal-based proteins.

Lee et al. [55] investigated the potential of *Fusarium venenatum*-based microbial protein as an anti-obesity supplement through multi-omics analysis in *Caenorhabditis elegans* and mice models. The study found that *F. venenatum* significantly extended the lifespan of *C. elegans* by reducing fat accumulation through the downregulation of fat synthesis genes (e.g., *POD-2*, *FASN-1*) and the upregulation of fat breakdown pathways. In mice, dietary supplementation with *F. venenatum* improved lipid profiles, reduced hepatic fat, and increased anti-inflammatory cytokines, demonstrating its potential as a sustainable protein source with anti-obesity effects.

Risner et al. [75] conducted a techno-economic analysis on mycoprotein production using *Fusarium venenatum*, evaluating the high-scale SCP production with fermentation vessels, using glucose as a carbon source and heat treatment for RNA reduction content. Utilizing airlift bioreactors, the study highlights that continuous operation over batch fermentation improves productivity fivefold. The projected production cost for mycoprotein was approximately USD 3.55 per kilogram, comparable to beef on a protein basis, although less competitive than poultry. Sensitivity analysis identified the cost of growth media and minor growth factors like biotin and zinc sulfate as key cost drivers, with potential savings if replaced by lower-purity ingredients. This study underscores the economic feasibility of mycoprotein as a sustainable protein source, emphasizing continuous bioreactor operation to meet global protein demands with reduced environmental impacts.

Putri et al. [58] used food processing wastes as a medium for *Chlorella* sp. cultivation to produce SCP. The study tested tofu, tempeh, and cheese whey wastes, varying concentrations (10–50%) in seawater as the growth medium. The highest cell concentration (42.5×10^6 cells/mL) and protein content (52.32%) were achieved using 50% tofu waste. Tempeh waste at 30% yielded similar protein levels (52%) but lower cell density. Conversely, cheese whey at 10% produced significantly lower cell growth and protein (15.43%), likely due to its high lactose and nitrate content, which inhibited growth. This research supports tofu and tempeh waste as effective substrates for SCP production, offering an eco-friendly solution to reducing waste.

Beet filter cake extract (BFCE) from the beet sugar industry may be a cost-effective growth medium for *Spirulina platensis* cultivation [68]. In batch experiments, a maximum dry weight of 0.34 g/L at 75% BFCE concentration was achieved, close to the 0.4 g/L yield with the standard Zarrouk medium (SZM). The highest protein content recorded in BFCE was 46.5% at 25% concentration, compared to 50% in SZM. Using response surface methodology, growth conditions were optimized with 33% BFCE, achieving a protein content of 52.5% and biomass yield of 0.56 g/L, nearly matching SZM performance, highlighting BFCE's viability as an alternative medium for sustainable SCP production.

Also, the production of protein-rich biomass from *Spirulina platensis* cultivated in beet vinasse-supplemented culture media was performed, focusing on both batch and continuous operations within an airlift tubular photobioreactor [34]. Optimal conditions included a light intensity of 72 μmol photons m^{-2} s^{-1} and 1 g/L vinasse concentration, yielding a maximum cell concentration of 6.5 g/L and protein productivity of 168 mg/L/day. Higher vinasse concentrations (2 g/L) reduced productivity due to decreased light penetration, while betaine within vinasse was efficiently utilized as a nitrogen source, enhancing mixotrophic growth. These findings demonstrate beet vinasse's viability as a substrate for sustainable *S. platensis* SCP production.

The algae *Chlorella* sp. cultivation using food processing waste was also efficient for SCP production to SCP [58]. The study tested tofu, tempeh, and cheese whey wastes,

varying concentrations (10–50%) in seawater as the growth medium. The highest cell concentration (42.5 $\times$ 10^6 cells/mL) and protein content (52.32%) were achieved using 50% tofu waste. Tempeh waste at 30% yielded similar protein levels (52%) but lower cell density. Conversely, cheese whey at 10% produced significantly lower cell growth and protein (15.43%), likely due to its high lactose and nitrate content, which inhibited growth. This research supports tofu and tempeh waste as effective substrates for SCP production, offering an eco-friendly solution to reducing waste.

The SCP, through the utilization of industrial agriculture, also presents advantages regarding water footprint, land use, and biodiversity impact [6,46,76]. Cultivating yeast and bacteria tends to consume less water than livestock, requiring less land use [76]. Also, the growth of microorganisms does not require the environmental impact caused by the cattle and fish industry, as it may be contained in laboratories and factories. Thus, new technologies have been also seeking new and more environmentally friendly approaches, such as photovoltaic-driven Single-Cell Protein (PV-SCP) production, which may use land and sunlight more efficiently [77]. PV-SCP achieves protein yields exceeding 1200 $g \cdot m^2 \cdot y^{-1}$, a ten-fold increase compared to soybeans, the leading protein-yielding crop at approximately 115 $g \cdot m^2 \cdot y^{-1}$. The studied systems presented efficient water and nitrogen utilization. Unlike crops, which have water and nitrogen uptake efficiencies (~50%), PV-SCP systems have also presented a minor water use, consuming ~100 and ~10,000 times less water than plants and animals, respectively [77].

4. Life-Cycle Assessment

Another important analysis is the Life-cycle assessment (LCA) which may provide an evaluation of the environmental impacts of Single-Cell Protein (SCP) production.

Kobayashi et al. [78] focused on yeast-based SCP using oat side-streams, highlighting that energy consumption significantly influences environmental performance. Although SCP production exhibited 61% lower land use compared to soy protein, it demonstrated higher impacts on water consumption and eutrophication due to energy-intensive drying processes. Thus, though SCP production is usually less harmful than other protein production methods, new technologies can enhance its potential even further.

Also, SCP production is derived from the filamentous fungus *Paecilomyces variotii*, cultivated on forest industry residues for aquafeed, resulted in a 73% reduction in biodiversity impacts and halved greenhouse gas emissions compared to soy protein, as reported by LCA analysis [79]. However, energy demand remained higher due to processing steps, such as drying and grinding, which may be attenuated by the application of renewable energy sources, such as photovoltaic-driven SCP production [77].

Thus, SCP production addresses food security and environmental sustainability. The use of alternative substrates and clean and renewable energy sources may reduce the environmental impact even further, with upstream processes often being the primary contributors [80]. Also, the type of substrate and pre-treatment significantly influence the environmental profile, with electricity being a common hotspot across all systems. Thus, future research should focus on improving SCP systems' efficiency and scalability, particularly for human food applications, to fully realize their potential in reducing the environmental footprint of protein production.

5. Challenges in Industrial-Scale SCP Production

Global protein demand is expected to reach 1250 million tons of meat and dairy by 2050, necessitating efficient, sustainable protein sources like Single-Cell Protein (SCP). Derived from microorganisms such as bacteria, fungi, and algae, SCP production is more efficient than traditional animal protein, where 6 kg of plant protein yields just 1 kg of

meat. SCP can offer 30–80% protein content, with algae like *Arthrospira platensis* (Spirulina) reaching 60–71% and fungi like *Fusarium venenatum* (used in Quorn products) around 44%. Additionally, SCP production from algae can consume CO_2, while methane-utilizing bacteria convert greenhouse gases, achieving productivity levels of up to 4 kg/m^3/h. Despite promising efficiency and environmental benefits, the industrial feasibility of SCP production is still discussed, as reducing nucleic acid content and scaling cost-effectively are fields that still need to be enhanced by new technologies or alternative methods [81].

The protein content in SCP typically ranges between 30–65% dry weight, with bacteria like *Methylococcus capsulatus* reaching up to 65% and fungi like *Fusarium venenatum* between 30–45%. Innovations in genetic engineering, such as CRISPR-Cas and metabolic pathway optimization, allow for enhanced protein yield and quality, adapting SCP for food and feed uses [46]. Industrial-scale production of SCP has garnered significant attention as a sustainable solution to meet the escalating global demand for protein. The scalability of SCP production hinges on factors such as microbial strain selection, substrate availability, fermentation technology, downstream processing, and economic viability.

5.1. Choice of Microorganism

The choice of microorganism is fundamental in industrial SCP production. Yeasts like *Candida utilis* and *Saccharomyces cerevisiae* are favored due to their high protein content, rapid growth rates, and ability to utilize diverse substrates. Filamentous fungi, such as *F. venenatum*, contain approximately 45% protein by dry weight. Bacterial strains, including *M. methylotrophus*, have been explored for their capacity to metabolize methanol, achieving protein yields of up to 70% of their dry biomass. Microalgae, notably *Spirulina* and *Galdieria*, are also utilized, with protein contents ranging from 60–70% dry weight.

Recent advancements in genetic engineering microorganisms may also enhance productivity; for example, *Pichia pastoris* has significantly enhanced Single-Cell Protein production. The engineered strain demonstrated high protein content (0.506 g/g dry cell weight, DCW) and efficient methanol conversion (0.43 g DCW/g) in a pilot-scale fed-batch culture at 33 °C. Biomass levels reached 63.37 g/L DCW, achieving 86% of the theoretical maximum yield. Comparative data highlight that this SCP yield surpasses traditional sources: SCP from *P. pastoris* contains 50.6% protein, while soy, fish, and meat typically contain 38.6%, 17.8%, and 21.2% protein, respectively [23].

Factors like temperature, pH, nutrient concentration, and oxygen levels are key to optimizing SCP production, directly influencing yield and protein quality. However, challenges still need to be addressed regarding large-scale production, safety, and cost for ongoing research and development [64]. For example, the presence of mycotoxins, such as citrinin and fumonisins, may be evaluated [54,56]. The high RNA content in fungi and algae has to be addressed. Thus, a thermic treatment may be applied, reducing it to less than 1% to be considered safe [53,82]. Finally, cell wall digestibility is also a problem to be addressed; for example, yeast and algae usually present low digestibility, though they are easier to separate [83] thus, genetic modifications or strain selection may be made regarding the protein accessibility by human and animal guts, for example, *G. sulphuraria* presents a high digestibility, as its amino acids bioaccessibility is higher than 70% [29].

5.2. Substrate Usage

Substrate costs significantly influence the economic feasibility of SCP production. Utilizing inexpensive and readily available substrates, such as agricultural residues, industrial by-products, and waste streams, can reduce production expenses. For instance, the use of lignocellulosic biomass, which is abundant and cost-effective, has been investigated for SCP production [51]. However, the complex structure of lignocellulosic materials

necessitates pretreatment processes to enhance microbial accessibility, which can add to production costs. Methanol and methane have also been employed as carbon sources, with certain bacteria capable of converting these substrates into protein-rich biomass. The utilization of industrial off-gases through a coupled fermentation approach has been explored, demonstrating the potential for SCP production from steel mill off-gas via acetate, with techno-economic analyses indicating feasibility under optimized conditions.

Producing SCP from industrial off-gas via a coupled fermentation process that utilizes acetate as an intermediate demonstrates promising techno-economic viability. Key metrics show that in a pilot model, SCP production achieved a total acetate productivity of 1.0 g/L/h and a final SCP biomass productivity of 2.0 g/L/h, with *C. necator* reaching a biomass concentration of 14 g/L in the acetate-to-SCP fermentation stage [84]. The capital investment required for a facility capable of producing 20,000 metric tons per year is estimated at USD 320 million, with a production cost of 4.15 USD/kg. Sensitivity analyses reveal that increasing acetate productivity to 4 g/L/h and concentration to 45 g/L can reduce costs to 2.78 USD/kg, yielding substantial economic benefits and optimizing the use of resources and energy. This model exemplifies the potential of SCP production from renewable resources, offering a sustainable alternative for protein-rich feed in food and agriculture industries.

Using *Rhodococcus opacus* strains DSM 1069 and PD630, Single-Cell Protein (SCP) production from agro-waste such as citrus (lemon and orange) and corn stover was evaluated. Notably, strain PD630 grown on lemon waste achieved a cell dry weight (CDW) of 3.28 g/L and protein content of 52.1%, while the same strain on orange waste reached a CDW of 2.32 g/L and a higher protein content of 56.9%. Corn stover provided a CDW of 2.20 g/L with a protein content of 52.7%. Strain DSM 1069 demonstrated lower protein yields across these substrates, with its highest protein content of 47.0% observed when grown on corn stover [85].

5.3. Reactor Design and Downstream

The design and operation of bioreactors are significant in the production of Single-Cell Proteins (SCP), necessitating meticulous attention to oxygen transfer rates, mixing efficiency, and contamination control. In aerobic fermentation processes, it is important to control oxygenation in the medium [20], and the same may be said of anaerobic production [21], as CO_2 feeding is still one of the most costly steps for the scalability of certain protein production, from algae for example [60].

The scalability of these systems is a fundamental factor in transitioning from laboratory to industrial-scale production. Post-fermentation processing is essential to recover and purify SCP, which may also be optimized, enhancing the protein extracted and its bioavailability [27,60]. Energetic costs are also necessary, such as in the case of mycoprotein; as a heated step, the removal of RNA is usually applied [75]. Innovations in downstream processing aim to enhance efficiency and reduce costs, thereby improving the economic viability of SCP production.

Substrate costs, fermentation efficiency, downstream processing expenses, and market demand influence the economic viability of industrial SCP production. Techno-economic analyses have been conducted to assess the feasibility of SCP production from various substrates and processes. For example, the production of SCP from industrial off-gas through acetate has been evaluated, with findings suggesting that process intensification of the gas-to-acetate fermentation can lead to significant cost reductions. Optimization strategies, such as metabolic engineering and process optimization, have been explored to enhance SCP production efficiency and reduce costs [12,84].

Continuous fermentation systems, such as Continuous Stirred Tank Reactors (CSTRs), are commonly employed to maintain steady-state conditions, thereby enhancing productivity. Modeling and economic optimal control strategies have been developed for laboratory-scale CSTRs to optimize SCP production, focusing on parameters like biomass growth and pH tracking [19].

The optimization of protein extraction and purification processes is also important for enhancing the quality and functionality of SCP. For example, in the case of *Galdieria sulphuraria*, protein content varied significantly based on cultivation and extraction methods, reaching up to 64% (*w*/*w*) under mixotrophic conditions [60]. Similarly, in *Spirulina* (*Arthrospira platensis*), ultrasound-assisted extraction under alkaline conditions yielded a protein-rich extract (50–70%) with high emulsifying capacity and stability, which may be used in the food industry [27]. Protein yields were improved significantly when employing isoelectric precipitation as part of the purification process. In the case of mycoprotein, the downstream may occur by centrifugation and filtration, or in the case of recombinant protein, by spray drying and ultrafiltration [86].

6. Sensory Aspects and Consumer Acceptance

Research on consumer acceptance of alternative proteins identifies several primary factors influencing acceptance. Key drivers include motivations for food choices, such as health and environmental benefits, along with factors like food neophobia, cultural norms, and familiarity with novel protein sources. Interventions aimed at increasing acceptance often involve educational efforts, persuasive messaging, and experiential exposures designed to reduce neophobia and increase consumer familiarity with these options. The review highlights the effectiveness of framing information positively and pairing educational content with actionable incentives or social modeling. Additionally, legislative frameworks for alternative proteins vary globally, with regulatory inconsistencies posing challenges to market growth and consumer trust in these novel foods [87].

Lähteenmäki-Uutela et al. [88] discuss the legislation surrounding Single-Cell Proteins (SCPs) for food, particularly within the European Union, which is primarily governed by the Novel Food Regulation (EU/2015/2283) [89]. This regulation mandates pre-market approval for foods not significantly consumed in Europe before 1997. SCP products, such as those derived from microorganisms, are evaluated for food safety, addressing concerns, such as high RNA content, which must be reduced, and potential contaminants. Additionally, if the microorganisms used are genetically modified, the Genetically Modified Food Regulation applies [90], requiring proper labeling and scientific assessment of its safety.

Consumer acceptance of plant-based meat substitutes hinges on sensory experience, health perceptions, environmental awareness, and pricing. While advances have been made about the taste of SCP, with plant-based products often rated less favorably than traditional meat in flavor and texture, consumers motivated by health and environmental concerns show greater openness to trying these alternatives. Food neophobia (which is an aversion to new foods) and attachment to meat are key barriers, especially among older consumers and those less familiar with plant-based options. Effective marketing strategies emphasize health benefits, clear labeling, and environmental impact, mainly when products achieve price parity with meat [91].

Protein standardization methods may include mass cytometry, immunofluorescence, and surface-enhanced Raman spectroscopy, each with distinct strengths and limitations for in situ protein analysis at the single-cell level. Mass cytometry offers high multiplexing capability, allowing for the study of complex cellular processes, although it is limited in providing detailed spatial information due to its destructive cell processing. Immunofluorescence enables more accessible and cost-effective analysis but faces challenges related

to multiplexing capacity and spectral overlap. Surface-enhanced Raman spectroscopy is notable for its high spectral resolution, though it has limited multiplexing ability and high sensitivity to experimental conditions. These advancements present promising potential for clinical diagnostics and prognostics, enhancing the understanding of core biological processes with applications in cell biology, pathology, and biomedicine [92].

You et al. [93] evaluate the potential of mycoprotein as an ingredient in high-protein nutrition bars. Replacing whey protein with mycoprotein at different levels (10%, 20%, and 30%) showed that while mycoprotein adds valuable dietary fiber and a fresh, mushroom-like odor, its effects on texture and digestibility varied. Bars with higher mycoprotein levels exhibited significant hardening over time, which could affect consumer acceptability. Additionally, bars with higher mycoprotein content demonstrated lower protein digestibility, especially during the intestinal phase, likely due to the fibrous structure of the mycoprotein. Sensory evaluations revealed that while bars with lower levels of mycoprotein substitution maintained desirable sensory qualities, higher levels impacted texture and taste. The findings suggest that moderate levels of mycoprotein can improve nutritional value and maintain sensory quality, offering a sustainable alternative protein option in nutrition bars.

The preparation of SCP may also influence its final sensory characteristics, as different drying methods may result in different flavored and odored algae proteins [94]. Agitated thin film drying (ATFD) intensified the earthy aroma due to lipid oxidation products like 1-octen-3-ol. In contrast, pulse combustion drying (PCD) enhanced cacao-like odors through the Maillard reaction, producing Strecker aldehydes and furans. Freeze drying (FD) resulted in a milder flavor profile with the lowest odor intensity and volatile organic compound (VOC) concentrations. These findings highlight the importance of tailoring drying methods to optimize sensory properties for specific applications of microalgal proteins in innovative foods.

The usage of SCP may also influence the sensory characteristics of animals fed with it. Moroni et al. [95] demonstrated that replacing fishmeal with Single-Cell Protein (SCP) from *Methylococcus capsulatus* influenced the sensory attributes of European sea bass fillets, with notable differences between wild-type (WT) and genetically selected (HG) fish. Sensory analysis using an electronic tongue revealed that WT fillets exhibited more significant variability in taste profiles across diets compared to the more consistent profiles of HG fish, highlighting the stabilizing effect of genetic selection. Despite dietary changes, the electronic nose detected no significant differences in volatile compounds, suggesting that aroma, a key sensory attribute, was unaffected. These findings emphasize that when combined with genetically selected fish, SCP diets can maintain sensory quality while advancing sustainability in aquafeeds.

Also, a comparison between meat and Single-Cell Protein presents nutritional differences. Beef presents a high-calorie density and microfibrillar proteins in comparison to microbial protein, which may result in a higher consume to match the same caloric intake [75]. However, SCP presents higher amino acids content [60], which results in a higher nutritional potential, which is important for consumer acceptance [96].

7. Recent Technological Advances and Industrial Applications

SCPs, produced from microorganisms such as algae, yeast, and bacteria, are notable for their high protein yield, rapid production cycle, and minimal environmental impact. The paper emphasizes optimizing production variables—such as temperature, pH, nutrient profile, and oxygen levels—to maximize yield and product quality. Techniques like Response Surface Methodology and Design of Experiments are highlighted for their efficacy in fine-tuning these parameters, enabling efficient SCP production with reduced experimental trials. The use of computational models, particularly Monod kinetics, further

supports SCP production by providing predictive insights into microbial growth dynamics, essential for scale-up processes. This comprehensive optimization approach positions SCP as a viable, sustainable alternative protein source, addressing industrial challenges like nucleic acid reduction to improve human and animal consumption digestibility by applying treatments [75] or enhancing the extraction process [29].

Metabolic engineering is central to optimizing SCP productivity and quality. It employs rational strategies like pathway optimization and non-rational methods like adaptive evolution to enhance biomass and protein yield. These approaches enable microbial strains to efficiently utilize diverse carbon sources, including methane and carbon dioxide, further minimizing environmental impact. SCP demonstrates versatility across food, feed, and industrial applications, positioning it as a promising alternative to meet global protein demands sustainably and efficiently [97].

SCP production from microorganisms and algae leverages sustainable carbon sources, such as CO_2 and methane, and uses various industrial waste streams, such as food waste [17,58], thus reducing environmental impact. Precision fermentation methods also have enhanced SCP feasibility, for example, metabolic engineering have improved the protein yield and the bioavailability [46]. Emerging technologies now support using mixed microbial populations rather than pure strains, enhancing process efficiency, for example, *Lactobacillus acidophilus* and *Aspergillus niger* [36], and also the coproduction of other bioproducts parallel to SCP synthesis, for example, xylitol, a polyol used as sweetener [42], and polyhydroxyalkanoates. Additionally, metabolic and genetic engineering improve microorganism efficacy in converting substrates into high-value proteins. These approaches make employing renewable and inexpensive resources feasible, establishing SCP as a viable alternative for human consumption and animal feed. SCP holds substantial potential to meet the global demand for protein sustainably and economically [81].

However, some industrial advances must be made to further incentive the production of SCP; for example, the risk of contamination is constant, as undesirable microorganisms may produce toxic or allergenic substances, as citrinin and other mycotoxins for example [56]. These problems may be solved by the use of GRAS microorganisms [32,76]. Also, the structure of microbial protein may be different from meat ones which may result in a lower bioavailability, which may be increased by precision fermentation [30], enzymatic treatments [98], and optimization methods [64]. Additionally, the industrial production of SCP may impact animal husbandry, as it may decrease meat consumption, reducing environmental stress [11]; however it may also be used as animal feeding [40].

8. Patents

An important factor in developing an industrial sector is the number of patents deposited, which indicates the recent advances regarding a process's scalability and industrial feasibility. Thus, several patents have been addressed in the past decades regarding applying microorganisms for protein production (Table 2). These scientific advances have enhanced the industrial scale of SCP production, the nutritional profile, and the protein flavor.

Table 2. Recent patents regarding the production of Single-Cell Protein for food applications.

Patent	Applicant Origin	Microorganism	Source	Year	Application	Reference
EP0074123A2	United States	*Pichia pastoris*	Yeast	1981	Increasing the nutritional potential of protein from yeast	[99]
WO2018029353A1	The Netherlands	Thermophilic fungi	Filamentous fungi	2018	Methods for the production of SCP from thermophilic fungi	[100]
US10856560B2	United States	*Clostridium*	Bacteria	2020	Utilization of anaerobic microorganism for protein production by gas fermentation	[101]
US20210392908A1	United States	Cupriavidus necator	Bacteria	2021	Production of high-protein foods from bacterial strains capable of fermenting CO_2	[102]
EP4309505A2	United States	*Fusarium*, *Rhizopus* and *Pleurotus*	Filamentous Fungi	2024	Production of a 40% (*w*/*w*) protein source from different fungi strains, maintaining low nucleic acid levels.	[103]

Shay and Wegner [99] describe a method for enhancing SCP production using mutant *Pichia pastoris* strains. These strains were developed to exhibit significantly increased methionine content, addressing the typical nutritional limitations of yeast proteins. The invention uses oxygenated hydrocarbon substrates like methanol in aerobic fermentation to cultivate these high-methionine yeasts. This eliminates or reduces the need for methionine supplementation in SCP products, lowering production costs and improving nutritional value. By disrupting regulatory mechanisms through mutagenesis, the yeast strains overproduce methionine, a breakthrough in SCP technology for industrial applications, particularly in food and feed industries. Also, Laat and Murillio [100] introduce an innovative process for producing Single-Cell Protein (SCP) from microalgae under optimized cultivation and processing conditions. This method integrates efficient harvesting and extraction techniques, ensuring a high purity level and preserving the extracted proteins' nutritional properties. The proposed application primarily targets the food industry, offering a sustainable and protein-rich alternative to address the growing global demand for alternative nutritional sources. Additionally, the patent emphasizes the commercial feasibility of large-scale production, highlighting its industrial significance and potential for scalability.

Another patent describes a method for producing animal feed using microbial biomass cultivated through gas fermentation. The microorganisms utilized are primarily anaerobic, Gram-positive strains, including species from the genus *Clostridium*, such as *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, and *Clostridium ragsdalei*. These microorganisms are cultured using gaseous substrates like CO, CO_2, and H_2, derived from industrial waste gases or syngas, making the process sustainable and resource-efficient. The microbial biomass produced has high protein content, typically exceeding 85% on a dry solids basis, and includes essential amino acids like methionine. The invention outlines a scalable approach for sterilization, centrifugation, spray drying, and blending with excipients to create nutritionally tailored animal feed [101]. On the other hand, these organisms are cultivated

using gaseous carbon sources such as CO_2, making the process environmentally sustainable by capturing and repurposing greenhouse gases. The protein products, including isolates, hydrolysates, and extracts, are processed into structured food compositions designed to replicate traditional animal meat's texture, flavor, and sensory qualities [102]. For specific protein profiles, it is possible to include other microbial proteins, such as from species like *Fusarium venenatum* or *Rhizopus oligosporus*.

A recent patent [103] introduces an innovative approach to sustainable food production using filamentous fungal biomass as high-protein food materials. The described fungi, including *Fusarium venenatum* (strain MK7), *Rhizopus oligosporus*, and *Pleurotus ostreatus*, are cultivated to form dense, cohesive biomass rich in protein (exceeding 40% by weight) while maintaining low RNA content (less than 8%). This low RNA level is important for mitigating adverse health effects associated with purine-rich diets. The biomats are designed to be versatile, with applications ranging from direct protein ingredients to processed food products like yogurt analogs or vegan alternatives. The patent also highlights using a scalable, energy-efficient bioreactor system that minimizes resource usage by eliminating the need for active aeration or complex agitation mechanisms. This system produces biomass with desirable structural properties for easy harvesting and further processing.

9. Conclusions

The findings of the article highlight the significant potential of Single-Cell Protein (SCP) as a sustainable alternative to traditional meat production. SCP production from microorganisms, including bacteria, yeast, fungi, and algae, addresses environmental concerns by reducing greenhouse gas emissions and utilizing waste materials. These microorganisms offer high protein yields. Recent advancements in SCP production include optimizing fermentation processes and using renewable substrates, such as wastewater, CO_2, CH_4, food waste, and agro-industrial waste. Innovations such as incorporating compounds like myoglobin and natural polymers have improved the flavor and texture of SCP, simulating traditional meat. Despite these advances, challenges still need to be addressed in scaling production cost-effectively and ensuring consumer acceptance. Key obstacles include addressing sensory attributes, reducing nucleic acid content, and ensuring compliance with regulatory standards. SCP is an important circular economy assessment, utilizing agro-industrial waste and byproducts and promoting environmental sustainability. SCP production technologies, such as photobioreactors and bioreactors, continue to evolve, enabling more efficient and cost-effective systems

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