Trends in Food Science & Technology 138 (2023) 577–598

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

Overview of single cell protein: Production pathway, sustainability outlook, and digital twin potentials

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ARTICLE INFO

Handling Editor: Dr AR Jambrak

Keywords:
Single cell protein
Sustainability assessment
Digital twin
Circular bioeconomy
Second generation substrates
Fermentation

ABSTRACT

Background: Single-cell protein (SCP) is an evolving biotechnological concept that can potentially align protein production with the global sustainability commitment.
Scope and approach: This review presents an overview of SCP's current outlook, including production, commercial prospects, and sustainability status. It also elaborates on the potential of the evolving digital twin concept in improving SCP production's efficiency and sustainability performance.
Key findings and conclusion: An expanding body of work was identified, with well-explored fermentative approaches and varying substrates and microbes. Whereas interest in first-generation substrates such as methane is gradually fading due to their high competitiveness and acquisition cost, second-generation substrates such as lignocellulosic materials and agro-industrial wastes are rapidly evolving due to their availability, low cost, appreciable nutrient density, and alignment with the circular bioeconomy path. Sustainability assessment of current production attaches substantial environmental, mainly global warming and land use offset, and economic savings to SCP production. Moreover, it emphasizes conventional energy use as a hotspot contributor to all impact categories. However, research on life cycle costing, social life cycle assessment, and environmental nutrition concepts is limited. Current trends project rapid market growth for SCP due to expanding feed, food, and nonfood applications. The rapid influx of transformative innovations such as mixed culture biotechnology and the emerging digital twin concept that present catalytic advantages in achieving market growth and sustainability cobenefits are backing these projections. Several sensor and predictive technologies are available to enable an SCP-digital twin path, presenting an opportunity to enhance green and precision SCP production. Despite these innovations, significant efforts are required to overcome limitations concerning toxicity, legislative restrictions, technical constraints, and consumer neophobia to bolster commerce and market value.

1. Background

Current anthropogenic activities have threatened the integrity of the planet. In the food system, for instance, crop and animal production and successive processes that transform raw materials into edible and shelf-stable forms have driven resource demands and increased associated emissions that imperil the earth's impact-bearing capacity (Agyemang & Kwofie, 2021; Aidoo et al., 2022). Such is also the case for the protein supply chain, where increase in global population has coerced global proclivity toward producing more protein foods to satisfy the current needs of consumers. From empirical data, stakeholders have ascertained how conventional protein supply, prominently animal production, result in astronomical environmental impacts, deviating from the global intent to produce and consume sustainably. As a result, several remediation strategies have emerged, including transitioning to more sustainable protein alternatives, including plant-based alternatives, insects, cultured meat, and single cell protein (SCP). In this study, we draw attention to the progressing prominence of microbial SCP in enhancing sustainable protein production and consumption. With an estimated tripling in market value within the next decade (2020–2030), it is more important

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https://doi.org/10.1016/j.tifs.2023.07.003
Received 10 April 2023; Received in revised form 20 June 2023; Accepted 6 July 2023
Available online 8 July 2023

to understand current dynamics in SCP production to facilitate innovations toward meeting these projections and beyond (Global Market Insights, 2023).

SCP derived its name from being produced from unicellular microorganisms (Abdullahi et al., 2021; Nasseri et al., 2011). The microbes are cultivated from either first-generation substrates of high commercial value (for example, methane, ethanol, methanol, and gas oil) or from second-generation substrates (SGS) such as nutrient-rich agricultural and agro-industrial residues (Abdullahi et al., 2021; Banks et al., 2022; Owsianiak et al., 2022; Rajendran et al., 2018). Furthering the advantages of first-generation substrates in SCP production, which endeavored primarily to augment protein security in a socioeconomically diverse food system amidst a growing global population, second-generation substrates offer this advantage while benefitting the evolving circular bioeconomy and food system sustainability agenda. It does so through the renewable bioconversion of waste or underutilized coproducts into high-value microbial protein products. Solid, liquid, and semi-solid fermentation technologies have been designed and explored in cultivating several yeast, bacteria, algae, and filamentous fungi strains with appreciable process outputs recorded relative to fermentation conditions, substrate characteristics, and microbial type (Abdullahi et al., 2021; Banks et al., 2022). Among these microbes, yeast is the trajectorial pioneer, having a longstanding historical association with World Wars I and II (Abdullahi et al., 2021; Nasseri et al., 2011). At the emergence of World War I, yeast served as a suitable unconventional alternative to complement protein demand in the war zones. Its nutritional quality subsequently inspired the drastic reduction in protein importation among the Germans during World War I, replacing almost 50% of imported proteins with locally cultivated yeast. What was seen as an economic buffer and a transient protein resort in a period of commotion and national instability has evolved into a revolutionary food system pursuit for enhancing protein-energy security (Abdullahi et al., 2021; Nasseri et al., 2011).

Aside from the nutritional, health, and environmental benefits SCP can provide, extensive research and innovations have attached techno-economic and socioeconomic benefits to their production and consumption. For instance, whereas most conventional proteins have been associated with high resource demand, generation time, and capital investment, SCP production is noted for the contrary. The consumption of readily available and inexpensive residual feedstocks minimizes resource demand, and the shorter generation time of microbes (bacteria: 30–120 min, yeast, 40–180 min, algae: 180–360 min) (Nasseri et al., 2011; Sharif et al., 2021) presents comparably attractive product-process efficiency benefits. For capital investment, the argument prevails to associate such benefits with the technology or technique used, with solid-state fermentation (SSF) identified as a relatively cheaper technological option due to less water and energy demand (Areniello et al., 2022). These benefits have enabled a gradually surging preference for SCP (Areniello et al., 2022; Ritala et al., 2017; Wada et al., 2022). However, major technical and process inefficiencies, such as difficulty in scaling up the SSF process, high nucleic acid contents, and other metabolic constraints, have limited the commercial potential of SCP production. Thus, there has been a resurging necessity for radical collaboration, continuous research, and technological advancements to improve performance and maximize commercial benefits.

In this study, we review the current outlook of the SCP technology. The paper is divided into three major broad parts. The first part (Sections 2 and 3) captures the production outlook, highlighting the production approaches, general production steps, and some microbes and substrates for the production process, including emerging advances. The second part (Section 4) discusses the commercial status of SCP, capturing the benefits of bolstering commercial entry, the current market dynamics, and current applications. Taking the emerging proclivity to the SCP technology as sustainable protein source, the third part of this review (Section 5) delves into exploring the progress of sustainability research, including Life Cycle Assessment (LCA), Techno-economic Assessment (TEA), Life Cycle Cost Assessment (LCCA), Social Life Cycle Assessment (TEA), and Environmental Nutrition. It also elaborates on the novel digital twin concept as an Industry 4.0 technology for improving SCP production and associated sustainability performance. Relevant technologies (sensors) and computer models that could be leveraged are briefly discussed, and specific applications of these technologies are highlighted. For emphasis and clarity, this paper does not capture the actual simulation of a digital twin for SCP production. It instead presents insights into the current potential that could be deployed to enable precision SCP production in an Industry/Technology 4.0 era. The study concludes with a summary of the significant takeaways from the review, drawing into perspective what future research should focus on and recommending pathways for progressing sustainable SCP production.

2. SCP production

SCP production follows a fermentation pathway that utilizes nutrient-rich feedstock to multiply a microbe into protein-rich biomass under optimal nutrient and substrate concentration conditions and other critical fermentation parameters, such as oxygen, temperature, and pH. This section captures two crucial aspects of SCP production, the fermentation approaches, and a general flow of the production steps.

2.1. Fermentation approaches

2.1.1. SSF

SSF involves the cultivation of microbes on a solid substrate without a free aqueous phase in varying fermenter designs (Bajpai, 2017). The microbes depend on the intrinsic moisture composition of the moist-solid substrate, requiring little to no additional water. Thus, SSF is restricted to microorganisms that grow efficiently under low water activity, including yeast and some filamentous fungi, requiring pure solid substrates with approximately 60–65% moisture (Sharif et al., 2021). The microbes access nutrients through adsorption or penetration of the solid substrate, allowing them to multiply into a protein-rich biomass. A nutrient concentration gradient exists in SSF; thus, nutrient diffusion is required to ensure optimal access to nutrients for microbial doubling. The fermentation process requires an adequate oxygen supply in the liquid phase, achieved through aeration and intermittent media stirring, and optimal temperature, pH, ionic strength, and nutrient conditions for optimal yield (Areniello et al., 2022). SSF is advantageous for its relatively low capital investment and minimal waste generation, offering better economic and environmental compensation (Aggelopoulos et al., 2014). Therefore, it is no surprise that evolving fermentation-based research is rapidly inclining toward the SSF approach, intending to enhance techno-economic and sustainability benefits (Aggelopoulos et al., 2014; Muniz et al., 2020; Webb, 2017). However, SSF faces critical challenges that demand significant innovations to activate its full commercial potential. Prominent among these are the current difficulties in technical scale-up, impeding commercial scale production, and limitating online monitoring and process control, which demands urgent interventions to spur commercialization and survival of the SSF approach in a rapidly evolving digital economy (Areniello et al., 2022; Bajpai, 2017). Additionally, difficulty in stirring and removing metabolic heat in the SSF approach has been critical to process efficiency; however, recent developments promise better system designs to surmount these limitations (Areniello et al., 2022; Jach et al., 2022).

2.1.2. LSF

Unlike SSF, liquid-state fermentation (LSF) requires microbial cultivation in a continuous liquid-phase substrate containing more than 95% moisture. The fermentation process is carried out in a closed bioreactor, usually in continuous mode, with proper control of temperature, pH, nutrients, and oxygen supply. LSF is widely adopted in industrial fermentation processes due to its advantages in easy technical scale-up, uniform distribution of nutrients and oxygen facilitated by its

continuous liquid phase, and high protein yield. It is also easy to remove metabolic heat and monitor or control the fermentation process online (Areniello et al., 2022; Sharif et al., 2021). Despite these advantages, the characteristic high capital demand and high waste generation of LSF are gradually reducing its attractiveness in an evolving sustainability-sensitive economy. While research and technology continue to progress toward optimizing these limitations and enhancing system performance, intensified traction toward the SSF approach, primed by its sustainability prospects, seems to suggest the possibility of overtaking LSF in future industrial adoption. However, this possibility will be challenged in an industry/technology 4.0 era unless present complexities in technical scale-up, heat, mass transfer, and digitization evolve simultaneously with growing interest.

2.1.3. Semisolid-state fermentation

Semisolid-state fermentation is an intermediary between SSF and LSF. Here, the free-flowing liquid content is increased to facilitate the distribution of nutrients and oxygen (Sharif et al., 2021). This marks its preference as an intermediate approach for microbes that require slightly high-water activity but perform better on a solid substrate. However, as an intermediate approach, its advantages and disadvantages fall between SSF and LSF. For instance, while it offers moderate metabolic heat removal facilitated by its slightly higher liquid phase relative to SSF, it is characterized by high capital investment relative to SSF and lower protein yield relative to LSF (Areniello et al., 2022).

2.2. General production steps

A graphical representation of the process flow for SCP production is illustrated in Fig. 1. Production of SCP starts with substrate preparation: the primary step for transforming substrates into a useable carbon source. The substrate preparation method depends on the type of substrate and fermentation approach. In recent literature, second-generation substrates (SGS) have been prepared using wet, direct, and dry preparation methods (Abdullahi et al., 2021) interlinked with LSF, SSF, and semisolid-state fermentation, respectively (Abdullahi et al., 2021; Pereira et al., 2022; Ritala et al., 2017). The wet preparation method is usually used for fresh fruit, vegetable waste, or substrates with high moisture content. Generally, it involves a series of water or acidic washes, pulverization, filtration, and sterilization to obtain a sterile liquid medium. The dry method is mostly used for low-moisture substrates. It employs a drying procedure to further remove moisture , followed by a diminution and sifting step that produces a fine powder of defined particle sizes. The fine powder is then blended with water, filtered, and sterilized to obtain a sterile moist-solid medium with a prominent aqueous phase (Abdullahi et al., 2021; Areniello et al., 2022).

The direct method is used when the SSF approach is preferred. It involves water washing and hydrolytic procedures, including acid, biohydrolysis, or thermal treatment, to convert the substrate into a medium ready for fermentation. Given their low moisture content and bulkiness, lignocellulosic materials usually follow dry and direct preparation methods. However, grinding and digestion of the substrate cannot be compromised in their preparation due to the complex interaction in their chemical composition and the need to enhance the accessibility of embedded nutrients. Sterilization of the resulting media is usually performed with an autoclave at a temperature of 121 °C for approximately 15–60 min. The autoclaving time depends on the substrate and the measure of contamination. However, the sterilization step

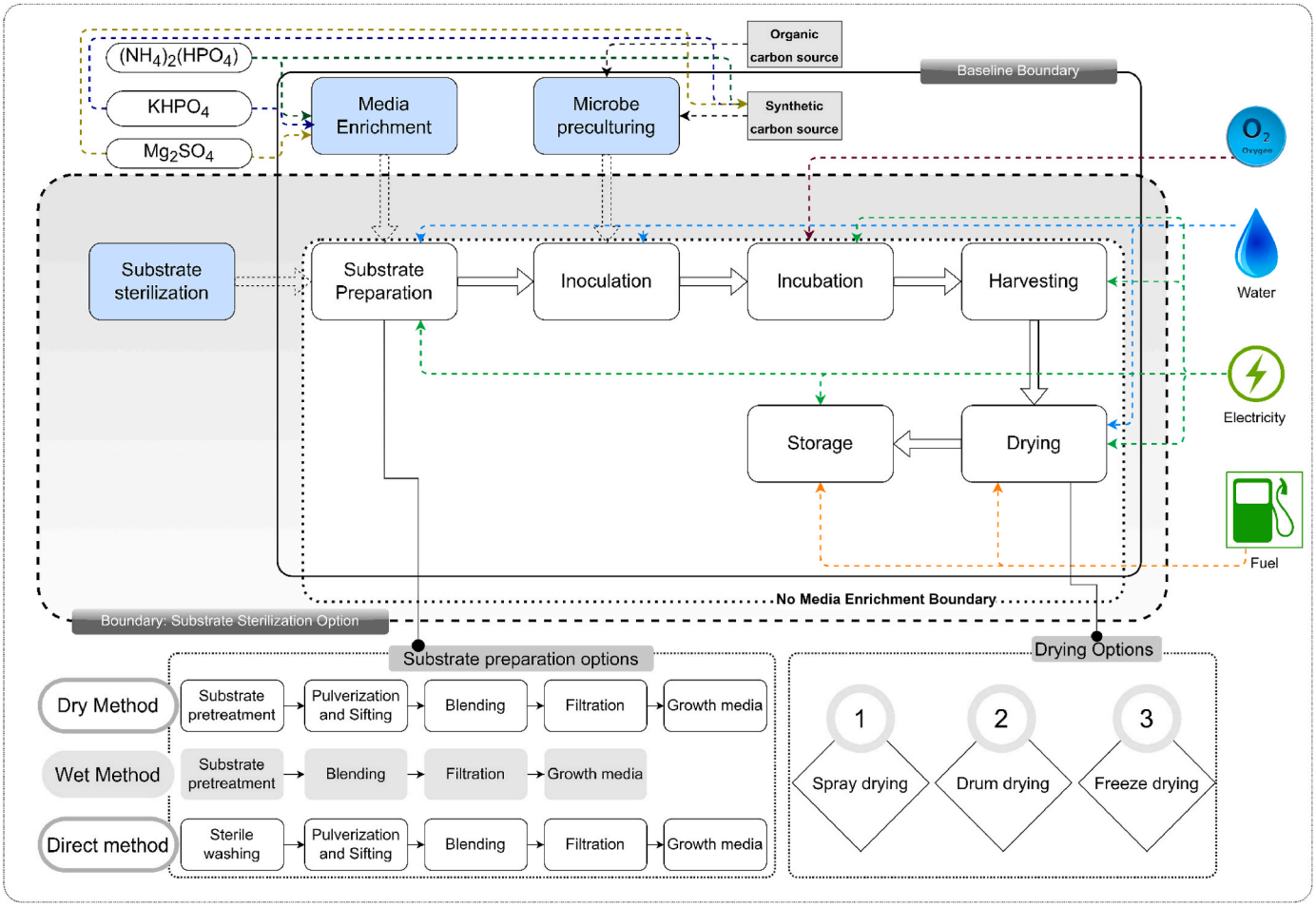

Fig. 1. Production flow of SCP.

could be omitted during substrate preparation, taking its significance in the fermentation process and effect on substrate integrity (Gervasi et al., 2018).

Following the substrate preparation step is media enrichment, performed to augment the nutritional capacity of the resulting media. In some cases of the application of second-generation substrates in SCP production, sole dependence on the substrate as the source of nutrients for the fermentation process fostered optimal microbial doubling without enrichment. This highlights the possibility of skipping the media enrichment step. However, it is relevant to understand the nutritional characteristics of the substrate and its capacity to augment the nutritional needs of the microbe before making such a decision.

Next is the inoculation stage, which could involve isolating or culturing microbial cells to attain a substantial load before transferring them to the prepared media. Otherwise, a viable inoculum can be purchased from sellers and used directly, or the back-slopping procedure could be employed accordingly. Next, the inoculated medium is incubated for a defined period. Within this period, the fermentation environment is intermittently regulated to enhance the multiplication of microbes.

Harvesting follows the incubation step. Here, centrifugal (yeast and bacteria) and filtration (filamentous fungi) technologies (Nasseri et al., 2011) are employed to separate SCP from the resulting biomass. The SCP is then dried to approximately 10% moisture content to enhance storage life. Spray, drum, and freeze-drying techniques have been widely used in SCP drying. Based on the use of SCP, techniques such as protein purification (Thiviya et al., 2022), cell wall degradation (Ugbogu & Ugbogu, 2016), protein extraction (Sharif et al., 2021), and nucleic acid removal (Thiviya et al., 2022; Ugbogu & Ugbogu, 2016) may be required before final stage drying and storage.

3. Substrates and microorganisms for SCP production

The previous section gave an overview of SCP production, establishing variations in substrate preparation and technologies for the production process. This section briefly discusses some substrates and microorganisms explored in SCP production, highlighting their characteristics, advantages, and disadvantages.

3.1. Substrates

3.1.1. Overview of substrates

SCP substrates have generally been classified into first and second-generation (SGS) categories (Abdullahi et al., 2021; Banks et al., 2022; García Martínez et al., 2022). First-generation substrates include methane (García Martínez et al., 2022), methanol, gas oil (Nasseri et al., 2011; Raziq, 2020), and staple food ingredients such as corn, cassava, and rice flour, which are highly competitive for their wide adoption in commercial processes. However, despite advantages such as high production rate and protein yield, challenges such as high cost, technical constraints, high toxicity and carcinogenicity, commercial competitiveness, and adverse environmental implications continue to limit commercial utility (García Martínez et al., 2022; Nasseri et al., 2011), which has necessitated rethinking their use. This has triggered interest in inexpensive and environmentally friendly SGS, materials that have lost their primary value or are commercially unattractive in their raw forms (Hulsen et al., 2022; Janssen et al., 2022; Pihlajaniemi et al., 2020). SGS are characterized by appreciable nutritional densities and other favorable characteristics that can completely displace first-generation substrates in SCP production while offering exclusive economic and ecological benefits (Banks et al., 2022). A broad list of SGS for SCP production has been identified in the literature and captured under broad categories: on-farm agricultural wastes, including manure and lignocellulosic materials (Bratosin et al., 2021; Spalvins et al., 2018; Thiviya et al., 2022), agro-industrial processing wastes (Bajpai, 2017; Türker et al., 2022), and lost agro-products (Areniello et al., 2022; Gervasi et al., 2018; Ritala et al., 2017).

3.1.2. Rising interest in starch-rich pulse coproducts as SGS for SCP production

Pulses are protein-rich crops with numerous advantages as food, feed or ingredients for food and feed products. In present deliberations, their roles as sustainable protein alternatives have been heightened. Therefore, the drastic progress experienced in the pulse industry over the past few years is no surprise. One such is the dramatic expansion in production in response to doubling raw material needs for plant protein extraction. For instance, within the past two decades, from 1998 to 2018, pulse production has increased by approximately 63% (Ben-Belhassen & Rawal, 2023). The leading pulse crops in this expansion are peas, lentils, chickpeas, and faba beans (Ren et al., 2021).

There has been tremendous production growth in the pea industry, with an approximately 100% increase in global production reported between 1961 and 2020 (FAO, 2022). The regional distribution of this increase indicates an approximation of 26000% in Estonia, 15500% in Canada, 1500% in West Africa, and 510% in the United States (US). These values precisely communicate the booming interest in pea and pulse production. However, although this massive expansion is desirable in augmenting the raw material supply for alternative protein production, the skewed interest in protein extraction curtails the realization of the full sustainability potential of the pea industry.

Pea contains approximately 13–40% proteins and 30–50% starch (Daba & Morris, 2021), emphasizing the considerable mass of starch-rich coproduct generated during pea protein extraction. In common practice, pea starch slurry is dried into pea starch flour, which is struggling in commerce due to its undesirable functionalities presented by the high amylose:amylopectin ratio (Daba & Morris, 2021; Ren et al., 2021). Additionally, fiber and remnant proteins minimize purity, hindering the use of pea starch flour in other non-food applications (Ren et al., 2021). Current technologies have employed modification strategies to enhance functionalities and improve applicability (Gebremedhin & Admassu, 2022). However, these have not charted any satisfactory commercial success. Therefore, enabling the full sustainability potential of the pea and pulse industries would require finding high-value, market-ready upcycle solutions for utilizing these coproducts. As biotechnological innovations gradually populate governmental and industrial strategies toward addressing waste challenges and enhancing protein security, fermented foods have been identified as a momentous circular solution for utilizing these starch-rich pulse coproducts to achieve economic, environmental, and food security cobenefits, and SCPs fall within this context (Adebo et al., 2017, pp. 77–109.). Nonetheless, these have not received the necessary engagements, especially for SCP production, presenting a significant gap that could be explored in future alternative protein production.

3.2. Microbes

3.2.1. Overview of microbes

Various fast-growing, nutritious, and generally recognized as safe (GRAS) microbial strains from bacterial, yeast, fungal, and algal sources have been identified and explored in SCP production (Raziq, 2020). Microbes exhibit varying structural, chemical, and doubling characteristics that determine process dynamics and influence the quality and use of the final microbial biomass. For instance, bacteria and yeast possess high nucleic acid contents and bear the potential to release toxins during fermentation, obstructing interest in human food applications (Abdullahi et al., 2021). It would take process optimization and advanced technologies that can overcome these constraints to spur SCP food application (García Martínez et al., 2022; Ritala et al., 2017).

Regarding microbial doubling or generation time, a decreasing rate order of bacteria, fungi, and algae has been reported, making bacteria and yeast production comparably more yielding than algae (Abdullahi et al., 2021; Sharif et al., 2021; Thiviya et al., 2022). However, contrary

to algae and filamentous fungi, harvesting bacterial cells is difficult due to their small size and low density. Therefore, expensive, and sophisticated centrifugal technologies are required to optimize harvesting efficiency for bacterial SCP. These dynamics emphasize the need to consider microbial selection as a critical factor in SCP production. Table 1 summarizes some recently explored substrates and microbes with details of operational variables, biomass yield, and protein content.

3.2.2. Analysis of compiled SCP production studies

To better understand the trends in the compiled studies presented in Table 1, further analysis was performed to ascertain variation in the average time, temperature, pH, and protein content of the microbes explored in these studies, as presented in Fig. 2. The aggregation of results for fermentation conditions from the compiled studies showed that bacteria on average require higher pH (more alkaline conditions) than yeast and fungi, with yeast demonstrating the capacity to thrive and multiply under highly acidic pH, rightly aligning with the established dynamics in microbial characteristics. For average fermentation time and temperature, it was conspicuous that bacteria required shorter generation times and relatively lower temperatures to reach optimal growth, corroborating existing trends. Subsequent studies should consider such dynamics in process design, optimization, and selection. Considering a linear correlation between product mass and protein content, the average protein content was approximately 48–50% for all microbes. However, Table 1 clearly distinguishes these numbers for substrate type and operational conditions. A distinguishing insight from Fig. 2 regards the dearth of information regarding the use of bacteria in SSF relative to the compiled studies. As emphasized in the literature, SSF favors microbes that can thrive under low water activity or moisture content. Thus, it is understandable that bacteria that thrive mostly under high water activity have not been extensively explored using the SSF approach. This directs a preference for yeast and filamentous fungi in the evolving SSF approach for SCP production.

3.2.3. Mixed culture biotechnology

Microbes' cultivation rate and performance in SCP production depend on their ability to thrive in a dynamic environment of varying pH, temperature, substrate composition, and toxicity, among other kinetics. Most microbes die out and fail to yield the required output when process conditions become unfavorable. The evolution of mixed culture biotechnology is rising as a solution. In mixed culture technology, different microbes synergize in a fermentation environment to overcome restraining antagonism or recalcitrance and yield desired process outputs (Bajpai, 2017). Several reports have highlighted the benefits of mixed culture, or coculturing, in SCP production. Using *Kluyveromyces marxianus* and *Candida kusei* on whey could alter the removal efficiency of chemical oxygen demand (COD) to an optimal level while minimizing the susceptibility of media to contamination (Bratosin et al., 2021). The outcome was an improved production rate with enhanced SCP quality. A synergistic association was also identified when chemo-organoheterotrophic bacteria were cocultured with purple nonsulfur bacteria (PNSB). The former facilitated the fermentative breakdown of sugar into volatile fatty acids and alcohols, serving as organic carbon sources for the latter (Wada et al., 2022). Similarly, coculturing prominent fungal strains such as *Aspergillus niger* and *Saccharomyces cerevisiae* rendered a desirable synergy. Herein, the former facilitated an enzymatic breakdown of cellulose in fruit peels into fermentable sugars, and the latter utilized the product as a carbon source for growth (Thiviya et al., 2022). The mixed culture approach is noted for its cost-saving benefits in SCP production, considering compensations for cost and energy-intensive processes such as sterilization offered by mixed-microbial culture (Sakarika et al., 2022). It is important to emphasize that the intent of mixed culture biotechnology is not necessarily to displace pure culturing in SCP production. Instead, it is designed to provide a reliable and sustainable alternative to overcome avoidable challenges such as antagonism in producing SCP from some microbial strains, especially when a symbiotic association is beneficial. However, the technology is still in its infancy and has not been widely explored for most SGSs in SCP production. This could be an interesting area to focus future SCP research.

4. Commercial status of SCP

There is a generic accession to the significant roles that incorporating SCP into the protein supply chain would play. The rich protein composition of SCP biomass (up to 80%) can contribute significantly to addressing the ever-increasing protein demand amidst global population growth. Others, specifically sustainability enthusiasts, recommend SCP for commerce mainly because of the environmental and economic benefits they confer through the circularization of cheaper waste resources and value recovery from renewable substrates such as sunlight and carbon capture. In this section, we summarize the commercial status of SCPs, starting with what benefits are triggering commercial potential, then a brief outlook of the current and future market, and a final discussion on the current and emerging applications.

4.1. Benefits to commercial entry

Researchers and other food system stakeholders have embraced confidence in SCP's capacity to complement nutritional needs and reinforce regenerative economic models when given a commercial space (Durkin et al., 2022). Regarding nutritional capacity, appreciable protein content (approximately 30–80%), including limiting amino acids such as methionine, lysine, threonine, and cysteine, has been characterized for SCPs, emphasizing their potential to serve the needs of the rising protein consumer market. These have given SCP recognition by international organizations such as the Food and Agriculture Organization (FAO) and the National Aeronautics and Space Administration (NASA) (Altmann & Rosenau, 2022; García Martínez et al., 2022). For instance, FAO deems *Aspergillus oryzae* a well-balanced protein source for satisfying protein needs (García Martínez et al., 2022; Ritala et al., 2017). Moreover, NASA has already employed *Spirulina* as a complete protein food for astronauts during space missions, illustrating an emerging exploration of SCP in space foods. Aside from proteins, SCPs are packed with significant amounts of carbohydrates, lipids, dietary fiber, minerals, and vitamins (Can Karaca et al., 2022; Pereira et al., 2022; Thiviya et al., 2022). They also contain considerable amounts of essential fatty acids such as eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), linolenic acid, and palmitic acid, which have been quantified in *Spirulina* and *Chlorella* SCPs (Gogna et al., 2022; Ragaza et al., 2020), and substantial quantities of fat-soluble vitamins such as A, D, E, K (Alagawany et al., 2021; Gogna et al., 2022; Pereira et al., 2022) and vitamin B-complex (Altmann & Rosenau, 2022; Can Karaca et al., 2022; Jach et al., 2022; Türker et al., 2022, pp. 201–244). SCPs are also loaded with trace minerals such as phosphorus, calcium, sodium, magnesium, and manganese (Gogna et al., 2022; Jach et al., 2022) and contain appreciable quantities of bioactive compounds such as carotenoids and chlorophyll A (Barka & Blecker, 2016; Carter & Codabaccus, 2022; Gogna et al., 2022), providing potential therapeutic or nutraceutical capacities.

Aside from the nutritional benefits, the availability and utilization of inexpensive second-generation feedstock for SCP production makes it economically attractive (Elyasi et al., 2021; Matassa et al., 2020), which explains the increasing exploration of fruit and vegetable waste, lignocellulosic materials, and industrial wastewaters as substrates in production at different scalar levels (Pereira et al., 2022; Rajendran et al., 2018; Raziq, 2020). Contrary to conventional animal and plant protein production, the synergy of shorter generation time and high doubling rate of microbes makes SCP production highly efficient and relatively profitable. Reports have established that for the same fold of land, the caloric and protein yields of SCPs could be tenfold and twofold, respectively, higher than those of protein-rich pulses, meats, and grains

Table 1
Studies on the utilization of second-generation substrates.

Substrate	Microbes	Microbial type	Operational variables			Experimental Setup	Media Enrichment	Yield	Protein content %	Reference
			Fermentation	Harvesting	Drying					
LIQUID STATE FERMENTATION										
Agro-industrial waste (Whey, Molasses, Potato pulp, Orange Pulp; 3:1:1:10)	*Kluyveromyces marxianus*	Yeast	Temp: 30 °C Time: 96 h pH: 7 Airflow rate: 0.5 L/min Stirring speed: [a]	Centrifugation Speed: 5000 rpm Time: 10 min	[a]	Lab scale: Erlenmeyer flask	NE	0.87gCWM/g	30.23	(Aggelopoulos et al., 2013, 2014)
Agro-industrial waste ((Whey, Molasses, Potato pulp, Orange Pulp; 0:1:0:2)	*Saccharomyces cerevisiae*	Yeast	Temp: 30 °C Time: 96 h pH: 5.5 Airflow rate: 0.5 L/min Stirring speed: [a]	Centrifugation Speed: 5000 rpm Time: 10 min	[a]	Lab scale: Erlenmeyer flask	NE	0.80gCWM/g	23.58	(Aggelopoulos et al., 2013, 2014)
Agro-industrial waste (Whey, Molasses, Potato pulp, Orange Pulp; 10:1:1:3)	*Kefir*	Yeast	Temp: 30 °C Time: 96 h pH: 5.5 Airflow rate: 0.5 L/min Stirring speed: [a]	Centrifugation Speed: 5000 rpm Time: 10 min	[a]	Lab scale: Erlenmeyer flask	NE	0.48gCWM/g	31.02	(Aggelopoulos et al., 2013, 2014)
Corn stover effluent	*Rhodococcus opacus*	Bacteria	Temp: 28 °C Time: 48 h Shaking speed: 150 rpm pH: 7.0	Centrifugation Speed: 5000 rpm Time: 10 min	Freeze drying	Lab scale: shaking flask	NH_4NO_3: 0.005 or NH_4Cl: 0.005	0.27–0.33 gCDM/100 ml	47.0–52.7	Mahan et al. (2018)
Food waste: a mixture of fish waste (fish head, viscera, skin, and bones), pineapple, banana, apple, and citrus peels	*Saccharomyces cerevisiae*	Yeast	Temp: 30 °C Time: 72–120 h pH: 4.5 Airflow rate: 0.5 L/min Stirring speed: 300 rpm	[a]	Freeze drying: -18 °C	5 L batch fermenter (Biostat Biotech B, Sartorius Stedim Biotech, Goettingen, Germany)	Urea phosphate salt: 2.3 g/L KCl: 0.2 g/L MgSO4·7H2O 3.8 g/L Ca-pantothenate: 0.0833 mg/L Biotin 0.0833 mg/L.	[a]	34–42	Tropea et al. (2022)
Lemon waste blend	*Rhodococcus opacus*	Bacteria	Temp: 28 °C Time: 48 h Shaking speed: 150 rpm pH: 7.0	Centrifugation Speed: 5000 rpm Time: 10 min	Freeze drying	Lab scale: shaking flask	NH_4NO_3: 0.005 or NH_4Cl: 0.005	0.22–0.33 gCDM/100 ml	45.8–52.1	Mahan et al. (2018)
Oat bran hydrolysate	*Candida tropicalis*	Yeast	Temp: 30 °C Time: 72 h Shaking speed: 200 rpm pH: 4.5–5.0	[a]	Temp: 105 °C Time: Overnight	Lab scale: shaking flask	$(NH_4)_2SO_4$: 0.002 KH_2PO_4: 0.002 (g/L)	7.2 g/100 g	[a]	Dimova et al. (2014)
	Candida utilis	Yeast	Temp: 30 °C Time: 72 h Shaking speed: 200 rpm pH: 4.5–5.0	[a]	Temp: 105 °C Time: Overnight	Lab scale: shaking flask	$(NH_4)_2SO_4$: 0.002 KH_2PO_4: 0.002 (g/L)	7.9 g/100 g	[a]	Dimova et al. (2014)
Orange peel	*Candida utilis*	Yeast	Temp: 30 °C Time: 96 h Shaking speed: 150 rpm pH: 3.7–4.2	Centrifugation Speed: 5000 rpm Time: 10 min	Temp: 60 °C Time: 24 h	Lab scale: shaking flask	$(NH_4)_2SO_4$: 1.5 KH_2PO_4: 0.75 K_2HPO_4: 0.75 $MgSO_4$: 0.05 (g/L)	1.65 g/100 ml	[a]	Carranza-Méndez et al. (2022)

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Table 1 (*continued*)

Substrate	Microbes	Microbial type	Operational variables			Experimental Setup	Media Enrichment	Yield	Protein content %	Reference
			Fermentation	Harvesting	Drying					
	Candida utilis	Yeast	Temp: 30 °C Time: 96 h Shaking speed: 150 rpm pH: 3.7–4.2	C *Candida utilis* centrifugation Speed: 5000 rpm Time: 10 min	Temp: 60 °C Time: 24 h	Lab scale: shaking flask	$(NH_4)_2SO_4$: 0.6 KH_2PO_4: 0.2 $FeSO_4$: 0.002 KCl: 0.8 $MgSO_4$: 0.07 (g/L)	1.03 g/100 ml	[a]	Carranza-Méndez et al. (2022)
	Candida utilis	Yeast	Temp: 30 °C Time: 96 h Shaking speed: 150 rpm pH: 3.7–4.2	Centrifugation Speed: 5000 rpm Time: 10 min	Temp: 60 °C Time: 24 h	Lab scale: shaking flask	$(NH_4)_2SO_4$: 0.6 Na_2HPO4: 0.640 $FeCl_3$: 0.029 KH_2PO_4: 0.427 $FeSO_4$: 0.002 $CaCl_2$: 1.793 $MgSO_4$: 0.492 CuSO4: 0.002 MnSO4: 0.009 ZnSO4: 0.011 (g/L)	0.77 g/100 ml	[a]	Carranza-Méndez et al. (2022)
Orange waste blend (pulp, peel, and juice	*Rhodococcus opacus*	Bacteria	Temp: 28 °C Time: 48 h Shaking speed: 150 rpm pH: 7.0	Centrifugation Speed: 5000 rpm Time: 10 min	Freeze drying	Lab scale: shaking flask	NH_4NO_3: 0.005 or NH_4Cl: 0.005	0.23–0.30 gCDM/100 ml	42.2–56.9	Mahan et al. (2018)
Pineapple skin and rice washing water	*Saccharomyces cerevisiae*	Yeast	Temp: 30 °C Time: 56 h Shaking speed: [a] pH: 3.8–4.5	[a]	[a]	Lab scale: Beaker	$(NH_4)_2SO_4$: 1.5 KH_2PO_4: 0.7 NaCl: 0.07 $MgSO_4$: 0.38 $CaCl_2$: 0.07 (g/L)	0.4752 gCDM/ 100 ml	[a]	Mujdalipah and Putri (2020)
Pineapple peel waste	*Saccharomyces cerevisiae*	Yeast	Temp: 29 °C Time: 24–48 h Shaking speed: [a] pH: 4.5	Centrifugation speed: 3000 rpm Time: 10 min	Temp: 60 °C Time: 5 h	Lab scale: shaking flask	Fructose or Sucrose: [a]	0.72–0.84 g CDM/100 ml	65–94	Nurmalasari and Maharani (2020)
Potato peel extract and glucose	*Rhizopus oligosporus*	Filamentous fungus	Temp: 35 °C Time: 72 h pH: 5.5	Whatmann filter	Temp: 80 °C Time: 24 h	Lab scale: shaking flask	KH_2PO_4: [a] $MgSO_4$: [a] NaCl: [a] Yeast extract: [a]	0.52 gCDM/ 100 ml	45–55	Nadeem (2021)
	Rhizopus oligosporus	Filamentous fungus	Temp: 35 °C Time: 72–96 h pH: 5.5 Airflow rate: 1.0vvm	Whatmann filter	Temp: 70–75 °C Time: Until constant weight	Stirred tank bioreactor	KH_2PO_4: [a] $MgSO_4$: [a] NaCl: [a] Yeast extract: [a]	0.45 gCDM/ 100 ml	~50	Nadeem (2021)
	Rhizopus oligosporus	Filamentous fungus	Temp: 35 °C Time: 72–96 h pH: 5.5 Airflow rate: 1.0vvm	Whatmann filter	Temp: 70–75 °C Time: Until constant weight	Bubble column fermenter	KH_2PO_4: [a] $MgSO_4$: [a] NaCl: [a] Yeast extract: [a]	0.55 gCDM/ 100 ml	~50	Nadeem (2021)
Rice husk hydrolysate	*Candida tropicalis*	Yeast	Temp: 30 °C Time: 72 h Shaking speed: 200 rpm pH: 4.5–5.0	[a]	Temp: 105 °C Time: Overnight	Lab scale: shaking flask	$(NH_4)_2SO_4$: 0.002 KH_2PO_4: 0.002 (g/L)	4.7 g/100 g	[a]	Dimova et al. (2014)
	Candida utilis	Yeast	Temp: 30 °C Time: 72 h Shaking speed:	[a]	Temp: 105 °C	Lab scale: shaking flask	$(NH_4)_2SO_4$: 0.002 KH_2PO_4: 0.002 (g/L)	5.8 g/100 g	[a]	Dimova et al. (2014)

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Table 1 (*continued*)

Substrate	Microbes	Microbial type	Operational variables			Experimental Setup	Media Enrichment	Yield	Protein content %	Reference
			Fermentation	Harvesting	Drying					
			200 rpm pH: 4.5–5.0		Time: Overnight					
Wasted Date Molasses (WDM)	*Hanseniaspora guilliermondii*	Yeast	Temp: 30 °C Time: 48 h Agitator speed: 150 rpm pH: 4.0	[a]	Temp: 80 °C Time: 24 h	Lab scale: shaking flask	Peptone: 4.0 (g/L)	55.30 gCDM/ 100 g WDM	52.0	Hashem et al. (2022)
	Hanseniaspora guilliermondii	Yeast	Temp: 30 °C Time: 48 h Agitator speed: 150 rpm pH: 4.0 Airflow rate: 0.25 vvm	[a]	Temp: 80 °C Time: 24 h	Bioreactor BioFlo/ CelliGen 115 (7 L capacity)	Peptone: 4.0 (g/L)	55.82 gCDM/ 100 g WDM	53.21	Hashem et al. (2022)
Wasted Date Molasses FT: LSF	*Hanseniaspora uvarum*	Yeast	Temp: 30 °C Time: 48 h Agitator speed: 150 rpm pH: 4.0	[a]	Temp: 80 °C Time: 24 h	Lab scale: shaking flask	Peptone: 4.0 (g/L)	44.89 gCDM/ 100 g WDM	50.0	Hashem et al. (2022)
	Hanseniaspora uvarum	Yeast	Temp: 30 °C Time: 48 h Agitator speed: 150 rpm pH: 4.0 Airflow rate: 0.25 vvm	[a]	Temp: 80 °C Time: 24 h	Bioreactor BioFlo/ CelliGen 115 (7 L capacity)	Peptone: 4.0 (g/L)	47.53 gCDM/ 100 g WDM	51.53	Hashem et al. (2022)
Wasted Date Molasses	*Issatchenkia orientalis*	Yeast	Temp: 30 °C Time: 48 h Agitator speed: 150 rpm pH: 4.0	[a]	Temp: 80 °C Time: 24 h	Lab scale: shaking flask	Peptone: 4.0 (g/L)	75.00 gCDM/ 100 g WDM	54.45	Hashem et al. (2022)
	Issatchenkia orientalis	Yeast	Temp: 30 °C Time: 48 h Agitator speed: 150 rpm pH: 4.0 Airflow rate: 0.25 vvm	[a]	Temp: 80 °C Time: 24 h	Bioreactor BioFlo/ CelliGen 115 (7 L capacity)	Peptone: 4.0 (g/L)	75.82 gCDM/ 100 g WDM	54.34	Hashem et al. (2022)
Wasted Date Molasses	*Cyberlindnera fabianii*	Yeast	Temp: 30 °C Time: 48 h Agitator speed: 150 rpm pH: 4.0	[a]	Temp: 80 °C Time: 24 h	Lab scale: shaking flask	Peptone: 4.0 (g/L)	46.90 gCDM/ 100 g WDM	45.0	Hashem et al. (2022)
	Cyberlindnera fabianii	Yeast	Temp: 30 °C Time: 48 h Agitator speed: 150 rpm pH: 4.0 Airflow rate: 0.25 vvm	[a]	Temp: 80 °C Time: 24 h	Bioreactor BioFlo/ CelliGen 115 (7 L capacity)	Peptone: 4.0 (g/L)	47.53 gCDM/ 100 g WDM	48.72	Hashem et al. (2022)
Wheat bran hydrolysate	*Candida tropicalis*	Yeast	Temp: 30 °C Time: 72 h Shaking speed:	[a]	Temp: 105 °C Time: Overnight	Lab scale: shaking flask	$(NH_4)_2SO_4$: 0.002 KH_2PO_4: 0.002 (g/L)	7.9 g/100 g	[a]	Dimova et al. (2014)

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Table 1 (*continued*)

Substrate	Microbes	Microbial type	Operational variables			Experimental Setup	Media Enrichment	Yield	Protein content %	Reference
			Fermentation	Harvesting	Drying					
	Candida utilis	Yeast	200 rpm pH: 4.5–5.0 Temp: 30 °C Time: 72 h Shaking speed: 200 rpm pH: 4.5–5.0	[a]	Temp: 105 °C Time: Overnight	Lab scale: shaking flask	$(NH_4)_2SO_4$: 0.002 KH_2PO_4: 0.002 (g/L)	8.6 g/100 g	[a]	Dimova et al. (2014)
Yam starch	*Yeast*	Yeast	Temp: 28.5 °C Time: 60 h Shaking speed: 200 rpm pH: 4.5	Centrifugation Speed: 3500 rpm Time: 10 min	[a]	Lab scale: shaking flask	[a]	241.54 ± 0.15 g wet weight/ 100 g dry starch	[a]	Chen et al. (2016)
SOLID STATE FERMENTATION										
Agro-industrial waste (Whey, Molasses, Potato pulp, Orange Pulp)	*Pleorotus ostreatus*	Filamentous fungus	Temp: 25 °C Time: 120–168 h pH: 4.0–7 Airflow rate: 0.5 L/min Stirring speed: 300 rpm	[a]	[a]	Lab scale: Petri dish	NE	3.95–5.94 g/ 100 g	27.96–38.35	Aggelopoulos et al. (2018)
Agro-industrial waste (Whey, Molasses, Potato pulp, Orange Pulp; BSG, MSR; 3:1:1:10:0:8)	*Kluyveromyces marxianus*	Yeast	Temp: 30 °C Time: 96 h pH: 7	Centrifugation Speed: 5000 rpm Time: 10 min	[a]	Lab scale: Petri dish	NE	0.21gCWM/g	[a]	(Aggelopoulos et al., 2013, 2014)
Agro-industrial waste (Whey, Molasses, Potato pulp, Orange Pulp; BSG, MSR; 10:1:1:3:2.5:6)	Kefir	Yeast	Temp: 30 °C Time: 96 h pH: 5.5	Centrifugation Speed: 5000 rpm Time: 10 min	[a]	Lab scale: Petri dish	NE	0.10gCWM/g	[a]	(Aggelopoulos et al., 2013, 2014)
Agro-industrial waste (Whey, Molasses, Potato pulp, Orange Pulp; BSG, MSR; 10:1:1:3:2.5:6)	*Saccharomyces cerevisiae*	Yeast	Temp: 30 °C Time: 96 h pH: 5.5	Centrifugation Speed: 5000 rpm Time: 10 min	[a]	Lab scale: Petri dish	NE	0.26gCWM/g	[a]	(Aggelopoulos et al., 2013, 2014)
Cashew bagasse	*Saccharomyces cerevisiae*	Yeast	Temp: 30 °C Time: 9 h pH: 4.2 Setup: Lab scale (Tray type)	[a]	Temp: 55 °C Time: Until constant weight	Lab scale: Tray	NE	11.1 gCDM/ 100 g	15.8	Muniz et al. (2020)
Guava peels	*Saccharomyces cerevisiae*	Yeast	Temp: 30 °C Time: 9 h pH: 3.6	[a]	Temp: 55 °C Time: Until constant weight	Lab scale: Tray	NE	11.3 gCDM/ 100 g	28.1	Muniz et al. (2020)
Rice straw pulp	*Trichoderma reesei*	Filamentous fungus	Temp: 30 °C Time: 288 h pH: 5.0	[a]	[a]	Lab scale: flask	NE	[a]	19.71	Novita et al. (2019)
Wheat bran	*Candida utilis*	Yeast	Temp: 30 °C Time: 96 h pH: 6.5	[a]	[a]	Lab scale: Erlenmeyer flask	KH_2PO_4: 0.25 $MgSO_4$: 0.05 Soluble starch: 0.5 Peptone: 0.25 (NH_4NO_3: 0.25 (g/L)	[a]	48.01	Irfan et al. (2011)

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Table 1 (*continued*)

Substrate	Microbes	Microbial type	Operational variables			Experimental Setup	Media Enrichment	Yield	Protein content %	Reference
			Fermentation	Harvesting	Drying					
Wheat bran	*Rhizopus oligosporus and Candida utilis*	Mixed culture	Temp: 28 °C Time: 48 h pH: 3.5	[a]	[a]	Lab scale: Freehold plastic bag	$(NH_4)_2SO_4$: 40 g/kg Chloramphenicol: 0.4 g/kg	[a]	41.02	Yunus et al. (2015)
Yam peel mash	*Aspergillus niger*	Filamentous fungus	Temp: 28 °C Time: 168 h pH: 3.5	[a]	[a]	Lab scale: Erlenmeyer flask	$(NH_4)_2SO_4$: 2.0 g/L	[a]	16.78	Akintomide and Antai (2012)
	Saccharomyces cerevisiae	Yeast	Temp: 28 °C Time: 168 h pH: 3.5	[a]	[a]	Lab scale: Erlenmeyer flask	$(NH_4)_2SO_4$: 2.0 g/L	[a]	21.30	Akintomide and Antai (2012)

Legend: CDM – Cell dry mass, NE: No enrichment.
[a] Not specified, Temp: Temperature.

such as millet and wheat (Bajpai, 2017; Leger et al., 2021). This presents SCP as a scalable technology solution to a sustainable protein supply and accentuates the economic benefits accompanying SCP commerce. The following subsection briefly captures current commercial engagements in the SCP arena.

4.2. Market dynamics

4.2.1. Current and projected market value

In thhe previous subsection, some significant benefits of accelerating SCP commercialization were delineated. Here, we elaborate on the current SCP market size while highlighting projections of its market value in the next decade. A significantly growing market and consumer base have been identified for SCP, driven by the increasing demand for sustainable protein alternatives, resurging interest in biotechnological technologies, and the expanding scope of its food and feed applications (Global Market Insights, 2023; Market Research Intellect, 2022). The industry was valued at USD 8 bn in 2021, according to a Global Market Insight survey involving 21 countries across five continents. The industry's value is anticipated to surpass USD 18.5–18.8 bn by 2030 and USD 20.64 bn by 2032 at an estimated compound annual growth rate (CAGR) of 9–9.7% (Global Market Insights, 2023; Transparency Market Research, 2023). A country-wise analysis shows dramatic growth in Malaysian and Vietnam SCP markets, with 2020 market values of USD 9.7 mn and USD 26.7 mn, respectively. These values are projected to go beyond USD 24.5 mn and USD 69.4 mn, respectively, by 2030, and markets in China, the US, and parts of Western Europe are expected to rapidly rise as well (Transparency Market Research, 2023). Regional insights also present a tremendous boom in SCP on the European market because of the increased pressure for feed protein supply. The current market is valued at USD 1.11 bn, representing approximately 30% of the 2023 global market share. The European market could surpass USD 4.5 billion by 2030 if expectations are backed with innovations, technological development, and advanced research (Global Market Insights, 2023). North America is also performing well, dominated by the US, with a regional market share of approximately 92%, and is also expected to grow at a CAGR of over 5% within 2022–2030 (Global Market Insights, 2022; Persistence Market Research, 2023).

Regarding the microbial category, algae and fungi are leading global commerce, adding up to 60% of total microbial protein extraction. Algal SCPs are valued at USD 1.41 bn, representing approximately 38% of the global SCP market value (Persistence Market Research, 2023). Production volume is expected to surpass 410 kt by 2030 due to booming private and public sector interest (Global Market Insights, 2023). A segmentation of SCP application shows a 2.7%, 3%, 4%, and 6% rise in food, dietary supplement, cosmetic, and animal feed applications, respectively (Maximize Market Research, 2023). These trends are expected to continually rise due to burgeoning industrial and research interest, technological innovations, and consumer demand (Global Market Insights, 2022). However, achieving this perceived growth would require radical collaboration between governments, industry, and researchers. Such collaboration could be linearized as willingness on the part of governments to fund industries or start-ups that are interested in SCP production and efforts by these industries or start-ups to engage experts and researchers in finding sustainability-sensitive technological solutions.

4.2.2. Global distribution of SCP commercial engagement

A global-wide literature search was performed to consolidate available information, summarized in Fig. 3, to demonstrate the spatial dispersion of SCP-related commercial activities. The trend highlighted a high density of SCP commerce in the US and China, representing approximately 50% of ongoing commercial engagements. Additionally, a fair distribution was identified across Europe, especially in Germany, France, the UK, and the Netherlands, which cumulatively hold approximately 23% of commerce. It was evident that commercial exploration

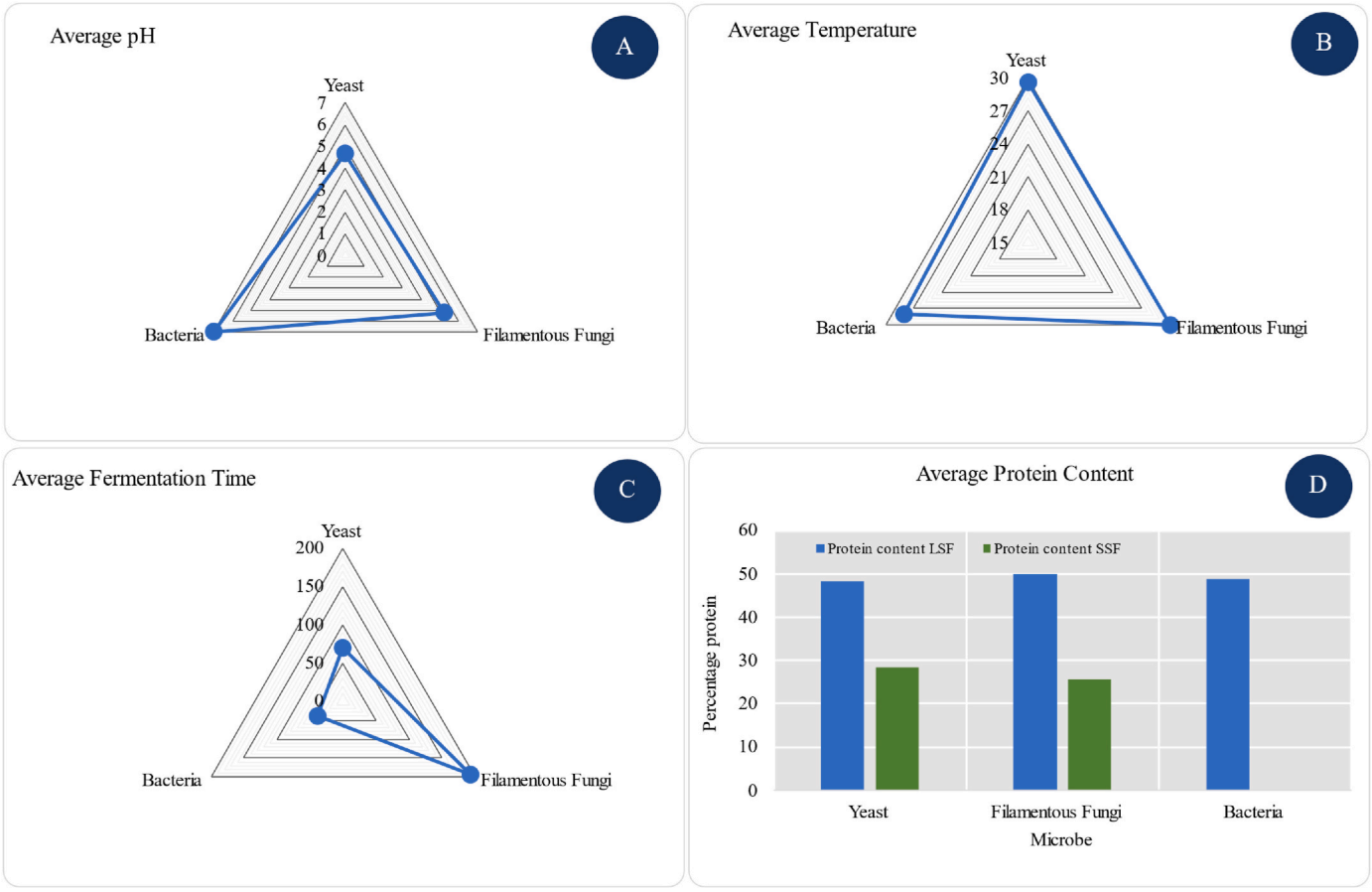

Fig. 2. Dynamics in SCP production for different microbial types: Average fermentation conditions and protein content.

has not progressed much in Africa, South America, and some parts of Europe. However, existing reports signal a gradually emerging interest in these regions, particularly South Africa, Nigeria, Brazil, and Argentina, demonstrating the potential to realize massive engagements in the rising urgency to adopt biotechnological solutions for sustainable agriculture and agro-industry (Maximize Market Research, 2023). The resounding message here is that several regions have explored SCP. However, many others are yet to explore this opportunity. Therefore, these incoming regions should learn from existing commercial trends to facilitate sustainable adoption. A typical recommendation for these regions is to explore the business incubator strategy to support and direct the interest of young entreprenuers towards the SCP technology, which could brood regional capacity to accelerate practice and commerce. In a global case where demand for sustainable protein sources rapidly evolves in pursuit, such adoption would be timely for marrying improved regional protein supply with planetary health and social development.

4.3. Current and evolving applications

4.3.1. Feed and food applications

There is a rapidly growing exploration of different varieties of SCP in feed production. To some, utilizing SCP in animal production is a suitable nutritional alternative and cost relief to farmers, given its protein adequacy and affordability. Additionally, its low requirement for land space situates its ability to decouple animal production from huge pastureland requirements, further triggering interest in SCP based feed production. Already, SCPs have been used to produce feed or feed supplements for pets, swine, cattle, poultry, and aquaculture (Altmann & Rosenau, 2022; Global Market Insights, 2022; Persistence Market Research, 2023). Its expanding application in aquafeed production is particularly fascinating, catalyzed by the exponentially growing aquaculture industry and associated increase in demand for high quality-low cost protein feed (Owsianiak et al., 2022; Ragaza et al., 2020; Transparency Market Research, 2023; Yang et al., 2021). Recent exploration has realized successful and beneficial substitution of aquafeed ingredients such as fishmeal and soybean meal with yeast SCPs such as brewer's yeast (Guo, Qiu, et al., 2019; Jin et al., 2018) andyeast hydrolysate (Jin et al., 2018), and bacterial SCPs such as *Corynebacterium ammoniagenes* (Hamidoghli et al., 2018) for shrimp, salmon, trout, and carp production. Nonetheless, these studies correlate SCP's best substitutional benefits to using optimal proportions in feed rations (Guo et al., 2019a, 2019b; Hamidoghli et al., 2018).

Utilization in food formulations is gradually dispersing across the food and beverage industry (Banks et al., 2022; Bratosin et al., 2021), with adoption in meat analogs, bakery, dietary supplements, dairy alternatives, cereals, snacks, and beverages dominating the current food application trend (Global Market Insights, 2022; Persistence Market Research, 2023). For instance, given its remarkable functional, nutritive, and radical scavenging capacities, Razzaq et al. (2020) inferred its suitability in food emulsions, minced meat, baked foods, and frozen desserts for improving product characteristics and health benefits. In this regard, SCP from food waste (banana peel, citrus peel, potato peel, and carrot pomace) has been successfully utilized in breadmaking for improving dough characteristics and enhancing the nutritional benefits of the resulting bread, wherein 4% addition was identified as the optimal concentration for achieving desirable functionalities (Khan et al., 2022). The North American and ASEAN (Association of South East Asian Nations) markets are constantly innovating ways to ameliorate algae (*Spirulina and Chlorella*) and fungi (*Fusarium*) production as a "superfood" to enhance regional commitment to convenient and healthy living (Transparency Market Research, 2023). Additionally, Health

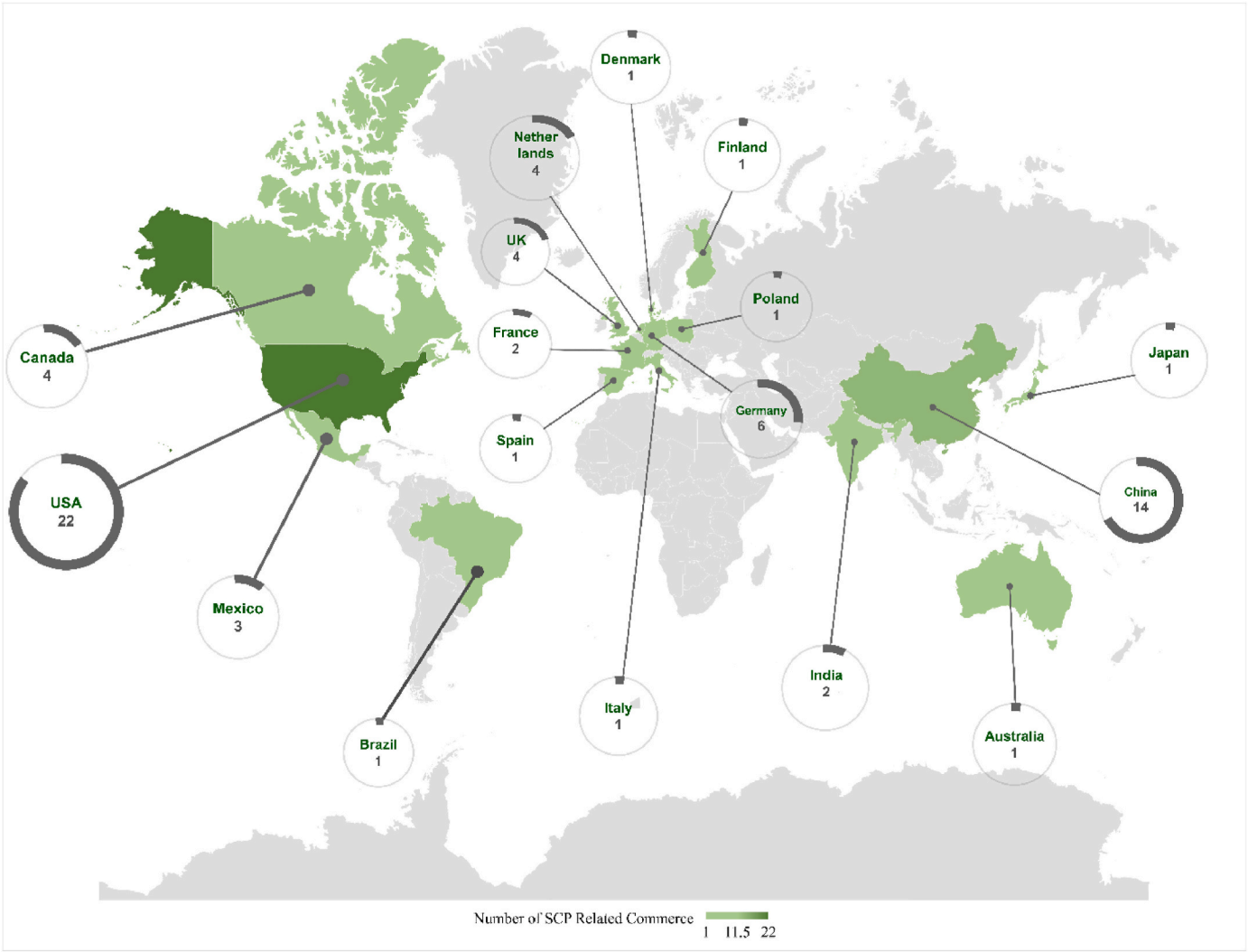

Fig. 3. Global distribution of SCP-related commercial engagements.

Canada has permitted and is keenly regulating the use of whole algal SCP as an alternative protein source in foods, signaling a future boom in food application (Global Market Insights, 2022). Nonetheless, the application of SCP in food and feed applications is limited by reported toxicity and metabolic constraints, elaborated upon in the following subsections.

4.3.1.1. Nucleic acid and toxicological limitations. The toxicological status of products is necessary for approving their use in food and feed. Despite the emerging interest in food and feed application, the presence of undesirable amounts of nucleic acid in most SCPs has limited acceptance as a food and food ingredient, given the health risks associated with a high intake of nucleic acid (Bajic et al., 2022; Carter & Codabaccus, 2022). Nucleic acid is synthesized into uric acid in humans, an undesirable chemical that instigates vulnerability to health detriments such as carcinogenesis, urinary diseases, and renal diseases such as renal calculus and gout – rich man's disease (Ugbogu & Ugbogu, 2016). Unfortunately, humans lack uricase, a uric acid-degrading enzyme; therefore, they stand the risk of uric acid bioaccumulation upon consumption of SCP (Nangul & Bhatia, 2013; Ritala et al., 2017). At undesirable levels, uric acid instigates vulnerability to acute and lethal health detriments, making SCP particularly unattractive for human consumption and sometimes for feed application (Ugbogu & Ugbogu, 2016). Nonetheless, techniques have evolved to reduce the nucleic acid content of SCP to the fairest and safest minimum, implicating the potential to spur its adoption as a food and feed ingredient (García Martínez et al., 2022; Ritala et al., 2017). Additionally, the toxicological orientation of some microbes has presented limitations in leveraging SCPs as human food. Gram-negative bacteria and filamentous fungi can produce endotoxins and mycotoxins, respectively, which attaches safety risks to SCPs from these microbes and decreases their suitability and acceptability for human consumption (Ugbogu & Ugbogu, 2016). In subsequent developments, scientists propose process optimization and deployment of aseptic techniques as key considerations to overcome such toxicological impediments (García Martínez et al., 2022).

4.3.1.2. Metabolic constraints. Concerns about the metabolic biochemistry of food and feed materials have elicited remarkable research interest. In this space, contemporary nutrition science and gastroenterological studies have aimed to understand the correlation between the nutritional properties of materials or products and digestive performance. The outcome of these studies has supported the prominent claim that the "classification of material or product as nutritious hinging on the in vitro quantification of their nutritional compositions is erroneous." They do not argue the relevance of assessing the nutritional content. However, they prioritize the digestive chemistry of a food or feed material/product as the "main deal" in dietary applications. This context, coupled with surging interest in leveraging SCP in food and feed applications to augment protein supply, has inspired several studies that sought to understand the digestibility of SCP. Existing results emphasize

that SCP from algal and some bacterial cells has lower digestibility, induced by poorly digestible cell walls, making it difficult for humans to access and metabolize the available proteins and nutrients (Nasseri et al., 2011; Ritala et al., 2017; Ugbogu & Ugbogu, 2016). Consequently, there has been a drastic contention against using bacteria-based SCP as human food. If this persists, the current prospection of these SCPs in addressing future protein demand and food shock will contradict expectations despite the evolving development and innovations in their processes and production. Fortunately, several methods have been developed for SCP cell wall degradation, with varying efficiencies and desirable outcomes, highlighting substantial progress in surmounting the present constraint.

4.3.2. Non-food applications

Aside from the overarching interest in SCP for food and feed, the substantial composition of biobased coproducts such as polyhydroxyalkanoates (PHAs) in some bacterial species has bolstered interest in biopolymeric material production (Kunasundari et al., 2013). The major advantage of this pathway is the possibility of requiring little to no strong chemicals in biopolymer synthesis while cobenefiting access to microbial protein to satisfy protein needs. For instance, in an animal-based model for SCP production, it was possible to recover indigestible polyhydroxybutyrate (PHB)- a useful biopolymer in the biorevolution of bioplastic films and packages, from the fecal matter of rats fed with *Cupriavidus necator* SCP, requiring no strong chemical in the extraction process (Chee et al., 2019; Kunasundari et al., 2013). In this regard, SCP stands as one of the evolving technological solutions for addressing the current climate emergency, among other environmental burdens accompanying the production of synthetic plastics, while providing sustainable plastic alternatives with desired mechanical, thermal, and biodegradable qualities (Areniello et al., 2022). However, the high cost of producing SCP-based biopolymers is stalling the scalar adoption of this procedure, requiring advanced research to improve current techniques (Kunasundari et al., 2013).

The agricultural industry has also found the application of SCP as an organic fertilizer for recovering the soil quality of farmlands (Kantachote et al., 2016). Microbes have been grown on agricultural biomass to recover essential nutrients such as nitrogen, phosphorus, and potassium, which are later applied to farm soils for bioenrichment and consequent improvement of plant growth (Areniello et al., 2022). In practice, the possibility of skipping resource-intensive operational conditions such as drying and using multiple substrates make the process cost-efficient. However, biofertilizers' current market value seems to curtail the potential for expanding production and verging into commercial sales (Areniello et al., 2022). Research is therefore needed to understand current limitations and uncover opportunities for commercialization.

Overall, SCP is expanding in application, and the influx of novel and innovative technologies instigated by extensive research and development promises an opportunity to further expand and extensively explore the vast potential of the SCP industry.

5. Sustainability outlook of SCP production

The previous sections established SCP as a rapidly evolving biotechnological solution to biomass conversion, underpinning its production status and rapidly growing commercial prospects in the face of increasing protein demand. In this section, we delve into understanding the sustainability outlook of SCP production, highlighting the application of sustainability metrics and associated findings for further improvement. A thorough bibliometric search on Google Scholar, Web of Science, and Scopus using query words that combine selected sustainability metrics and microbial protein or SCP showed that LCA and TEA has been extensively used in SCP sustainability assessment. However, other sustainability metrics, such as LCCA, SLCA, and environmental nutrition, have not yet been investigated. This section is divided into three parts. The first subsection summarizes available LCA studies, including their methodological structures, significant findings, and meaningful recommendations. It also discusses the sustainability prospects of a novel power-to-food (PtF) technology in SCP production. The second subsection discusses relevant TEA studies, and the third subsection elaborates on current gaps and recommendations for future studies.

5.1. LCA

5.1.1. Summary of LCA studies

Table 2 summarizes relevant LCA studies gathered from the literature. It captures the scope, methodologies, findings, gaps, and recommendations from these studies. The table displays a notable trend of SCP bearing significant environmental benefits in the global warming and land use categories. It generally informs SCP as a sustainable alternative to animal-based proteins, particularly dairy and beef, inferring an opportunity to replace these animal products with SCP wherever such substitution benefits product quality. Moreover, in generic terms, SCP offers substantial impact offsets relative to livestock and aquacultural feed such as soybean meal, fishmeal, and palm kernel meal, hinting at an opportunity forrelated industries to maximize performance through sustainable feed formulations. However, such benefits vary with the substitution level and other operational factors such as electricity use and analytical dynamics such as the boundary of the environmental impact assessment. Overall, renewable energy is identified as a sustainable energy source for augmenting environmental benefits, with substantial global warming and land use offsets identified in several recent studies (Khoshnevisan et al., 2020; Kobayashi et al., 2023; Owsianiak et al., 2022; Upcraft et al., 2021). Some interesting recommendations from these studies include the adoption of precision fermentation and virtual support systems, integrating multicriteria decision analysis in process-product design, deploying genetic engineering in microbial improvement, and expanding performance analysis to include nutrition, economic and social metrics.

5.1.2. LCA: application of the innovative power-to-x technology in SCP production

Power-to-X (PtX, where X could be any biobased product) technologies are reportedly self-sustaining alternatives for producing products from renewable energy sources via water electrolysis and other complimentary processes (Secreters European Union's Horizon Programme, 2022). Using this concept, a recent study compared a power-to-food (PtF) SCP pathway to soybean and other SCP pathways. The study indicated a 60% offset of global warming footprint by the PtF-SCP approach (0.81–1.00 $kgCO_{2eq}/kg_{protein}$) relative to soybean production (0.89–3.74 $kgCO_{2eq}/kg_{protein}$). This signifies the potential PtF-SCP bears in addressing the current climate emergency and, from the endpoint view, decoupling SCP production from human health damages (Sillman et al., 2020). Regarding land use, water use, and eutrophication, the PtF-SCP offered approximately 84.00–99.50% impact savings compared to soybean production, further signaling its sustainability inclination. Relative to other SCP pathways, such as methane-based SCP and Quorn mycoprotein (fungal SCP, *Fusarium venenatum*), PtF-SCP maintained its low GWP advantage. However, both reference pathways outweighed the PtF-SCP in eutrophication impact (Sillman et al., 2020; Smetana et al., 2015). The authors related global warming offsets of the PtF-SCP system with renewable electricity sources such as wind and solar, contrary to the use of conventional fossil electricity sources in the other pathways (Sillman et al., 2020). Additionally, a sensitivity analysis of the impact of different renewable energy sources on the performance of the PtF-SCP approach laid an exciting trend, highlighting wind electricity as a better alternative to solar for improved energy utilization.

Overall, land and global warming savings are the major environmental benefits driving the adoption of SCP production. Energy modeling is also honed as a critical activity for improving SCP

Table 2
Summary of SCP-LCA studies.

Scope of Study	Methodological Structure			Major Findings	Gaps/Recommendations	Reference
	Functional Unit	System Boundary	Inventory Data/ Modeling			
Empirical attributional LCA assessment of SCP production with expanded system boundary and impact categories Microbe: *Hydrogen-oxidizing bacteria (HOB)* Fermentation Approach: LSF	1 kg of MP product prior to pack with a 5% moisture content at the factory gate	Cradle-to-gate with scenarios for electricity consumption created for sensitivity analysis.	Pilot scale production data (Plant area was 1580 m^2, and byproducts were cutoff in the analysis) Database: Ecoinvent 3 Impact method: ReCiPe 2016 v1.1 Midpoint (H), AWARE (water use), and CED v1.11 (energy use) Sofware: SimaPro 9.1.0.11 Secondary analysis: Sensitivity and Uncertainty Analysis	• Consuming SCP (65% protein assumed) instead of the dairy herd or bovine meat would offset an average of **16 m^2** and **36 m^2** of LU per 100 g of protein, respectively. • Electricity consumption contributed the most to all impact categories. • Hydropower could offset up to **87.5%** GWP and approximately **25 times less** LU relative to electricity mix with a high percentage of nuclear power	• Consequential LCA of SCP production to ascertain expected changes when SCP is commercialized. • Expand impact categories to include others like biodiversity, which closely correlates with conventional protein production	Jarvio et al. (2021)
Consequential LCA of different biorefinery pathways using a mixture of organic fractions of municipal waste and supermarket waste as substrates to identify the most sustainable valorization pathway. Substrate: Organic Fraction of Municipal Waste and Supermarket Waste Microbe: *Methane-oxidizing microbe*	Management of 1 tonne of biopulp with an average TS of 18.3%	Gate-to-gate	Lab-scale experiments Database: Ecoinvent (v3.3) Impact method: Impact 2002 + Software: SimaPro 8.5 Secondary analysis: Sensitivity	• SCP-based pathways could save up to **155 kg CO_2 eq per tonne** of biopulp compared to conventional protein sources like fish, soybean, and palm kernel meals. • Renewable energy options increased environmental savings of design pathways. • The environmental benefit from each scenario depends not only on the biorefining pathway but also on the selected downstream process	• Employ multicriteria decision support tools for SCP sustainability assessment considering energy performance, economics, environmental, consumer, and regional legislations.	(Khoshnevisan et al., 2020) (Elyasi et al., 2021)
Attributional LCA to assess the environmental impact of SCP from oat-side stream. Substrate: Oat-side stream	1 kg of dried SCP product	Cradle-to-gate	Experimental data, literature, and technical reports Database: Ecoinvent 3.8, Agribalyse 3, Agri-footprint 5.0). Impact method: ReCiPe 2016 Midpoint (H) Software: SimaPro 9.3.0.2 Secondary analysis: Sensitivity	• Impact contribution was based on the nature of substrates, with wet-side streams offering better impacts than the dried-side stream. • The dried-side stream had **approximately 8 and 18%** increases in FC and GWP values relative to the wet-side stream • Regional sensitivity demonstrated fossil energy-dense regions to contribute highly to most impact categories. • Approximately 6**1%** LU offset was achieved for SCP relative to soy protein concentrates	• Genetic modification of yeast for improving biomass yield, generation time, substrate use efficiency, and nutritional value. • Integration of renewable energy systems to enhance the environmental savings of SCP production systems	Kobayashi et al. (2023)
Comparative study of an optimized closed-loop mycoprotein framework with animal-based proteins Microbe: *Fusarium venenatum*	kg of microbial protein	Cradle-to-gate	The average global profile of Quorn™ fermentation process data and global average feedstock and energy profiling Impact Method: ReCiPe Midpoint (H) Software: OpenLCA	• Closed-loop SCP system offsets up to **96%, 99%,** and **85%** impact values of CC, LU, and WC, respectively, relative to beef. • Substituting future beef consumption with an equivalent quantity of	• Further optimization of microbial protein systems to provide more environmentally sustainable and scalable SCP technology solutions to meet future protein demands	Durkin et al. (2022)

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Table 2 (*continued*)

Scope of Study	Methodological Structure			Major Findings	Gaps/Recommendations	Reference
	Functional Unit	System Boundary	Inventory Data/ Modeling			
				SCP would reduce the impact on CC and LU from **45** and **24%** to **2** and **0.2%,** respectively		
A static LCA approach for future projections of the environmental impacts of substituting ruminant meat with sugar-based SCP using spatially explicit land-use model MAgPIE Substrate: Sugar	Per capita replacement of ruminant meat with SCP in forward-looking land-use scenario	Cradle-to-gate (A middle-of-the-road scenario for future population, income, and food demand)	Literature data Impact Model: Model of Agricultural Production and its Impact on the Environment (MAgPIE)	• Global forest loss based on the current agricultural system is estimated at 175 Mha by 2050. • **20, 50, and 80%** per capita substitution can offset **56, 82, and 93%** of deforestation; **56, 83, and 87%** of net carbon dioxide emissions; and **11, 26, and 39%** of methane emission by 2050, respectively	• Include consequences of reducing the production of commercially viable ruminant production byproducts like hide skin, fats, organs, bones, and blood in LCA. • Precision fermentation as a future technology for promoting alternative protein production	Humpenoder et al. (2022)
A life cycle assessment to compare four food waste management scenarios. Substrate: Food waste Microbe: *Purple nonsulphur bacteria (*PNSB) Fermentation approach: LSF	Food waste produced by a city of 50,000 people per day at 0.31 kg FW/ day/per person	Gate-to-gate (Waste collection to manufacturing gate)	Literature data Database: Ecoinvent 3.1, LCA Food DK Impact method: TRACI Software: SimaPro Secondary analysis: Sensitivity and Uncertainty Analysis	• The environmental benefits associated with SCP are based on the product it is replacing. • Replacing soybean with PNSB production presents better environmental compensations than fishmeal replacement.	• Future PNSB technology could be improved by optimizing growth rate, organic loading, and degree of substitution	LaTurner et al. (2020)
Quantify the relative and absolute environmental performance of a pilot-scale SCP production from starch-rich process water and use it as feed relative to conventional feed sources. Substrate: Starch-rich potato process water Microbe: *Aerobic heterotrophs* Fermentation approach: LSF	Provision of nutritional value to edible white leg shrimp (Litopenaeus vannamei) required to produce 1 tonne per year of shrimps in an Intensive aquaculture production system at a feed conversion ratio between 1.2 and 1.8 and yield of at least 61 t/ha of pond	Gate-to-gate (From the supply of substrate to the management of aquaculture biowaste)	Pilot scale and flowsheet simulation data Impact method: Multiplying elementary flows by characterization factors and summing resulting indicator scores (Compared with ReCiPe 2016 Midpoint (H) Software: SimaPro 9.2.0.2 Secondary analysis: Sensitivity and uncertainty	• The environmental impact of SCP-based feed depends on the substitution level and the type of meal being replaced. • SCP feed outperformed soybean meal in terms of GW and LU • Greener energy modeling is not sufficient to make SCP sustainable in absolute terms	• Using the bioreactor off-gas as the carbon source for hydrogen oxidizing bacteria, purple phototrophic bacteria, or green microalgae SCP feed would be a more sustainable technological alternative to improving SCP resource use efficiency.	Owsianiak et al. (2022)
Attributional life cycle assessment is used to compare the environmental impact of replacing soy ingredients with SCP in salmon feed. Microbes: Methanotrophic bacteria and Yeast Substrates: Fossil methane (Bacteria), Wheat byproduct (Yeast)	Per 660 g of protein (1 kg of soy protein, 0.94 kg of bacteria meal, and 1.07 kg of yeast protein concentrate)	Cradle-to-gate	Literature data, Norwegian imports data Impact method: ReCiPe (v.1.11) Impact calculation: According to Pauly and Christiansen's (1995) equation	• Yeast SCP had the lowest impacts in all categories and overall. • Bacteria SCP had similar CC and FWC impact results like soy protein but performed moderately in other categories. • Overall, replacing soy protein with bacterial or yeast SCP can significantly reduce environmental impacts	• Using diverted methane instead of natural gas for bacteria production can substantially offset environmental impacts. • Industrial-scale production developments are required to improve the benefits of SCP in aquaculture	Couture et al. (2019)
To assess the environmental sustainability of the lignocellulosic SCP and compare it with food-derived SCP and conventional proteins Microbe: *Fusarium venenatum* Substrate: Rice straw	1 kg mycoprotein paste at biorefinery gate, with a solids content of 25% based on a production capacity of 40,000 tonnes per year	Cradle-to-factory gate	Field and literature data Impact Method: ReCiPe 2016 Midpoint (H) Software: SimaPro V9 Secondary analysis: Contributional analysis, Sensitivity analysis	• External electricity use and production represents **72.9% and 58.4%** of GWP and TA • Straw production contributes approximately 75% and **67.58%, and 95%** of WC, ME, and LU • Cutting-off emission from rice production	• Integrating renewable energy resources into lignocellulosic SCP would be a reliable decarbonization solution. • SCP could be a transformative solution to future protein security due to manufacturing in a controlled environment	Upcraft et al. (2021)

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Table 2 (*continued*)

Scope of Study	Methodological Structure			Major Findings	Gaps/Recommendations	Reference
	Functional Unit	System Boundary	Inventory Data/ Modeling			
				reduces SCP emissions substantially • Substituting soybean meal with yeast SCP possesses environment and nutrition cobenefits. • Replacing soybean meal with yeast SCP could offset **55% and 32%** of GWP and LU • Environmental impact was sensitive to SCP yield and level of impact allocation	and short generation time. • Work is required to upscale feed production from novel SCP. • Breaking technical and legislative barriers is next in achieving success in commercial adoption	Tallentire et al. (2018)
To examine the environmental implications of replacing soybeans with novel ingredients in chicken feed formulations Microbes: Yeast, Bacteria	One bird grown to a live weight of 2.2 kg	Gate-to-gate	Literature data Database: Agri-footprint, Ecoinvent Impact method: ReCiPe Software: SimaPro Secondary analysis: Comparative, Sensitivity, and Uncertainty analyses			
Comparative analysis of meat substitutes' environmental performance to estimate the most promising options	1 kg of a ready-to-eat meal	Cradle-to-gate	Field and literature data Impact methods: ReCiPe and IMPACT 2002+ Secondary analysis: Sensitivity analysis (Calorific energy content of product used)	• SCP had a similar impact on human health compared to chicken meat but performed better than lab-grown meat. • Energy demand contributed **approximately 45% and 25%** of the impact of SCP processing and frying. • Chicken meat outweighed SCP in terms of calorie-based FU	• Comparison of meat substitutes in the same production conditions with sole dependence on field data needed. • The functional unit definition should be core in LCA since it could dramatically alter impact results	Smetana et al. (2015)

Legend: CC: climate change, GWP: global warming potential, LU: land use, WC: water consumption, FWC: fresh water consumption FU: functional unit, ME: marine eutrophication, TA: terrestrial acidification, PNBS: purple nonsulfur bacteria.

sustainability, with recommendations underpinning renewable energy and waste heat recovery systems as more sustainable alternatives to electricity grids that rely heavily on fossil energy. It is, however, notable how SCP fails to satisfy the absolute limitations of some planetary boundaries, reinstating the vitality of hotspot analysis and impact category expansion to understand the trade-offs and improvements required for augmenting future SCP technological scenarios.

5.2. Techno-economic assessment (TEA)

A brief description of TEA would place the perspectives discussed in this study into context. TEA is mainly used to analyze technical hotspots and viabilities of a product, process, or service (Giacomella, 2021). It has experienced a continuous escalating interest within academia and industry due to its support in *a priori* and *a posteriori* technical decision-making (Kumar & Tewary, 2021; Kurambhatti et al., 2021), revealing the cost variability in a system, project, and investment. Also, it relies on technological performance to contemplate the economic conditions of varying technical adoptions, which often triggers proclivity toward high-performing technologies or technical solutions with optimal economic impacts (Giacomella, 2021). These advantages establish the cruciality of TEA in enhancing the technical feasibility and performance of SCP production. Although comprehensive standalone studies on the TEA of SCP production are limited, quite a few studies have considered the integration of SCP production as an economically advantageous upcycle pathway in complex industrial settings, some of which are elaborated below.

Whole stillage is a major nutrient-dense byproduct of bioethanol production (Bulkan et al., 2020). In the business-as-usual, whole stillage is processed into distiller's dried grains with solubles (DDGS) through energy-intensive centrifugation and evaporation. Empirical estimations highlight such steps to contribute an average of 35% and 43% to the electrical and thermal energy demands of a typical dry mill ethanol production plant, respectively, vitally affecting capital investment and intensifying environmental consequences (Bulkan et al., 2020). A techno-economic study targeting the identification of economic and energy-saving pathways asserted the substitution of conventional whole stillage processing with a downstream process that produces additional ethanol and protein-rich fungal biomass (practically SCP production) as a turnkey solution (Rajendran et al., 2016). Although this choice demanded capital investment to increase by USD 1.2 million to reach USD 70.2 million, the resulting increase in net present value (NPV) by USD 31 million and an attractive profit margin boom underlined its preference over the conventional production approach. Energy-wise, the novel approach resulted in approximately 2.5% energy savings, enhancing the overall technical efficiency of the process. An expansion of the system boundary of this study by Bulkan et al., (2020) further buttressed the economic advantage of an integrated SCP pathway in ethanol production, also projecting an approximate 6% additional increase in NPV when fungal biomass is sold in the human food market.

Simulation of SCP production from grass silage via steam explosion, enzymatic hydrolysis, and alkaline pretreatment techniques was assessed for its techno-economic viability (Pihlajaniemi et al., 2020). For a processing capacity of 60,000 tons of silage (dry matter), a capital investment of approximately € 38.8–55.8 million was needed, equivalent to USD 42.02–60.42 million at present (January 2023), at an exchange rate of approximately 1 € = 1.082874 USD (Forbes, 2023). The steam explosion process was cost-intensive, placing the alkaline (ammonia) pretreatment technique as a feasible alternative for localized, small-scale SCP production. The authors highlight variable costs such as enzyme, silage protein, and protein quality as limiting factors to commercial success. Therefore, they recommend optimizing the processes in favor of these variables to enhance technoeconomic feasibility and progress commercial entry.

As accentuated by Giacomella (2021), the absence of a defined standard for TEA practice diversified the cost inclusion and exclusion criteria, indicator selection, and scope of the assessment for the studies considered in this review, subjecting generalized economic assumptions from present assessments to uncertainties. On that premise, it is strongly recommended that efforts toward developing a robust TEA standard be intensified to enhance the reproducibility, reliability, and generalizability of TEA studies and findings. Additionally, the unavailability of economic data for novel technologies has stalled techno-economic scrutiny of novel SCP pathways such as photovoltaic-driven SCP (in which microbes utilize chemical energy generated from bioconversion of solar energy) (Leger et al., 2021), PtF (Sillman et al., 2020), and microalgae (Janssen et al., 2022). Subsequently, generating such data would enhance the TEA of evolving pathways and promote a smooth transition to sustainable protein in an economically beneficial manner.

5.3. Current gaps in SCP sustainability assessment

Recently, LCCA has gained traction in economic analysis due to its alignment with the LCA standards (ISO 2006:14040,14044) and consideration of a broader perspective of relevant system elements in cost analysis (Giacomella, 2021). It is distinguished by its intricate consideration of economics, cash flows, and other externalities, such as cost of greenhouse gas emissions (Giacomella, 2021; Ioannidou et al., 2022). This presents the current cost meanings of systems with little to no technological influence and situates cost modeling and decisions within the eco-economic decoupling frame (Allotey et al., 2023). SLCA is another critical component of the reformation in life cycle sustainability thinking, which aims to expound the relevance of social burdens or benefits associated with a product, service, or process in typical sustainability decisions (Caruso et al., 2022; Tsalis et al., 2022). It considers several social indicators, complexly characterized, usually qualitatively, to represent the accrued social footprint of a defined system on associated workers, the local community, consumers, and other value chain actors (Allotey et al., 2023; Caruso et al., 2022; Tsalis et al., 2022). While LCCA and SLCA have evolved to drive sustainability within economic and social contexts, rapid penetration of the environmental nutrition concept, especially into food and nutrition assessments, has also been witnessed (Aidoo et al., 2023). Holding this evolution is the desire to negotiate for sustainability pursuits with environmental and nutrition cobenefits (Agyemang et al., 2022; Aidoo et al., 2023). In this regard, decision-making targets solutions that can facilitate the achievement of optimal environmental and nutritional benefits (Aidoo et al., 2023). Life cycle sustainability assessment presents a holistic and robust perspective on sustainability assessment, combining all sustainability metrics and applying trade-off or multicriteria decision analysis to identify optimal solutions (Allotey et al., 2023; Wada et al., 2022). Despite the apparent significance of these concepts in robust sustainability assessment, a dearth of studies has explicitly applied them in SCP production systems according to our present knowledge. Thus, we foresee their integration as an opportunity to improve the SCP process and product design and provide a reliable baseline for making sustainable micro, meso, and macro decisions.

5.4. The digital twin concept for improving production and sustainability

Digital Twin is an Industry 4.0 technology that uses a virtual reality concept to incorporate computer simulation into actual system operations (Dyck et al., 2022). By this,complex system operations are brought into real-time virtual view by coupling sensor technologies with other graphical, mathematical, or predictive computer models. Dyck et al. (2022) mentioned three dynamic elements of a digital twin: a physical product in a physical space, a virtual product in a virtual space, and a mediative element that ties the physical product to its virtual representation using data and information. Whereas the adoption of digital twins into the food industry is still in its early stages, the benefits have been colossal, with massive improvements in productivity and the greenness of the systems that have applied the concept (Hassoun et al., 2022). In the recent application of digital twins in agriculture, the virtual representation of farms has been modeled to enhance data transmission, processing, and optimization of physical processes to maximize efficiency and reduce energy use, improving the overall sustainability of farms (Nasirahmadi & Hensel, 2022). Additionally, integrating digital twins in food and other industrial production lines has eased traceability and hotspot identification and accelerated the input of corrective actions to improve system sustainability performance (He & Bai, 2020). Therefore, embracing a digital twin system in the gradually evolving SCP production trend appears an outstanding opportunity to enable a unique trajectory of smart protein production in an Industry 4.0 era while aligning SCP production with sustainability targets. A summary of some available models or technologies that could sponsor a digital twin trajectory in SCP production is presented in Table 2 and the following subsections.

5.4.1. Available technologies and models for establishing an SCP-digital twin

Table 3 captures some available sensor technologies that could be utilized in building an SCP-digital twin. It briefly describes their mode of operation, current application, and relevant findings from their use.

5.4.2. Data-driven models

Data-driven models have gained core relevance in industrial processes, including fermentation. Its role in practical real-time analysis of sensor data and prediction of process variables, fault detection, and design optimization is outstanding. Recent developments have focused on overcoming process constraints such as complexity, nonlinearity, and time variations (Zhu et al., 2020). A detailed review of selected modern soft sensing models with application in fermentation is briefly highlighted in the following subsections.

5.4.2.1. Support vector machine (SVM) model. This standard data-driven soft sensing model has been used for predicting the output of complex nonlinear processes or systems even with small sample data. In practice, it is considered ideal for fermentation processes with small sample data to self-learn and accurately predict characteristics for accurate generalizations (Zhu et al., 2020). For large datasets, using SVM models can be very cost prohibitive. The coupling of SVM with other predictive and optimization methods such as generalized predictive control (GPC), particle swarm optimization (PSO), least square (LS), and multiple output variable least square (MLS) models rendered better prediction offermentation parameters such as biomass and substrate concentration (Robles-Rodriguez et al., 2016, pp. 175–183; Wang & Ji, 2015).

5.4.2.2. Fuzzy-Logic (FL) models. FL has also been deployed in process optimization, parameter or pattern identification, and system control. Having the potential to imitate the reasoning prowess of humans, FL has gained utility prominence in making intelligent data-driven guesses of the dynamics in fermentation systems. A typical example is the fuzzy neural network (FNN) model for predicting variables such as biomass, substrate, and product concentration of a penicillin fermentation process (Yonghong et al., 2012). For input variables such as dissolved oxygen, carbon dioxide, and sugar concentration, a uniform incidence degree algorithm has been employed for their identification with a high degree of performance (Zhu et al., 2020).

5.4.2.3. Deep learning (DL) models. DL-based soft-sensing models are also data-driven machine-learning models that have evolved in modern science and engineering design, optimization, and control. Unlike SVMs, deep learning models portray the benefits of effectively handling nonlinear structures, big process data cases, and better parameter approximations at a relatively affordable cost (Shang et al., 2014).

Table 3
Sensor technologies for monitoring fermentation process.

Technology	Mode of Operation	Current Application	Major Findings	Reference
Microbial Potentiometric Sensors (MPS)	Considering fermentation as a complex redox reaction, the potentiometric sensor measures the potential difference between the sample and a reference electrode probe which produces a signal to represent the stage of fermentation	To monitor the completion time of kefir-facilitated milk fermentation	• The MPS technology could monitor kefir fermentation in real-time with high reproducibility. • The regression analysis approach was able to discern a correlation between the fermentation completion time and the mass of kefir inoculum	Hristovski et al. (2022)
Thermodynamic Sensors (TDS)	Based on the energy measurement, which is supplied to the circuit to temperature setting and equilibration of temperature element with the ambient. The signals read are translated as the state of microbial activity	Preliminary studies have tested the performance of TDS in monitoring some phases like the end of the fermentation process in dairy fermentation, beer brewing, yogurt fermentation, and baking processes	• Need for advanced studies to verify its validity and performance at large industrial scales	Adamek et al. (2022)
Electrochemical Glucose Biosensors	Enzymatic oxidation of glucose to gluconic acid followed by the reoxidation of flavin groups to form H_2O_2 generation. H_2O_2 undergoes anodic oxidation on the surface of a working electrode which produces signals that are translated into a glucose concentration	Glucose quantification for batch-fed fermentation of yeast	• Fast and accurate measurement of glucose concentrations in fermentation • Glucose detection ranges up to 150 mM	Pontius et al. (2020)
Fluorescence-based optical sensors	Luminophor (a polymeric matrix) absorbs photons to reach a higher energy state with excited electrons. The interference in energy absorption influenced by the medium's physical (temperature and pressure) and chemical properties (nutrient composition) alters the energy gain of electrons in the luminophore, translated as the level of luminescence. Such variation in electron energy before and after oxygen interference is a measure of luminescence.	Oxygen concentration in winemaking	• Bears advantages in small and large-scale applications	Trivellin et al. (2018)
Combined Internet of Things and CO_2 Sensor System	The system consists of a CO_2 tunnel that collects CO_2 produced during fermentation. A sensor in the tunnel detects CO_2 concentration which is quantified and measured against a threshold. A fan is connected to the system, which activates and deactivates if CO_2 is above or below the threshold. A wireless system transmits data from the sensors for instant visualization and feedback.	Real-time monitoring of an alcoholic fermentation process proved an efficient solution for optimizing and controlling wine fermentation at a laboratory scale	• The instant visualization and feedback of the system minimize the need for human intervention. • The system can determine the state of fermentation and identify whether it is in the natural course, beginning, tumultuous, or completing using CO_2 signals	Canete-Carmona et al. (2020)

DL-based models such as deep neural networks (Ke et al., 2017, pp. 1–6) and hierarchical extreme learning machines (HELM) (Yao & Ge, 2018), among others, have been satisfactorily used in the parameter estimation of penicillin fermentation process and other multivariable bioprocesses with extensive process data (Gopakumar et al., 2018). Recently, Xu et al. (2022) also harnessed the integrative capacity of mechanistic modeling and deep learning models, such as the recurrent neural network (RNN), for predicting and optimizing the media enrichment step in a bioenergy production process involving polyhydroxyalkanoate (PHA)-forming bacteria. The optimization procedure was to identify the best combination of additives to achieve optimal PHA with desirable functionalities. These models could be duly deployed in an SCP-digital twin system for real-time monitoring or estimation of such parameters for rapid process decisions.

6. Future perspectives and conclusions

The review highlights SCP as an evolving protein alternative with the potential to enhance nutrition security without compromising environmental integrity. Expanding its commercial visibility emulates a trajectory for advancing circular bioeconomy goals in the dominant linear protein business model while producing enough protein to complement deficits in current supply. A summary of some potentials and limitations to SCP production is shown in Fig. 4. Based on the revamped interest in process and technological improvement, we envision a rapid influx of innovations to facilitate SCP biorevolution in response to the need to provide sustainable protein alternatives. For instance, current projections estimate an increase in SCP market value to USD 18.5 billion by 2030 (Global Market Insights, 2023), which provides enough motivation to recommend reinforced research and development in this emerging industry. Governmental and nongovernmental agencies are equally enthused about this trajectory, signaling the availability of comprehensive funding sources to support the current and projected SCP business scale. Certainty prevails that treading the SCP pathway also presents essential sustainability benefits, especially in reducing global warming and land use impacts. However, significant gaps in applying LCCA, SLCA, and environmental nutrition are noted, which could subsequently be explored together with LCA and TEA in a multiobjective manner to provide justifiable baselines for sustainability decisions.

The expansion of SCP production within a global scope of diversified consumers comes with several limitations, with consumer disorders such as food neophobia - the distrust and reluctance to try new foods, strict and limiting regulations, and some eliminable safety concerns such as high nucleic acid contents, standing prominent among the cluster of limitations. Food neophobia has been associated with the dwindling bacterial SCP market share and has contributed colossally to the existing resistance to SCP commerce. To some, boosting the penetration of bacterial SCP on the consumer market would demand intense sensitization and education to reconscientize the sheer number of consumers who uphold asceticism as a path to escaping the alarming pathogenicity of

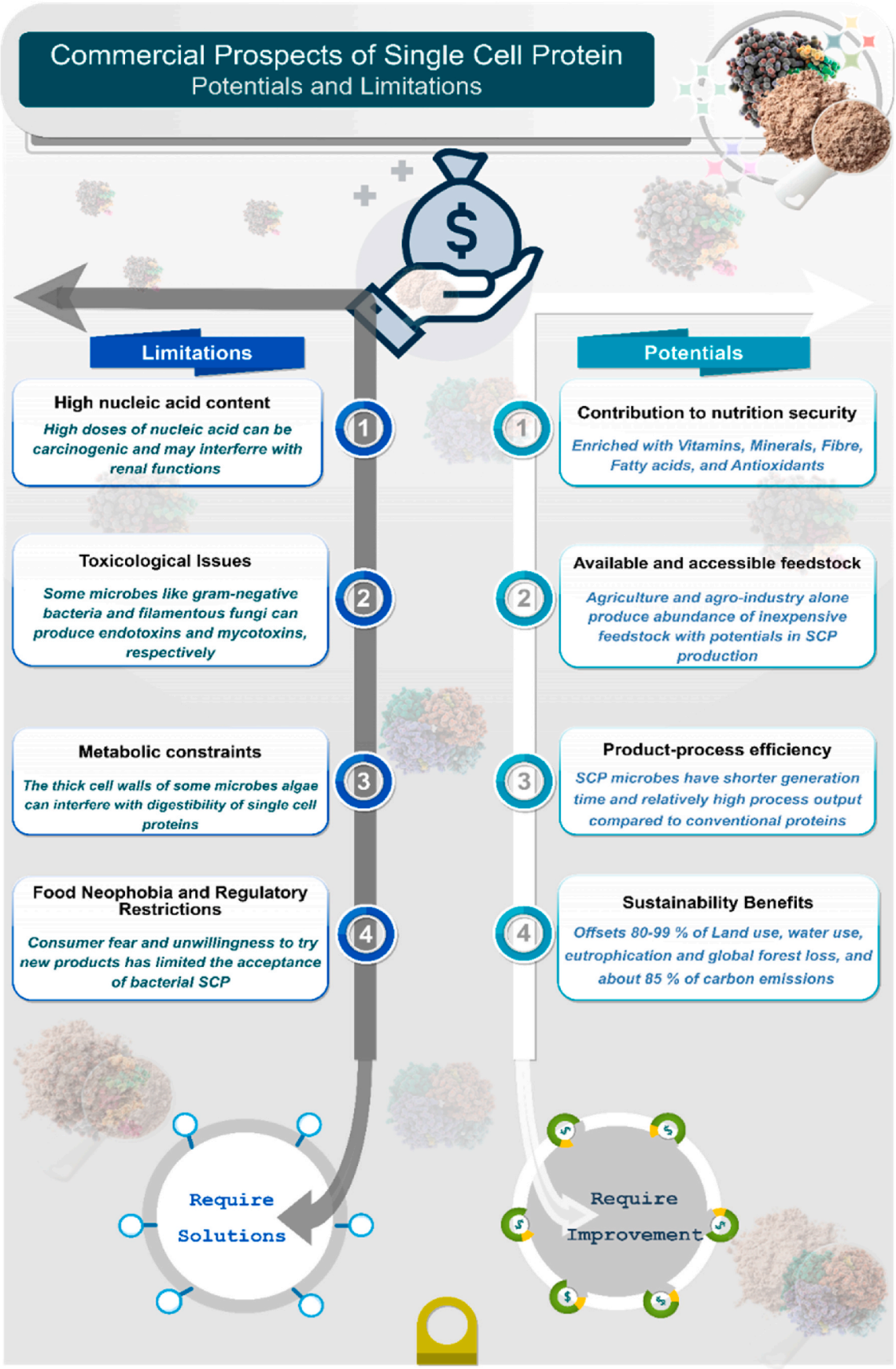

Fig. 4. Potentials and limitations to the commercial entry of SCP.

bacteria (Mazac et al., 2022). In proposition, subsequent SCP food design and development should prioritize food snob or fear factor as a significant parameter to predetermine consumer resistance. This can guide decisions on optimization and further market actions that would enhance overall patronage. Some novel SCPs, such as microalgal SCP, have also suffered delays in commercial launch due to complex and costly regulatory demands. For instance, it is time-consuming and cost-intensive to fulfill the regulatory demands for introducing entirely new products on the food market, resulting from revamped efforts to maximize compliance with safety and quality requirements. Consequently, SCP commerce has become unattractive for small and medium enterprises (Janssen et al., 2022). Thus, strategies to address these limitations while progressing innovations toward maximizing system efficiency must be engaged to activate the full commercial potential of sustainable SCP production.

In a world where digitization has become an indelible norm, engaging the digital economy concept in SCP production could increase the competitiveness, efficiency, and sustainability performance of SCP systems. Regarding this, the digital twin concept seems to hold high prospects in advancing digitalized SCP production, which could be activated by exploring the technologies and models discussed in real-time use. Success in enabling the SCP-digital economy would strengthen its resilience in a digitally evolving food system and ease process design, optimization, monitoring, and control of current and future designs.

Funding

The funding for this research was provided by the Sustainable Protein Production Program of the National Research Council Canada (SPP130-A1-019784). The manuscript represents National Research Council Communication #58401.

Declaration of competing interest

The authors declare no internal or external intent or association that can instigate conflicting interests.

Data availability

No data was used for the research described in the article.

Acknowledgment

The authors would like to express their profound appreciation to Dr. Jim Johnston and Dr. Pierre Fobert for their expert input and opinions that have shaped this paper and placed it in a better context. We also thank members of the Sustainable Food Systems Engineering Lab of McGill University, Macdonald campus, for internally reviewing this paper and providing perspectives that have placed it in its current form.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tifs.2023.07.003.

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