Environmental Science and Pollution Research (2024) 31:26378–26414
https://doi.org/10.1007/s11356-024-33004-7

REVIEW ARTICLE

A comprehensive report on valorization of waste to single cell protein: strategies, challenges, and future prospects

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Received: 10 June 2023 / Accepted: 16 March 2024 / Published online: 27 March 2024
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Abstract

The food insecurity due to a vertical increase in the global population urgently demands substantial advancements in the agricultural sector and to identify sustainable affordable sources of nutrition, particularly proteins. Single-cell protein (SCP) has been revealed as the dried biomass of microorganisms such as algae, yeast, and bacteria cultivated in a controlled environment. Production of SCP is a promising alternative to conventional protein sources like soy and meat, due to quicker production, minimal land requirement, and flexibility to various climatic conditions. In addition to protein production, it also contributes to waste management by converting it into food and feed for both human and animal consumption. This article provides an overview of SCP production, including its benefits, safety, acceptability, and cost, as well as limitations that constrains its maximum use. Furthermore, this review criticizes the downstream processing of SCP, encompassing cell wall disruption, removal of nucleic acid, harvesting of biomass, drying, packaging, storage, and transportation. The potential applications of SCP, such as in food and feed as well as in the production of bioplastics, emulsifiers, and as flavoring agents for baked food, soup, and salad, are also discussed.

Keywords Microbial protein · Microorganisms · Fermentation · Downstream processing · Food source · Green protein

Introduction

The global population is predicted to increase to nine billion by 2050. In light of the present pattern of food consumption, we may probably require 1250 million tonnes of dairy and meat products per year to fulfill the demand of animal-derived proteins (Verstraete et al. 2016). In the future, requirement of additional proteins cannot be fulfilled with the existing food production strategies such as agriculture. However, the proteins are quite essential for cellular and metabolic activities and serves as a source of nitrogen for animals and humans to form their functional and structural components for survival. In recent decades, protein-calorie malnutrition (PCM) has been reported to affect children, resulting in poor mental growth and weak immunity (Junaid et al. 2020). The nutritional value of proteins depends on their constituent amino acids. Due to their inability to be synthesized by the cells, animal populations typically require essential amino acids (EAAs) from external food sources to achieve their daily demand (Junaid et al. 2020). Proteins derived from different fruits, vegetables, and typical grains are often out of reach of the average person; therefore, microbial protein can be an alternate source of food for economically deprived population worldwide. Hence, this is high time to concentrate on deriving alternate, innovative, affordable, and unconventional protein sources to satisfy the nutritional requirements of the growing population. In regard, single-cell proteins (SCPs), cultured meat, plant-based new proteins, macroalgae, seaweed, and insects are some of the examples of sources of alternate proteins. Production of SCP is one of such potential approaches.

Single-cell protein mainly consists of a dried mass of microorganisms with high protein content, carbohydrates, lipids, minerals, and vitamins. The term SCP was coined by Carol L. Wilson in 1966 to define microbial biomass products (Suman et al. 2015). It can be total biomass or proteins isolated from pure culture or a mixed culture of microbial populations such as bacteria, algae, and fungi. The SCP has countless significant advantages over other protein sources:

Responsible Editor: Ta Yeong Wu

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(i) usually, raw or waste material is utilized for the growth and development of microbial population; (ii) the microbes efficiently convert the substrate into high-yielding biomass; (iii) the efficiency of the process of SCP production is not influenced by seasonal variation; (iv) microbial cells multiply far more rapidly than the higher and more complex species; (v) the entire cell biomass is possibly edible (Srividya et al. 2013; Nyyssölä et al. 2022)

The SCPs are popularly used in animal and human feeds as a dietary supplement as these are rich in protein and relatively low in fat content (Srividya et al. 2013). The SCPs also contain vitamins such as thiamine, nicotinic acid, pyridoxine, riboflavin, ascorbic acid, pantothenic acid, biotin, cyanocobalamin, folic acid, α-tocopherol, and β-carotene; EAAs like lysine and methionine; lipids; minerals; and nucleic acids (Suman et al. 2015). The major benefits of using SCP over animal- and plant-based equivalents are its beneficial functions and independence of seasonal and climate change. The SCPs have already been employed for several purposes ranging from feed (cattle, pigs, fish, poultry) to food (vitamins, emulsifying acids, aroma carriers, etc.). They are frequently utilized in the food business as replacements of meat, texture-providing ingredients, vitamin emulsifiers, carriers, and taste enhancers, and to increase the nutritional content of baked goods, ready-to-eat meals, soups, and other nutritional products (Suman et al. 2015; Hezarjaribi et al. 2016).

Production of SCP is not a new evolution. Since 2500 B.C., a variety of microorganisms have been consumed in the form of fermented products (Frey 1930). Blue-green algae (Spirulina) was consumed as a source of protein even in the sixteenth century (Clément et al. 1967). During the First World War, consumption of *Saccharomyces cerevisiae* as a dietary supplement has been increased significantly in Germany, and during the Second World War, aerobic yeasts such as *Candida utilis* was grown as food supplements and were usually added into the sausages and soups. Since then, there has been a significant increase in the production of SCPs (Najafpour 2007; Upadhyaya et al. 2016). Although SCP has been effectively marketed in Russia, England, Japan, France, and Finland since decades (Ritala et al. 2017). Several researchers are still investigating the optimum conditions for the fermentation of a wide variety of substrates with the help of microorganisms. The core concept of SCP production is the search for inexpensive and plentiful source(s) of carbon/substrate materials. Agricultural wastes; industrial wastewater; and petroleum residues including n-paraffin and fuel oil, methane, methanol, heptane, biogas, ethanol, CO_2, brewery wastes, molasses, and cellulosic biomass; and several other agricultural and industrial wastes are potential feedstocks for SCP production (Mensah and Twumasi 2017; Jones et al. 2020). Since microorganisms can use ample amounts of substrates for their growth and biomass production, conventional raw materials such as agricultural, industrial, animal, and dairy wastes can serve as economical and low-cost substrates for production of microbial biomass. The selection of substrate material would also affect the design and technique to be opted for SCP production (Patthawaro and Saejung 2019; Khumchai et al. 2022).

In this context, this review goes into great length about the difficulties and utilization of newer possible waste materials for SCP production at cheaper cost. In this report, we have critically discussed the production of high-quality SCPs from different waste materials at low cost. Furthermore, limitations and critical factors affecting the efficiency of SCP production from waste materials, downstream processing, and its techno-economic system analysis have also been discussed. Lastly, applications of SCPs have been reviewed critically to aid researchers in producing newer SCPs for a range of future applications.

Sources of SCP

Microorganisms are a major source of SCP production because they can multiply quite rapidly over a range of substrates with high nutritional content (Table 1). The source of SCP is broadly classified into four major classes, i.e., algae, bacteria, fungi, and yeast, which utilizes several different carbon sources for SCP production. In general, wastes are used as sources of both carbon and nitrogen due to their inexpensive nature and abundant availability. Microbes utilize nitrogen and carbon to synthesize proteins with high nutritional value that may be used as a food supplement in the human and animal diet. Various microorganisms which can be potential sources of SCPs are discussed below.

Algae

Algae are single-celled, photosynthetic organisms and are a very good alternative for production of SCP due to their rapid growth, easy cultivation methods, and low-cost maintenance (Putri et al. 2018). Owing to their high nutritional and protein compositions, algae have been exclusively used as dietary supplements in Central Africa and East Asia (Srividya et al. 2013). Algal species such as *Spirulina* sp., *Chlorella* sp., *Soenedesmus* sp., and *Coelastrums* sp. have been identified to be suitable for large-scale cultivation and SCP production (Nasseri et al. 2011). Several studies have been revealed that the algae could produce high-quality SCPs (Table 2).

Algae generates high-protein feed additives for humans and animals (including cattle, fish, poultry, sheep, and swine) (Becker 2004). For the synthesis of SCP from algae, several culture techniques, including open ponds, tank culture, circular ponds with mixing arms, large bags,

Table 1 A comparative analysis of different sources of SCP

Sources	Protein content	Fat	Nucleic acid	Ash	Advantages	Challenges	References
Algae	40–60%	7–20%	3–8%	8–10%	Easy cultivation	Requires disruption of cells to release nutrients	Jones et al. (2020); Nyyssölä et al. (2022)
					Maintenance costs are low as sunlight is a free source of energy	Can accumulate heavy metals	
					Good tolerance towards water impurities	Requirement of large surface area	
					Production of omega-3 fatty acids.	Risk of contamination during their cultivation in open condition	
						Economical scale-up	
Bacteria	50–80%	1–3%	8–12%	3–7%	Rapid growth	Small size	Ritala et al. (2017); Nyyssölä et al. (2022)
					High protein content	Difficulty in harvesting	
					Can utilize variety of substrates	Palatability issue	
					Versatility in Genetic Engineering	High nucleic acid	
						Acceptability	
Yeast	45–65%	2–6%	6–8%	5–10%	Fast growth	Low digestibility	Nasseri et al. (2011); Nyyssölä et al. (2022)
					Can utilize variety of substrates.	High nucleic acid	
					Can grow at low pH		
					Long history of use		
Fungi	30–50%	2–8%	4–6%	9–14%	Higher cell density	Slow growth	Nasseri et al. (2011); Nyyssölä et al. (2022)
					Simple reactor design	Possibility of containing mycotoxins	
					Long history of use	Low digestibility	

Table 2 Algae and various substrates used for SCP production

Algae	Substrate	Protein (%)	Reference
Arthrospira maxima (*Spirulina maxima*)	Light + CO_2	60–71	de Oliveira et al. (1999)
Arthospira platensis Spirulina *platensis*	Light + CO_2	46–63	Rafiqul et al. (2005)
Euglena gracilis	Light + CO_2	50–70	Rodríguez-Zavala et al. (2010)
Aphanothece microscopica	Effluent of parboiled rice	42	Zepka et al. (2010)
Chlorella vulgaris	Municipal effluent + CO_2	42–55	Li et al. (2013)
Chlorella sp.	Potato starch processing waste	62–68	Liu et al. (2014)
Scenesdesmus obliquus	Light + CO_2	33	Duong et al. (2015)
Chlorella sorokiana	Industrial process water	46–65	Safafar et al. (2016)
Chlorella pyrenoidosa	Light + CO_2	45	Waghmare et al. (2016)
Scenesdesmus obliquus	Wet market wastewater	50.72	Apandi et al. (2017)
Chlorella sorokiniana	Industrial wastewater	52.5	Rasouli et al. (2018)
Chlorella sorokiniana and *Methylococcus capsulatus*		27.6	
Chlorella sp.	Tofu waste	52.24	Putri et al. (2018)
	Tempeh waste	52%	
Chlorella vulgaris	Liquid digestate of dairy wastewater	21.8	Qin et al. (2018)
Chlorella vulgaris and *Yarrowia lipolytica*		31.1	
Haematococcus pluvialis	Synthetic brewery wastewater	64.9	Yap et al. (2022)

raceway ponds, and heterotrophic fermenter systems, have been used (Ghasemi et al. 2011). The process of SCP production by algae begins with the cultivation of microalgae in large tanks or ponds (Ramli et al. 2020). The algae are typically grown in a nutrient-rich medium under optimal growth conditions, such as temperature, light, and pH. Once the algae have sufficient population density, they are harvested using methods such as centrifugation, filtration, or flocculation (de Assis et al. 2020). There are several methods for the production of SCP utilizing algae:

i. Photoautotrophic culture: This method involves growing microalgae in large tanks or ponds under optimal light conditions. The algae do photosynthesis to convert light energy into chemical energy, which is used to produce protein. This method is considered most sustainable and efficient to produce SCP utilizing algae (Montenegro-Herrera et al. 2022).
ii. Heterotrophic culture: Microalgae are cultivated in a medium that contains organic carbon sources, such as glucose or acetate. The algae use these sources to produce protein. This method is less sustainable than the photoautotrophic culture because it requires large amounts of organic carbon (Ende and Noke 2019).
iii. Mixotrophic culture: Microalgae are grown in a medium that contains both light energy and organic carbon sources. The algae use both photosynthesis and heterotrophic metabolism to produce protein. This method is considered a compromise between the two previously discussed methods, as it is more efficient than the heterotrophic culture but less sustainable than the photoautotrophic culture (Li et al. 2022b).

Furthermore, besides SCP, ongoing research involves development of algae as a versatile source for pharmaceuticals, biofuels, food additives, cosmetics, and biofertilizers (Bhatt et al. 2022). The selection of the method will be based on factors such as the type of microalgae to be used, the availability of resources (e.g., light, organic carbon, water), and the desired end product. The SCP of algae has several advantages over traditional proteins such as soy and animal-based proteins. Algae can be grown using non-arable land and brackish water, and do not require large amounts of energy or water (del Carmen Carranza-Méndez et al. 2022). Additionally, algae do not compete with food production and have a lower environmental impact than traditional protein sources. The disadvantage of algae as SCP is that they have a cellulosic cell wall which is not digestible by humans, and sometimes they accumulate heavy metals (Nasseri et al. 2011).

Bacteria

Bacteria have faster growth and contain high amounts of protein and sulfur-containing amino acids (Rudravaram et al. 2009; Khoshnevisan et al. 2019). They can also grow on a wide variety of substrates, including carbohydrates like starch and sugars, and liquid and gaseous, hydrocarbons such as petroleum components and methane (Bamberg 2000). In bacteria-based SCP, methanotrophs, which use methane as their sole carbon and energy source, are suitable microorganisms for animal feed production. Methane-oxidizing bacteria are highly efficient and ready-to-market microorganisms for synthesizing SCP from an industrial aspect (Strong et al. 2016). Several bacteria can synthesize SCP by utilizing a variety of substrates as shown in Table 3.

The bacterial SCP synthesized using the *Lactobacillus* strain with fruit waste produced 24.67% protein (Patel et al. 2019). In another study, the *Rhodococcus opacus* strains DSM 1069 and PD630 grown in different agro-wastes (orange waste, lemon waste, and corn stover effluent) revealed 42–57% of protein (Mahan et al. 2018). Similarly, *Bacillus subtilis*, *B. cereus*, and *Escherichia coli* have been grown on ram horn hydrolysate and were determined a good amount of SCP. The amounts of SCP for *E. coli*, *B. subtilis*, and *B. cereus* were 66%, 68%, and 71%, respectively (Kurbanoğlu 2001). The *Haloarcula* sp. IRU1 is capable of degrading and utilizing petrochemical effluent as a source of carbon produced SCP at the rate of 76.4% (Taran and Asadi 2014). In 2018, Al-Hadithi et al. cultivated *Raoutella ornithinolytica* over paper, potato, and corncob starch residue, which produced 24.4% protein, 17.9% fat, 24.6% carbohydrates, 21.8% ash, 88.6% relative humidity, 1.012% RNA, and 1.235% DNA. The SCP also had very high amounts of EAAs (Al-Hadithi et al. 2018).

Photosynthetic bacteria (PSB) are also considered a good source of SCP (Patthawaro and Saejung 2019). A non-sulfur bacterium *Rhodopseudomonas gelatinosa* when cultured on agricultural waste produced around 65.0% protein and 5.1% nucleic acid (Shipman et al. 1975). In 2014, Kornochalert et al. exploited *Rhodopseudomonas palustris* for the treatment of latex rubber sheet wastewater along with fermented pineapple extract and the biomass obtained afterward had 65% protein, 8% carbohydrate, 14% ash, 3% fat, and 10% moisture content (Kornochalert et al. 2014). Similarly, *Rhodopseudomonas faecalis* and other *Rhodopseudomonas* sp. from wastewaters of Thai Sugar Company (Saejung and Salasook 2020) and municipal corporation (Saejung and Thammaratana 2016) respectively obtained significant amounts of proteins: 50–60%. Recently, coculture of heterotrophic bacteria and purple non-sulfur bacteria generated substantial levels of protein (45–71%), amino acids, and fatty acids (Alloul et al. 2021). Similarly, Zha et al. (2021) employed *Methylomonas* and *Methylophilus* sp. and reported

Table 3 Bacteria and various substrates used for SCP production

Bacteria	Substrate	Protein (%)	Reference
Rhodopseudomonas gelatinosa	Agricultural byproducts	65.0	Shipman et al. (1975)
Rhodopseudomonas sp.	Biogas plant effluent	70.0	Vrati (1984)
Cellulomonas	Hempstalk waste	12.5	Jeder et al. (1987)
Bacillus substilis	Ram horn hydrolysate	71.0	Kurbanoğlu (2001)
Bacillus cereus		68.0	
Escherichia coli		66.0	
Haloarcula sp.	Petrochemical waste	76.4	Taran and Asadi (2014)
Rhodopseudomonas palustris	Latex rubber sheet wastewater	65.0	Kornochalert et al. (2014)
Hydrogen-oxidizing bacteria	NH_4 and CO_2	71.0	Matassa et al. (2016b)
Rhodopseudomonas sp.	Municipal wastewater	60.1	Saejung and Thammaratana (2016)
Rhodococcus opacus PD630	Orange waste	56.9	Mahan et al. (2018)
	Corn stover waste	52.7	
	Lemon waste	52.1	
Rhodococcus opacus DSM 1069	Orange waste	42.2	
	Corn stover waste	47.0	
	Lemon waste	45.8	
Raoutella ornithinolytica	Waste potato, paper, and corn cob media	24.4	Al-Hadithi et al. (2018)
Photosynthetic bacteria	Manure	62.7	Patthawaro and Saejung (2019)
Lactobacillus	Fruit waste	24.67	Patel et al. (2019)
Rhodopseudomonas faecalis	Sugar industry wastewater	50-51.5	Saejung and Salasook (2020)
Methanotrophic bacteria	Biogas and pasteurized supernatant of sewage sludge-based anaerobic digestion	41.0	Zha et al. (2021)
Streptomyces tuirus	Paper and Pulp Industry effluents	78.79	Khumchai et al. (2022)

that the protein content was more than 41% of the dry biomass, in addition to EAAs such as histidine, valine, leucine, isoleucine, phenylalanine, threonine, and lysine (Zha et al. 2021).

In addition to SCP production, bacterial biomass finds application in enzyme production, pharmaceuticals, biofertilizers, manufacturing of bioplastic, and food additives (Murali Sankar et al. 2023). The bacterial SCP has a more significant advantage over others due to its easy cultivation and lovingness towards a broad spectrum of substrate. However, the smaller size of bacterial cells and relatively lower density make their harvesting laborious and expensive. In addition, compared to yeast and fungus, bacterial cells have a relatively higher nucleic acid concentration (Najafpour 2007; Ritala et al. 2017); therefore, an extra step of processing is required to reduce the content of nucleic acids, which raises the production cost. Normally, people think that the bacteria lead to several diseases, hence are needed to be educated to disprove this myth and increase the acceptability of bacterial SCP (Nasseri et al. 2011).

Yeast

Yeasts are the most extensively acknowledged and used organisms for SCP production. Among the microorganisms, yeasts, particularly *Saccharomyces* sp. and *Candida* sp., were extensively used to produce SCP utilizing a range of waste materials (Mondal et al. 2012). Furthermore, yeasts are easier to use with inexpensive raw materials, more straightforward to harvest than any other microbes, and contain less nucleic acid than the bacteria (Bekatorou et al. 2006). Yeast cell proteins play an essential role in the probiotic composition due to their improved immunomodulating effects that improve animal health when used in feed (Sauerwein et al. 2007). Yeast can grow at lower pH and have high amounts of malic acid and lysine contents. However, yeast has drawbacks of a slow growth rate, less protein (45–65%), and methionine contents compared to bacteria (Nasseri et al. 2011). The yeasts employed for the production of SCPs are illustrated in Table 4.

The *S. cerevisiae* can be used as SCP by utilizing a variety of substrates. When grown on wastes of sweet orange, biomass containing 57% protein was produced (Nwabueze and Oguntimein 1987). In another study, 39% protein was produced when molasses and food waste were employed as substrate materials (Gervasi et al. 2018). Similarly, mango waste (Marius et al. 2017) and pineapple waste (Dunuweera et al. 2021) were used as substrates, and a significant amount of protein, i.e., 79.14% and 48.32%, respectively, were obtained. In another study, Tropea et al. (2022) determined

Table 4 Yeast and various substrates used for SCP production

Yeast	Substrate	Protein (%)	Reference
Candida utilis	Alfalfa process waste	32.6	Mudgett et al. (1980)
Saccharomyces cerevisiae	Sweet orange residue	57	Nwabueze and Oguntimein (1987)
Pichia pinus	Mango peel extract	62.2	Rashad et al. (1990)
	Methanol	52.2	
Candida lipolytica	Deproteinized leaf juice	50.5	Chanda and Chakrabarti (1996)
Torula utilis		54.3	
Saccharomyces cerevisiae	Deproteinized leaf juice	45.6	Chanda and Chakrabarti (1996)
Marine yeast	Prawn shell waste	70.4	Rhishipal and Philip (1998)
Candida tropicalis	Plastic waste	46.7	Karthigesan and Brown (2007)
Saccharomyces cerevisiae	Banana skin	58.62	Khan et al. (2010)
	Mango waste	54.28	
	Sweet orange peel	50.86	
	Rind of pomegranate	39.98	
	Apple waste	26.26	
Candida utilis 1769	waste capsicum powder	48.2	Zhao et al. (2010)
Candida tropicalis 1253	Waste capsicum powder	46.5	Zhao et al. (2010)
Saccharomyces cerevisiae 1335	Waste capsicum powder	40.5	Zhao et al. (2010)
Saccharomyces cerevisiae 1027		25.4	
Candida tropicalis	Millet bran	9.19	Abalaka and Daniyan (2010)
	Maize bran	8.94	
	Rice husk	8.69	
Saccharomyces cerevisiae	Agro-industry waste	49.29	Bacha et al. (2011)
Saccharomyces cerevisiae	Papaya extract	34.0	Maragatham and Panneerselvam (2011)
Candida utilis CGMCC 2.1180	Soy molasses	60.99	Gao et al. (2012)
Candida utilis CGMCC 2.120		57.32	
Candida tropicalis CGMCC 2.587	Soy molasses	56.42	Gao et al. (2012)
Saccharomyces cerevisiae	Cucumber peel	53.4	Mondal et al. (2012)
	Orange peel	30.5	
Geotrichum candidum CGMCC 2.498	Soy molasses	52.06	Gao et al. (2012)
Geotrichum candidum CGMCC 2.1035		51.96	
Pichia kudriavzevii	Cassava processing waste	66.8	Rachamontree et al. (2015)
Candida utilis and *Rhizopus oligosporus*	Wheat bran	41.02	Yunus et al. (2015)
Saccharomyces cerevisiae	Mango waste	79.14	Marius et al. (2017)
Candida tropicalis	Sugarcane bagasse	60.05	Magalhães et al. (2018)
Candida utilis	Mango waste	56.40	Marius et al. (2018)
Saccharomyces cerevisiae	Food waste	39	Gervasi et al. (2018)
Saccharomyces cerevisiae	Pineapple waste	48.32	Dunuweera et al. (2021)
	Papaya waste	42.14	
	Cashew apple	37.28	
	Mango waste	33.98	
	Jackfruit	28.68	
	Cacao	24.31	
	Prickly custard apple	19.58	
	Banana waste	15.32	
	Mangosteen	11.57	
	Pomegranate	9.64	

Table 4 (continued)

Yeast	Substrate	Protein (%)	Reference
Palmyrah Toddy Yeast	Papaya peel	52.4	Thiviya et al. (2021); Thiviya et al. (2022a)
	Pineapple peel	49.7	
	Watermelon peel	45.2	
	Banana peel	30.4	
	Sour orange peel	29.5	
	Mango peel	24.6	
Saccharomyces cerevisiae	Carbon and nitrogen recovered from waste streams	47.0	Zeng et al. (2022)
Saccharomyces cerevisiae	Multifood waste	40.19	Tropea et al. (2022)
Pichia pastoris	Methanol	67.21	Gao et al. (2023a)

40.19% protein by utilizing fruit waste as substrate (Tropea et al. 2022). Chanda and Chakrabarti (1996) utilized deproteinized juice of leaves along with *S. cerevisiae*, *Torula utilis*, and *C. lipolytica*, and obtained 45.6%, 54.3%, and 50.5%, respectively, of SCP. Likewise, when *C. utilis* was grown on wastes of mango (Marius et al. 2018) and alfalfa (Mudgett et al. 1980), about 56.40% and 39% respectively of crude protein was produced. In another study, rice, maize, and millet were fermented with *C. tropicalis*, which produced 8.69%, 8.94%, and 9.19% protein, respectively (Abalaka and Daniyan 2010). Gao et al. (2012) utilized soy molasses with *C. tropicalis* which gave 56.42% crude protein along with numerous EAAs (Gao et al. 2012). Similarly, when wheat bran was fermented with *C. utilis* and *Rhizopus oligosporus*, 41.02% of SCP was obtained (Yunus et al. 2015). Recently, papaya, pineapple, watermelon, banana, sour orange, and mango fruit peel have been used along with natural palmyrah toddy yeast, which provided 52.4%, 49%, 45.2%, 30.4%, 29.5%, and 24.6% protein, respectively (Thiviya et al. 2021; Thiviya et al. 2022a).

Fungi

Several fungal species have also been used for the production of SCPs (Table 5). Fungi possess 30 to 50% protein when grown, particularly for SCP production. They also contain lipids and fiber (e.g., cell wall chitin and glucan). Despite its low methionine concentration, the amino acid content of fungal SCP with high lysine and threonine content satisfies the guidelines of FAO (Nyyssölä et al. 2022). Along with being a valuable source of protein and high nutritional value, functional features such as texture, foaming, and emulsifying ability of food items can be enhanced by using fungal SCPs (Nyyssölä et al. 2022). Filamentous fungi have an advantage in terms of harvesting because of their larger size.

Aspergillus niger and *A. terreus* have been grown on wheat bran which had around 40% protein along with all EAAs except methionine (Gabriel et al. 1981). Pogaku et al. (2009) utilized three different fungi, i.e., *Trichoderma viride*, *A. oryzae*, and *A. niger*, with de-oiled rice bran, which resulted in 44%, 43%, and 39.2% protein, respectively. Similarly, *A. niger* when cultivated over wastes of sweet orange and orange pulp revealed 52.48% and 46.50% protein, respectively (Alemu 2014). In another study, *Kluyveromyces frajilice* and *F. oxysporum* were used with Kilka stickwater, which produced 57.47% and 54.39% protein, respectively (Babazadeh et al. 2021). Likewise, when *Arachniouts* sp. was added with corn cob, about 18.87% SCP production was achieved (Asad et al. 2000).

Fungal SCPs not only serves as a remarkable protein-rich nutritional source but also enhances the nutritional quality and functional attributes of the food products, including texture, emulsification, and foaming capacity (Nyyssölä et al. 2022). Notably, fungi have shown promising applications as substitutes of meat (Hüttner et al. 2020). However, fungi as SCP have drawbacks such as slow growth rate, low protein content, and less acceptance (Nasseri et al. 2011). It is crucial to consider the risk of mycotoxin formation with some species, such as *Aspergillus* and *Fusarium*, during their cultivation (Anupama and Ravindra 2000).

Mixed culture

Co-cultures are becoming increasingly popular as a method for producing SCPs (Jia et al. 2019). An advantageous aspect of this approach is its capacity to expand and enhance the range of hydrolytic activities that are essential for the utilization of substrates. For example, the efficient processing of lignocellulosic raw materials often requires a combination of enzymes that work synergistically. Furthermore, metabolic residues generated by one species may function as substrates for another, or the metabolic processes of these species may complement one another by utilizing distinct substrates (Areniello et al. 2023). To reduce the risk of contamination,

Table 5 Fungi and various substrates used for SCP production

Fungi	Substrate	Protein (%)	Reference
Chaetomium cellulolytica	Pulp and paper mill solid wastes	28	Pamment et al. (1979)
Aspergillus niger	Wheat bran	36.84	Gabriel et al. (1981)
	Corn cob	22.41	
	Rice husk	14.52	
Aspergillus terreus	Wheat bran	26.18	
	Corn cob	24.43	
	Rice husk	15,61	
Scytalidium acidophilum	Paper waste hydrolysate	44-47	Ivarson and Morita (1982)
Chaetomium globosum	Kinnow-mandarin waste	28	Kalra et al. (1989)
Sporotrichum pulverulentum		26	
Myrothecium verrucaria	Paper mill waste	23.0	Swaminathan et al. (1989)
Arachniouts sp.	Corn cob	18.87	Asad et al. (2000)
Aspergillus oryzae	Starch-processing wastewater	45.7	Jin et al. (2002)
Rhizopus oligosporus		49.7	
Trichoderma viride	De-oiled rice bran	44	Pogaku et al. (2009)
Aspergillus oryzae		43	
Aspergillus niger		39.2	
Aspergillus niger	Sweet orange wastes	52.48	Alemu (2014)
	Orange pulp wastes	46.50	
Aspergillus niger	Potato starch processing waste	24.86	Liu et al. (2014)
Rhizopus oryzae	Pea-processing byproduct	50.03	Souza Filho et al. (2018)
Neurospora intermedia		54.53	
Monascus purpureus		53.61	
Aspergillus oryzae		43.13	
Trichoderma viride	Pineapple waste	18.35	Anichebe et al. (2019)
	Banana peel extract	29.76	
Kluyveromyces frajilice	Stick water	57.47	Babazadeh et al. (2021)
Fusarium oxysporum		54.39	
Mucor indicus	Apple pomace	29.0	Borujeni et al. (2022)
Pleurotus ostreatus LGAM 1123	Agro-industrial hydrolysate	54.5	Bakratsas et al. (2023a)
Pleurotus ostreatus LGAM 1123	Fiber sludge	44.8	Bakratsas et al. (2023b)

pure cultures incur additional costs, whereas mixed cultures show more resistance to environmental factors (Li et al. 2023). It is difficult to accomplish a complete transformation of complex elements of the culture medium using a single strain (Li et al. 2022b). The utilization of co-cultures has been suggested as a means to improve nutritional value by ensuring a balanced distribution of EAAs or by adding vitamins or lipids to the product. However, there are certain challenges including the possibility of negative interactions resulting in the formation of antagonistic environments or the production of inhibitors, and complex process control (Nyyssölä et al. 2022).

In view to produce SCP, a study involved five yeast strains during fermentation of wastewater of potato starch processing industry. When *Geotrichum candidum*, *C. utilis*, and *C. tropicalis* were mixed in the ratio of 9:5:1, 3.06 g/L of SCP has been obtained (Tian et al. 2023). Similarly, a consortium consisting *Kluyveromyces lactis* and *Rhodotorula graminis* was employed to produce SCP from waste milk. Under optimized conditions, the co-culture yielded 43.8 g/L of SCP (Myint et al. 2020).

Substrate

Substrate and its composition play a key role in SCP production. It requires an appropriate make-up of carbon, nitrogen, and phosphorus supplements for optimum production of biomass in a short duration. Since the beginning of the biotechnological era, there has been a significant focus on developing microbial-based strategies for addressing global concerns such as food scarcity and management of hazardous wastes. This trend has given a momentum to microbial food supplements, such as SCP. Most of the developing

nations are nowadays targeting production of SCP along with managing wastes as a sustainable solution to the dual issues of food shortage and waste disposal. The possibility of creating biological products with a significant economic value from inexpensive waste materials promotes the idea of "wealth from waste" (Umesh et al. 2017). Additionally, the total economics of manufacturing and distribution is significantly reduced by the resilience of the production process and the use of wastes or inexpensive raw materials for SCP synthesis.

However, waste materials must meet the specific requirements to be suitable substrates for manufacturing microbial protein. They must be abundant, regenerative, non-toxic, non-exotic, and affordable, and they must facilitate the faster growth and multiplication of microbes with the generation of increased biomass (Reihani and Khosravi-Darani 2019). Incorporation of pre-treatment procedures at the time of production of SCP from agricultural and food wastes raises the operational cost, for example, filtering and shredding to eliminate solids, accompanied by a process that transforms the pulp into reducing sugars, such as heat treatment, enzyme hydrolysis, or acid hydrolysis. Utilization of carbon and nitrogen retrieved from wastes, rather than the sugar-rich substrate, for yeast-driven SCP manufacturing might increase the environmental and economic sustainability of the process (Zeng et al. 2022). Perceptions of the "waste-to-protein" concept significantly vary across countries and demands careful consideration. Microbial fermentation has already gained substantial acceptance in the Europe and North America, where products like Quorn™ have become popular mainstream food items. Expanding microbial protein technologies in these regions presents a promising opportunity. The public acceptability of waste-to-protein manufacturing processes is influenced by various factors, including sustainability, safety, flavor, education, cultural considerations, regulatory approval, cost, accessibility, environmental awareness, and support from major consumers (Stringer and Hall 2007; Meyer et al. 2017; Piercy et al. 2023). Ensuring global user acceptance requires that the upgraded "waste-to-protein" products should be universally recognized as high-quality and safer alternatives. Therefore, the conversion and upgrading processes must align with the conditions set by the feed/food chain alliance and adhere to hygiene, quality, and safety standards mandated by the regulatory authorities.

Wastes from several sectors, including agricultural, culinary, dairy, animal, and industrial, have been extensively used for the SCP synthesis in recent decades (follow Tables 2, 3, 4, 5 and Figure 1). The SCPs are also produced using wastewater as substrate.

Agricultural waste

The increased local production of agricultural residues such as vegetable and fruit peels, molasses, and bagasse significantly raises the pollution load. Therefore, this waste could be used to produce SCP, resulting in a decrease in pollution along with producing SCP biomass. Various substrates can make SCP, which is usually done to lower the effluent discharge and biological oxygen demand in agro-processing plants. The choice of suitable agricultural waste for SCP production is based on two primary approaches: low-class waste materials and relatively high-quality protein content

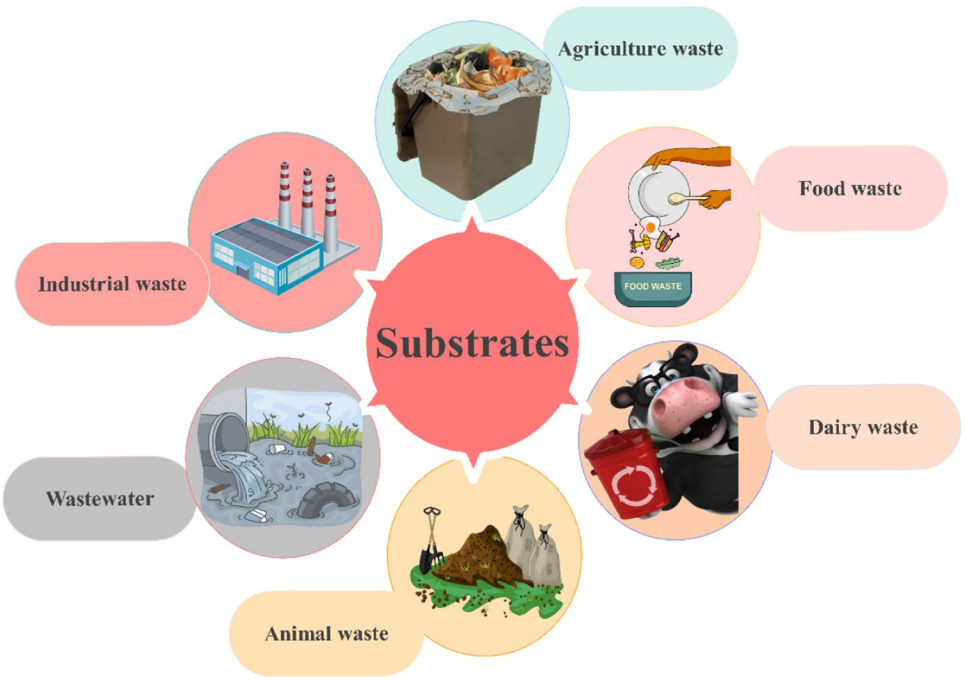

Fig. 1 Commonly used substrates for the production of single-cell protein.

(Reed and Nagodawithana 1995). The most commonly utilized substrate for SCP production is carbohydrate of sugarcane bagasse (Ugalde and Castrillo 2002). Other agricultural substrates frequently used for the SCP production are rice husk, paper industry waste, wheat straw, sugar beet waste, cassava waste, coconut waste, sweet orange residue, orange waste, grape waste, mango waste, etc. (Mensah and Twumasi 2017; Spalvins et al. 2018a). The agricultural wastes popularly used for SCP production are discussed in the subsequent sections.

Fruit waste

Fruit wastes are abundant in fermentable sugars as well as other essential elements that promote microbial growth and are appropriate substrates for the SCP production (Nasseri et al. 2011). Peels are the predominant by-products of certain fruits, accounting for about 30% of the overall weight (Romelle et al. 2016). Patel et al. (2019) cultivated *Lactobacillus* over fruit waste and reported 24.67% protein production. Similarly, when natural palmyrah toddy yeast was added with watermelon, papaya, and banana fruit peels, it revealed 45.2%, 52.4%, and 30.4% protein, respectively (Thiviya et al. 2021). Likewise, mango, prickly custard apple, pineapple, papaya, banana, mangosteen, cashew apple, jackfruit, and pomegranate wastes were employed as substrates for cultivation of *S. cerevisiae* (Dunuweera et al. 2021). Mondal et al. (2012) cultured *S. cerevisiae* on cucumber and orange peel and reported 53.4% and 30.5% protein production, respectively. Recently, citrus peels, banana, pineapple, apple, and fish have been simultaneously employed as a substrate during fermentation with *S. cerevisiae* (Tropea et al. 2022). Around 58.62%, 54.28%, 50.86%, 39.98, and 26.26% of crude protein were produced when *S. cerevisiae* was cultured with the wastes of banana, pomegranate, apple, mango, and sweet orange respectively (Khan et al. 2010). The co-fermentation of citrus pomace (CP) was investigated using indigenous probiotic bacteria, *Bacillus amyloliquefaciens* BF2 and *C. utilis* GIM 2.9. After fermentation, CP exhibited significant improvements in crude protein, soluble protein, and small peptide content, with increases of 40.01%, 923.53%, and 626.67%, respectively. Essential amino acids accounted for 47.04% of total free amino acids, with leucine levels reaching 4.04 mg/g dry weight, a remarkable increase of 1293.10%. Additionally, the fermentation process resulted in the production of histidine, valine, cysteine, and tyrosine (Sheng et al. 2023).

Lignocellulosic waste

The proportion of hemicellulose, cellulose, and lignin in the lignocellulosic wastes varies depending on their type and generation source. Wood, leaves, grasses, rice bran, wheat straw, wheat bran, sugarcane bagasse, groundnut shell, paper waste, and other agricultural wastes are examples of lignocellulosic materials (Nascimento et al. 2022). The availability and affordability of molasses, its composition and fermentation inhibitors, and the absence of harmful substances decide the quality of SCP (Bekatorou et al. 2006; Patel et al. 2019).

Pre-treatment of lignocellulosic waste is a crucial step in the production of SCP from lignocellulosic biomass. The purpose of pre-treatment is to break down the complex structure of lignocellulosic materials, such as straw, woody biomass, and agricultural wastes, into simpler sugars that can be utilized by microorganisms for SCP production (Mahmood et al. 2019). There are several methods of pre-treatment including mechanical, chemical, and biological (Bajpai 2017). Mechanical methods involve the physical breakdown of materials, such as grinding or hammer milling (Cai et al. 2021). Chemical methods involve the use of dilute acid or alkali (Asad et al. 2000), while biological methods involve use of enzymes, fungi, bacteria, etc. (Sharma et al. 2019; Su et al. 2020). For example, corn cob has been delignified by NaOH before its use for SCP production (Asad et al. 2000). Similarly, microwave-assisted pre-treatment is also an effective method for lignocellulosic wastes (Intanakul et al. 2003; Pellera and Gidarakos 2017). In addition, endoglucanase, xylanase, and pectinase have also been used for the pre-treatment of lignocellulosic materials (Ziemiński et al. 2012). Identification of optimal enzyme-cocktail-substrate combination allows efficient conversion of biomass into the fermentable sugars. The user-friendly and cost-effective attributes of this approach ensures accessibility for a diverse range of industrial engineers and researchers, promoting innovation and advancement in related fields (Gao et al. 2023b). Selection of the pre-treatment method is based on the type of lignocellulosic waste, the microorganism to be used, and the desired end product. For example, alkaline pre-treatment is often used for the SCP production with yeasts, while fungal pre-treatment is popularly used for SCP production with fungi (Mahmood et al. 2019). Process of pre-treatment needs to be optimized for specific feedstock and also to maximize sugar recovery and minimize generation of inhibitors (Sharma et al. 2019).

Rhodopseudomonas gelatinosa when cultured with agricultural waste produced 65% protein (Shipman et al. 1975). Abalaka and Daniyan (2010) employed rice, maize, and millet for fermentation with *C. tropicalis*. Carbohydrate-rich wheat bran was used to generate microbial biomass with *C. utilis* and *R. oligosporus* (Yunus et al. 2015). *Pichia kudriavzevii* was cultivated on cassava waste (Rachamontree et al. 2015). Three different fungi namely *A. oryzae*, *Trichoderma viride*, and *A. niger* have been used with de-oiled rice bran which revealed protein content of 43%, 44%, and 39.2%, respectively (Pogaku et al. 2009).

Food waste

Food waste is one among the most significant issues in developed and developing nations (Huang et al. 2015). Studies on effective and novel recycling techniques for converting food waste into valuable substances are becoming more attractive and economically feasible, which is also essential to maintain and protect public health. Utilization of food waste may help us minimize environmental pollution and generate products with added advantages, such as supply of protein for livestock feed (Gervasi et al. 2018). Wastes, particularly derived from the food sector, contain a considerable amount of sugar that can be either fermentable or non-fermentable and might be used to make SCP for animal feed using microorganisms (Puniya et al. 1995; Nasseri et al. 2011). In a study, when *S. cerevisiae* was grown over multi-food waste (Tropea et al. 2022), and candy industry waste (Bertasini et al. 2022), it produced 40.19% and 28% protein respectively. In another study, *Kluyveromyces marxianus* was when cultivated with food industry waste, it revealed 33.7% protein production (Aggelopoulos et al. 2014).

Dairy waste

Dairy waste can also be used as a suitable substrate for the production of SCPs. It contains significant amounts of lactose, minerals, nitrogenous compounds, and vitamins (Moeini et al. 2004). Dairy is among the most prominent industries around the world. Due to the elevated production of milk and demand for dairy products, this sector has been growing rapidly. Whey, buttermilk, and their derivatives are the typical by-products of milk. Milk industries discharges milk spills/effluents of drips, cleaning cans, tankers, utensils, equipment, bottles, and floors (Suman et al. 2015).

Whey is widely used for producing protein supplements due to its high-quality protein (Chourasia et al. 2022). However, because of its high nutritional value, it may produce a variety of products with added value, including bioactive peptides, bacteriocins, enzymes, and prebiotics (Gutiérrez-Cortés et al. 2018; Bustamante et al. 2021; Fischer and Kleinschmidt 2021; Shi et al. 2022). Lactic acid bacteria are abundant in milk products owing to their capacity for lactose fermentation and their auxotrophy for specific amino acids that are abundantly present in the milk proteins (Chourasia et al. 2021). Due to the availability of lactose as a carbon source and concomitant elimination of whey, effluent of milk processing serves as a typical substrate for SCP production (Yadav et al. 2014). Yeast is most often used for the bioconversion of whey into the SCP (Kaur et al. 2020). Food-grade yeasts, such as *Candida*, *Kluveromyces*, and *Saccharomyces*, have been used with whey to synthesize SCP (Ritala et al. 2017).

Animal waste

Production of SCP out of animal wastes helps to reduce the cost of protein feedstock up to an extent (Patthawaro and Saejung 2019). A major part of the animal waste comprised of excreta and urine of cattle, swine, and poultry. Excreta of animals can pollute ground and surface water, which could result in a severe disposal issue (Lin et al. 2017; Borowski et al. 2017). Due to its low cost, bioconversion of waste into SCP has become popular alternative. Animal manure has been recognized as a source of recyclable nitrogen, phosphorus, potassium, organic matter, and micronutrients (Gaind 2014). However, only a few studies have been reported and published on SCP generated from animal manure (Vrati 1984; García et al. 2019). Cattle, swine, and poultry-derived manure were used with PSB, and among these poultry manure has been found as the best substrate comprising 62.7% protein, including EAAs such as lysine, threonine, methionine, leucine, isoleucine, phenylalanine, histidine, and valine, and 4.52% nucleic acid (Patthawaro and Saejung 2019). Cow dung digestate has been used to culture PSB (Vrati 1984). However, the mixed methanotrophic culture had also been employed for the anaerobic digestion of animal waste, thereby biogas and biomass production (Zha et al. 2021).

Wastewater

Numerous wastewaters can be employed as a substrate for the production of protein utilizing microorganisms. Wastewaters of food industries are more interesting than the others because of their potential for microbial protein synthesis owing to the lower content of pathogens, harmful toxins, and heavy metals. For higher yield of microbial protein, a carbon-to-nitrogen ratio of 10:20 has been recommended (Vethathirri et al. 2021). Untreated wastewater has been utilized to produce SCP by converting nutrients into activated sludge, which is made up of flocs comprising both autotrophic and heterotrophic microbial populations developed mainly in an aerobic environment (Vriens et al. 1989). In addition, industrial effluents can be a significant substrate for the production of SCP because they contain relatively lesser amounts of nutrients, carbon, and water. During the production of SCP, wastewater may also be processed to meet environmental standards. The operational and capital expenses of the wastewater treatment unit can be balanced with the income from the SCP produced.

Vethathirri et al. (2021) determined 50% of SCP production out of wastewater from the soybean processing unit. *Rhodopseudomonas faecalis* has been grown in a photobioreactor filled with wastewater of a Thai Sugar Company (Saejung and Salasook 2020). Using Kilka stick water,

Fusarium oxysporum and *Claveromyces frajilice* were cultured (Babazadeh et al. 2021).

Industrial waste

After production of goods, industrial waste is left over which may include materials like sludge, product residues, kiln dust, slag, and burns. This garbage can come from various operations like cotton and wood processing, fuel and paper manufacturing, latex, and other industrial processes. This also contains large amounts of cellulose, hemicellulose, latex, lignin, and other polymers (Klemm et al. 2005). Polymers, particularly lignocellulosic wastes, polysaccharides, and some other complex molecules, require mechanical, enzymatic, or chemical pre-treatments (or a combination of these) before fermentation by SCP-producing microbes. Consequently, use of polysaccharides raises the cost of SCP production (Spalvins et al. 2018a). Other industrial wastes like methane, methanol, formic acid, and acetic acid are primarily produced by disintegration and biochemical processing of organic compounds. Thus, effective use or proper treatment and management of these compounds are essential (Spalvins et al. 2018b).

Methods of production of SCP

Production of SCP involves growth of microbial cells in a fermenter, and then fermented biomass is harvested and purified following downstream processing. Downstream processing consists of series of steps like washing to remove the unused medium, cell disruption to release the required product, protein extraction, purification, pre-concentration, and then drying and packaging of the product (Bekatorou et al. 2006; Thiviya et al. 2022b). For human consumption, the product should be rich in nutrients, highly soluble, light in color, and devoid of viable cells (Labuza et al. 1970). The three most common techniques for cultivation of microorganisms for SCP production are solid, semi-solid, and submerged fermentation (Bajpai 2017). In the following sections, these methods have been dealt briefly with their advantages and applications in the SCP production.

Solid-state fermentation

Growth of microorganisms on solid materials in the absence or near-absence of free water is known as solid-state (substrate) fermentation (SSF), although the substrate must be wet enough to enable growth and metabolism of microorganisms. Several microbial groups such as *Kluyveromyces marxianus*, *A. niger*, and *A. oryzae* have been reported to efficiently grow over solid surfaces (Pandey 1992; Pandey 2003; Pogaku et al. 2009). Several articles have been published outlining different types of bioreactors, microbes, and process parameters for the manufacturing of several value-added products like ethanol, SCP, enzymes, vitamin B complex, organic acids, flavors, and pigments, following the SSF (Ukaegbu-Obi 2016).

This technique has the potential to bring down cultured microbes near the substrate and achieve highest substrate concentration for fermentation. It probably provides natural environment to microorganisms to flourish and produce desired value-added products. The benefits of this method include simple technique, minimum energy and water demand, and requires less downstream processing. The protein-rich by-products are employed as dried supplements in the process; hence, drying after processing consumes less energy (Muniz et al. 2020). The SSF requires precise selection of microbes and substrates, optimum process conditions, and end-product purification, which is also a challenge for this technique. Further, requirement of moisture is determined by the microorganisms used as well as the type of substrate. Following the concept of water activity, yeast and fungi were identified as suitable organisms for SSF. Fungi requires less moisture, 40–60%; however, selection of substrate depends on various factors, including availability and cost, and may require screening of various agro-industrial residues (Singhania et al. 2009). Pogaku et al. (2009) cultivated *A. oryzae*, *T. viride*, and *A. niger* following SSF. Under SSF, *A. niger* has been grown on sweet orange and orange pulp wastes (Alemu 2014).

In SSF, the entanglement of microbial biomass with the substrate poses a considerable challenge in achieving complete separation and accurate estimation of the microbial biomass. Extraction of product from the solid fermented matter in SSF is challenging and typically involves solvents (aqueous or mixtures of other solvents). Following the fermentation process, the fermented matrix undergoes an extraction phase where selection of an appropriate solvent is crucial for efficiently extracting the product from the fermented broth (Kumar et al. 2021; Chilakamarry et al. 2022).

Semi-solid fermentation

Semi-solid fermentation often uses a solid substrate, such as cassava waste, green coconut husk, sugarcane bagasse, and cashew apple bagasse (Adedayo et al. 2011; Oliveira et al. 2018). Pre-treatment plays a pivotal role in enhancing the efficiency of the semi-solid fermentation process by breaking down complex structures into simpler ones within the substrate to alleviate barriers to microbial access, ensuring a more thorough and effective degradation of the raw material (Mohammadi et al. 2016). The operation of semi-solid fermentation involves mixing and stirring of multiphase system, supply of oxygen through gas bubbles to microbes in the liquid phase, and heat is exchanged with the

surroundings by the liquid phase. The U-loop fermenter is a unique bioreactor developed to detect energy and mass transfer phenomena (Prado-Rubio et al. 2010). The basic steps to be followed during the production of SCP are (i) selection of a fermenter, (ii) formulation of a suitable medium with an appropriate carbon source, (iii) selection of appropriate microorganism having desirable properties, (iv) keeping a check on the contamination, and (v) separation and processing of the synthesized biomass (Soland 2005). Carbon sources include n-alkenes, methanol, ethanol, gaseous hydrocarbons, and renewable sources such as CO_2, polysaccharides, molasses, brewery effluents, and other solid substances (Ukaegbu-Obi 2016). In this fermentation process, the recovery of SCP involves different methods based on the microorganism used. The SCP from bacteria and yeast is typically recovered through centrifugation, whereas SCP produced from fungi is usually recovered through filtration (Adoki and Adoki 1993; Bertolin et al. 1996; Oliveira et al. 2018). In addition to that, extraction buffers are also used to extract the product (Mohammadi et al. 2016). Following semi-solid fermentation, *Candida* sp. was grown on agricultural waste for SCP production (Adoki and Adoki 1993). In another study, rice husk was used for the growth of *Trichoderma* sp., *A. niger*, and *Phanaerochaeta chrysoporium* (Bertolin et al. 1996).

Submerged fermentation

The substrate used in submerged fermentation is always in a liquid state (Varavinit et al. 1996). This technique requires a higher initial cost and operating expenses. The substrate-containing fermenter is continuously operated, and the resultant biomass is continuously harvested using various techniques. The substance is then dried after being filtered or centrifuged. A high oxygen transfer rate encourages an elevated respiratory rate, and increased metabolic heat generation during cultivation (Srividya et al. 2013). Therefore, a cooling device is used to eliminate excess heat. Various approaches can be used to harvest microbial biomass (Kargi et al. 1980). The recovery of filamentous fungus is accomplished by filtration, whereas the recovery of single-celled organisms like bacteria and yeast is accomplished through centrifugation. It is necessary to retrieve water as much as possible before final drying, which must be done under clean and sterile conditions.

At an industrial scale, liquid-state fermentation takes place in tanks ranging in size from 1001 to 2500 m^2 (10,770 to 26,910 ft^2). Growing unicellular organisms such as yeasts or bacteria in liquid culture is more suitable. The bacterium must be continually supplied with oxygen to maintain liquid aerobic fermentation, often accomplished by stirring the fermentation media. Precise control over the production of desired metabolites involves regulation of soluble oxygen, temperature, ionic strength, nutrition, and pH (Fontana Capalbo et al. 2001). Recently, natural palmyrah toddy yeast was grown with peel wastes of pineapple, papaya, watermelon, sour orange, banana, and mango in a liquid-state fermentation (Thiviya et al. 2021; Thiviya et al. 2022a).

Evaluation of nutritional contents of SCP

Composition of SCP defines its nutritional value and possible utility. It constitutes proteins, carbohydrates, lipids, ash components, water, and other elements like phosphate and potassium. Before the product is utilized for feed or as food supplement, its minerals, nitrogen, vitamins, carbohydrates, lipids, cell wall components, amino acids, protein concentration, and nucleic acids should be determined (Anupama and Ravindra 2000). Additionally, issues like palatability, allergies, and digestibility should also be considered. The microbes that produce SCP are the primary factor determining its nutritional content. Besides this, the harvesting, drying, and processing methods also influences nutritional content of final product. Biological value, digestibility, net protein utilization, and protein efficiency ratio are nutritional criteria that determines the quality of SCP (Bajpai 2017).

The SCPs derived from different organisms vary in their nutritional composition as shown in Table 6. In the context of algae, it must be emphasized that, for technological and economic reasons, the purpose is not to isolate and use only the protein but to grow the entire algal biomass. Therefore, the word SCP is inaccurate, as micro-algal material is much more than just protein. It contains, in addition to protein, a wide variety of other nutritious substances, including peptides, lipids, carbohydrates, vitamins, minerals, pigments, and other vital trace elements (Becker 2007). On the other hand, SCP derived from yeast and fungus has 50–55% protein and a high protein-to-carbohydrate ratio (Kurbanoğlu 2001; Mchoi and Park 2003). It has more lysine while less cysteine and methionine (Suman et al. 2015). Fungus is rich in the B-complex group of vitamins. Mycoprotein produced by *F. venenatum* by fermentation is relatively low in fat, sugar, free of cholesterol, and rich in dietary fiber and EAAs (Whittaker et al. 2020). However, bacterial SCP has high protein and EAAs. The crude protein content is approximately 80% of the total dry weight. The nucleic acid concentration, particularly RNA, is relatively high, 15–16%. In addition, bacterial SCP contains 2.2–3.0% methionine, which is greater than that of the fungus (2.5–1.8%) and algae (1.4-2.6%) (Anupama and Ravindra 2000; Attia et al. 2003).

Factors affecting production of SCP

Production of SCP involves contribution of many factors, which also determines its merit of use as a dietary supplement. For instance, the microorganisms employed in the

Table 6 Comparative study of the nutritional composition of various conventional protein sources with different SCPs

Components	Milk	Meat	Fish	Soyabean	*Chlorella* sp. (microalgae)	*Schizochytrium* sp. (microalgae)	*Haematococcus pluvialis* (microalgae)	*Saccharomyces cerevisiae* (yeast)	*Candida utilis* (yeast)	*Rhodopseudomonas Faecalis* (bacteria)	*Escherichia coli* (bacteria)	*Pleurotus florida* (fungi)	*Agaricus bisporus* (fungi)
Protein	3.28	21.2	17.8	43.50	54.5	11.9	–	47.78	32.75	62	–	62.8	47.1
Nucleic acid	–	–		–	–	–	–	–	0.27 (RNA)	4.52	–	–	–
Carbohydrate	4.67	4.67	0	–	25.8	–	–	38.56	10.50 (Cellulose)	–	–	–	–
Fat	3.2	3.68	5.6	14.13–22.44	9.4	54.1	–	2.34	–	–	–	–	5.8
Ash	–	1.02	1.46	5.80	5.3	8.7	–	7.9	12.95	–	–	–	7.9
Fiber	0	0	0	6.60	–	2.4	–	3.42	11.50	–	–	–	–
	Essential amino acids												
Histidine	0.06	1.70	2.00	0.98	1.0	0.3	0.75	0.79	0.19	1.15	2.1	1.98	3.81
Isoleucine	0.12	2.41	2.71	1.61	1.5	0.4	2.58	2.12	0.81	1.00	5.1	7.32	2.48
Leucine	0.23	4.06	4.35	3.53	4.2	0.7	10.87	4.35	1.44	2.05	7.2	6.82	0.84
Lysine	0.13	4.45	5.16	2.79	4.6	0.5	11.05	3.14	1.24	3.36	4.9	9.55	3.62
Methionine	0.04	1.35	1.62		1.0	1.2	0.54		0.44	0.25	2.7	2.11	1.76
Phenylalanine	0.13	2.20	2.22	1.83	2.3	0.4	2.4		0.98	1.85	6.3	4.37	2.26
Threonine	0.08	2.29	5.59	1.68	2.0	0.4	5.2	2.49	0.60	1.40	6.2	0.64	2.36
Tryptophan	–	–	–	0.69	1.5	0.2	–	–	–	–	–	–	–
Valine	0.14	2.50	3.46	1.41	2.4	0.6	–				6.3	6.68	2.85
	Non–essential amino acids												
Alanine	0.08	2.92	3.39	–	–	0.8	12.68	1.84	1.18	1.44	7.0	6.23	1.86
Arginine	0.05	3.16	3.21	–	2.9	0.8	5.55	3.21	0.82	1.30	8.8	8.3	2.34
Asparagine	–	–	–	–	–	–	–	1.39	–	–	–	–	–
Aspartic acid	0.13	4.50	5.86	–	–	1.2	7.24	3.90	1.32	3.54	8.8	5.22	2.98
Cysteine	–	0.64	0.66	–	–	–	0.55		–	–	–	1.18	2.34
Glutamic acid	0.35	7.65	0.35	–	–	1.9	5.62	2.55	3.20	2.14	10.1	6.38	3.01
Glutamine	–	–	–	–	–	–	6.83	2.07	–	–	–	–	–
Glycine	0.04	2.43	2.73	–	–	0.5	9.38	1.52	0.75	1.42	6.1	4.21	1.84
Proline	0.15	1.89	2.08	–	–	0.5	9.96	3.9	0.74	1.54	3.9	3.2	1.64
Serine	0.10	2.02	2.45	–	–	0.4	7.7	1.44	0.64	0.47	5.4	3.6	1.68
Tyrosine	0.12	1.80	2.07	–	–	0.3	1.11		0.86	0.87	5.5	2.61	1.44

Table 6 (continued)

Components	Milk	Meat	Fish	Soyabean	*Chlorella* sp. (microalgae)	*Schizochytrium* sp. (microalgae)	*Haematococcus pluvialis* (microalgae)	*Saccharomyces cerevisiae* (yeast)	*Candida utilis* (yeast)	*Rhodopseudomonas Faecalis* (bacteria)	*Escherichia coli* (bacteria)	*Pleurotus florida* (fungi)	*Agaricus bisporus* (fungi)
Reference	Salazar-López et al. (2022)	Salazar-López et al. (2022)	Salazar-López et al. (2022)	Azaza et al. (2008); Molfetta et al. (2022)	Glencross et al. (2020)	Sarker et al. (2016)	Yap et al. (2022)	Razzaq et al. (2020)	Rajoka et al. (2004)	Patthawaro and Saejung (2019)	Yang et al. (2022)	Khanifar et al. (2011)	Kurbanoglu et al. (2004)

process should proliferate rapidly and utilize a wide range of materials as an appropriate substrate. Also, the nutritional factors include energy value, amino acid balance, and protein content, whereas technical aspects include culture type, separation method, and nutritional needs. Further, the SCP produced should not be pathogenic to animals and humans, and suitable for feed and food. Furthermore, they should have high nutritional value, be devoid of toxic materials, and have low production costs (Bajpai 2017). The essential requirements and processes for production of SCP from any substrate or microbe is the availability of a carbon source which may require physical and chemical pre-treatments. Other nutrients, like nitrogen and phosphorus, may be added to promote optimum growth of a particular microbe. A biomass fermenter is also required to grow the microbes (in their pure form), with strict sterilization protocols to avoid contamination. Because processes of SCP production are mainly aerobic (except in algae), proper aeration should be provided. The microbial biomass must be recovered and processed properly to increase its usefulness and storage ability (Ukaegbu-Obi 2016). Briefly, SCP production is affected by several factors, including the type of microbe, inoculum size and age, temperature and pH, incubation time, carbon and nitrogen sources, rate of oscillation and aeration (Reihani and Khosravi-Darani 2019), etc., which are summarized below.

Inoculum size and age

The inoculum age and size can affect yield, fermentation cost, and growth of an organism during SCP production (Pogaku et al. 2009; Yadav et al. 2014). According to a report, when culture sizes of 13% and 10% (v/v) of *F. venenatum* were inoculated, it led to a biomass yield of 4.84 g/L and approximately 47% protein production (Hosseini et al. 2009; Hosseini and Khosravi-Darani 2011). Conversely, Prakash et al. (2015) noticed a biomass yield of 5 g/L with a culture size of only 5% (v/v). Likewise, Yunus et al. (2015) achieved maximum production of protein, 41.02%, using *Rhizopus oligosporus* and *C. utilis* along with wheat bran with an inoculum size of 10% (v/v), and Marius et al. (2017) obtained protein yield of 79.14% when *S. cerevisiae* was inoculated as 8% (v/v) (Yunus et al. 2015).

Carbon source

Easily accessible food waste can widely be used in the production of SCP. However, cheap substrates and waste materials, particularly agricultural wastes, have already been identified as potentially valuable raw materials for the production of SCP through fermentation (Özyurt and Deveci 2004; Spalvins et al. 2018a; Gervasi et al. 2018; Najari et al. 2022). Typical substrates for manufacturing of SCP by various microbes have already been discussed in the "source and

substrate" section. Lignocellulosic biomass has been used as a suitable substrate for enhanced production of SCP (Bajpai 2017). Nonetheless, several chemical and structural properties resist biological degradation, consequently restricting the biotransformation of lignocellulosic materials (Reihani and Khosravi-Darani 2019). In addition, use of waste for SCP production is an effective method for minimizing environmental pollution and lowering the production cost of protein (Al-Farsi et al. 2019).

Previous studies have reported that use of different substrates as carbon sources revealed varied amounts of SCP production. For most of the bacterial species, agricultural wastes were used as the carbon source, such as fruit wastes for *Rhodococcus opacus* (Mahan et al. 2018) and *Lactobacillus* (Patel et al. 2019). Similarly, fruit wastes were also used as a carbon source for *S. cerevisiae* (Khan et al. 2010; Marius et al. 2017; Dunuweera et al. 2021), *C. utilis* (Marius et al. 2018), and *P. pinus* (Rashad et al. 1990). Other studies showed that the millet bran, maize bran, and rice husk were used as a carbon source for *C. tropicalis* (Abalaka and Daniyan 2010).

Nitrogen source

Owing to its structural properties, nitrogen source is regarded as one of the most significant components in microbial protein production. Nitrogen sources include ammonium salts, ammonia, nitrate, urea, and organic nitrogen, which are supplied by various substrates for the growth of microbes. Furthermore, addition of a mineral supplement to the growth media is often recommended to replenish the shortage of nutrients and to sustain microbial growth (Reihani and Khosravi-Darani 2019). Further, different nitrogen sources may result in varied production of SCP. Additional quantity of nitrogen in the form of peptone was provided to the *C. lipolytica* strain. Growth of yeast and SCP production were seen to be increased by progressive addition of peptone during fermentation. The maximum (17.55 g/L) concentration of SCP was obtained in a medium with 0.4% peptone (Rages et al. 2021). Ammonium phosphate was found to be the preferred nitrogen source for *C. tropicalis* to form SCP out of sawdust hydrolysate (Haider 2021). In another experiment, ammonium sulfate was determined to be better than other forms of nitrogen for *F. oryzae*, *F. graminearum*, and *C. utilis* for SCP production (Reihani and Khosravi-Darani 2019).

Aeration

Aeration is essential in submerged fermentation so that microbes can absorb oxygen (Nascimento et al. 2022). Generally, when the substrate is reduced more, the cell yield increases but requires higher oxygen demand for substrate oxidation (Reihani and Khosravi-Darani 2019). Morphology of microorganism has a valuable role in oxygen absorption (Zheng et al. 2005). Previous study revealed that the aeration rate for *E. coli* and *Bacillus* sp. was 1.5 vvm (the vvm stands for volume of air sparged (in aerobic cultures) per unit volume of growth medium per minute, and is a standard unit of measuring volume of air) (Kurbanoğlu 2001) and for *S. cerevisiae*, value of vvm was 3 (Curto and Tripodo 2001). Another study conducted with *K. marxianus* revealed optimal yield when airflow was 1 vvm (Anvari and Khayati 2011).

Temperature and pH

Temperature is an important influential parameter on the growth of microbes and, thus, on the efficiency and yield of SCP. The worthiest incubation temperature for many microbes was the ambient temperature, about 25–27°C (Reihani and Khosravi-Darani 2019). For certain yeasts, such as *C. utilis* and *K. fragilis*, the optimal temperature was observed to be between 33–35°C (Ghaly et al. 2005; Zhao et al. 2010). For bacteria such as *Bacillus* sp. and *E. coli*, 30°C was widely used (Kurbanoğlu 2001). For the growth of *S. cerevisiae*, 30°C was found to be suitable (Curto and Tripodo 2001).

On the other hand, pH also plays an important role in the growth and development of microbes. *S. cerevisiae* was cultivated on sweet orange residue in a 4% (w/w) citrus-waste medium at pH 5.5 and 36°C for 12 h, and the obtained biomass comprised of 57% (w/w) protein (Nwabueze and Oguntimein 1987). Similarly, different pH was used to determine the amount of SCP produced by *C. lipolytica*, and maximum yeast growth (16.8g/L) was observed at pH 6.5. In contrast, the maximum SCP production was 10.50g/L at a pH of 5.5 in the fermentation medium. The SCP accumulation was inhibited when the pH value exceeded 5.5 (Rages et al. 2021). In a study, *C. robusta* URM5293 was cultured at several pH levels, i.e., 6.0, 7.0, and 8.0, out of which 6.0 was found to be the optimum one (Nascimento et al. 2022).

Safety concerns and regulatory aspects

Foreign proteins like SCP may harm humans by imposing allergies, skin problems, or gastrointestinal issues consequently vomiting and nausea. Heavy metals or other metallic elements may also be found in the SCP that, even in trace amounts, can cause mutations. It may also contain antinutritional factors, i.e., nucleic acids. The SCP may also contain carcinogenic elements, such as impurities derived from the substrate utilized. Therefore, before utilization, final product should be properly decontaminated and purified following

standard procedures (Anupama and Ravindra 2000). The use of suitable substrate materials and manufacturing processes for SCP production are of prime concern from the feed safety perspectives. Therefore, standard protocols for identification and evaluation of SCPs are essential for ensuring the safety of feed and food products (Lähteenmäki-Uutela et al. 2021).

Contents of antinutritional factors such as nucleic acids in the SCPs vary depending upon the type of substrate and the microbes employed in the process. The nucleic acid content varies among microbes; in algae, bacteria, yeast, and fungi, its level has been reported to be 3–8%, 8–12%, 6–12%, and 6–10%, respectively (Najafpour 2007). When a nucleic acid-enriched diet is ingested, uric acid is produced as a by-product of the breakdown of nucleic acids. Because human beings lack the uricase enzyme, uric acid accumulates in the body. This necessitates lowering the nucleic acids in SCPs to appropriate levels if they have to be utilized as human food (Anupama and Ravindra 2000). Consumption of more than 2 g nucleic acid equivalent/day may result in gout and kidney stones (Calloway 1974; Kumar et al. 2022). Therefore, the total nucleic acid content in the SCP used for human food must be below 3% (Abou-Zeid et al. 1995). Endogenous RNase, chemical treatments, alkaline hydrolysis, modification in growth conditions, extraction, and autolysis have been shown to reduce nucleic acid content of SCP (Kumar et al. 2022). Algae usually have a low nucleic acid content compared to the rapidly growing fungi and bacterial species (Ritala et al. 2017). Various strategies for reducing the toxic concentration of RNA and DNA in SCP have already been developed and are still in use. These techniques include extraction of protein from microbial cells using concentrated urea, sodium hydroxide, and heat shock treatment to reduce RNA. The most effective approach is extraction of protein from yeast cells using sodium hydroxide, which increases the total protein content and removes around 75% of the RNA and 81% of the DNA (Abou-Zeid et al. 1995). A potential solution for the future could involve development of an inducible method engineered into the microbes itself, enabling them to autonomously remove excess nucleic acids. In another study, a decrease in nucleic acid content in potato peel grown *S. cerevisiae* was determined by 43%, 36%, 20%, and 17% following heat shock, base, acid, and salt treatment respectively (Khan et al. 2022).

A toxin is also a limiting factor in the SCP production which serves as a contaminant. Toxins are secondary metabolites formed during the growth of some of the fungi and bacteria. In general, algae do not produce toxins. Before commercializing the SCP product, an assessment of its toxicity is crucial. The tests and analyses are specifically tailored to ascertain the suitability of the final product for deployment as a feed additive, dietary supplement, or human foodstuff (Anupama and Ravindra 2000). Various toxins like aflatoxins (B1, B2, G1, and G2) are secreted by *A. flavus*, ergotamine by *Claviceps* sp., trichothecenes, and zearalenone by *Fusarium*, and citrinin by *Penicillium citrinum* (Kumar et al. 2022). Cyanobacteria, particularly *Microcystis aeruginosa*, have potent neurotoxins. One among them is a cyclic heptapeptide known as microcystin, which probably inhibits type 1 and type 2A phosphatase protein, which are essential for structural protein production, and cause liver cancer (Testai et al. 2016). Additionally, certain neurotoxins, like saxitoxins and anatoxins, are found in *Anabaena*, *Oscillatoria*, *Aphanizomenon*, and *Trichodesmium*, and are associated with human and animal poisoning (Lévesque et al. 2016). Another class of toxin is bacterial toxins. Bacteria produce either endotoxins or exotoxins. Exotoxins are secreted by the Gram-positive bacteria. These are proteins with molecular weights between 10 and 900 KDa (Anupama and Ravindra 2000). They do not induce fever but produce non-specific symptoms and various lesions in the host (Anupama and Ravindra 2000), while endotoxins are an intrinsic component of the Gram-negative bacterial cell walls and are released upon lysis. The cell wall of this bacteria is made up of lipopolysaccharide, where lipid A acts as a toxin. They usually cause fever in the host and, at slightly greater concentrations than exotoxins, are lethal to laboratory animals (Powar et al. 2005). When used as SCP, hazardous bacteria must be prevented, and SCPs produced from non-pathogenic bacteria should positively be decontaminated before their usage as sources of SCPs. As the exotoxins are soluble in media, they may be easily removed, but endotoxins are the cellular component of the bacteria; hence, their removal is difficult (Anupama and Ravindra 2000). Through careful selection of species, substrate, and medium conditions, this problem can be prevented. Some of the toxins can be eliminated through simple chemical or heat treatments. Microbial consortia can eliminate toxic compounds and restrain the growth of pathogenic species (Sharif et al. 2021). An illustrative example is the enzymatic reduction of Aflatoxin, a prevalent toxin present in food contaminated with *A. flavus*, observed in diverse fermentative processes. Through rigorous strain engineering, strain selection, and the application of suitable fermentation techniques, toxin production can effectively be avoided or minimized (Kovac et al. 2017; Liu et al. 2020b).

Allergic reactions or analogous symptoms have been documented in human beings following the ingestion of foods or supplements derived out of microorganisms. For example, a case of acute tubulointerstitial nephritis in a child who had used chlorella pills for 3 months has been reported (Yim et al. 2007). In another case, the ingestion of Spirulina (*Arthrospira platensis*) tablets triggered anaphylactic symptoms in an adolescent, with the presence of phycocyanin identified as the causative factor (Petrus et al. 2009). An additional protection could reduce the presence of

particular contaminants, allergens, or hazardous substances in the substrate through a rigorous screening procedure, especially if it originates from wastes. Hence, comprehensive animal feeding trials are necessary before distribution to thoroughly understand the potential health risks associated with the consumption of a specific microbial strain. Some of the examples of these trials as feed are outlined in Table 7.

SCPs find applications in both food and feed. However, it is crucial to emphasize that obtaining regulatory approval is a necessary step for novel SCP products designed for both animal and human consumption. This also applies to food and feed additives such as preservatives, colorants, and texture modifiers (Nyyssölä et al. 2022). Regulatory frameworks vary across regions, ensuring the safety of food and feed for consumption. These regulations differ based on the proposed persistence of the product; for example, SCP may be categorized as either food or feed. The United Kingdom (UK), the European Union, the United States (US), the Food Standards Agency (FSA), the European Food Safety Authority (EFSA), and the Food and Drug Administration (FDA) agencies play pivotal roles in this regard. Díaz et al. (2020) and Ioannidou et al. (2021) summarized the FDA and EFSA regulations, respectively, concerning food and food supplements. Campden (2021) provided valuable information and differences between the regulatory frameworks related to food and feed in various countries like Brazil, China, Canada, US, the European Union, Japan, Australia, and Argentina. It is important to note that the animal categorizations may vary across the regions; for example, pet food is categorized as feed in some areas but not in others. In India, the Food Safety and Standards Authority of India (FSSAI), the Ministry of Fisheries, Animal Husbandry, and Dairying, and the Department of Animal Husbandry and Dairying (DAHD) are the primary regulatory authorities governing food safety and standards for both human and animal food and feed (Shukla et al. 2014).

The yeast, *Yarrowia lipolytica*, is recognized as non-pathogenic, and numerous production processes involving it have received Generally Recognized as Safe (GRAS) classification from the FDA (Groenewald et al. 2014). Since 2010, the utilization of dried and heat-killed biomass of *Y. lipolytica* cultured using biofuel waste has been permitted as a feed additive. Notably, in 2020, *Y. lipolytica* yeast biomass enriched with selenium was officially listed among authorized novel foods (food supplements) under Commission Regulation (EU) 2020/1999 (2002/46/WE) (Jach et al. 2022). Similarly, *Fusarium* and *Torula* have gained acceptance for use as food within the EU (Weatherholtz and Holsing 1976; Wiebe 2002). Acellular Genetically Modified Organisms (GMOs), like *T. reesei* producing β-lactoglobulin, hold GRAS status in the US, permitting their use as food (Voutilainen et al. 2021). In the European context, Pekilo and recombinant protein would need to comply with novel food legislation (EU) 2015/2283 (Rychen et al. 2018). Adherence to novel food regulations necessitates rigorous safety testing, encompassing toxicity assessments, compositional analysis, and risk analyses. This includes providing a comprehensive description of the process and all original data supporting the approval (Turck et al. 2016). As of now, no GMO microorganisms have secured approval from the EU for use as a source of protein. However, GMO microorganisms are subject to specific guidance and additional requirements beyond novel food regulations. These include molecular characterization, comparative analysis to their non-GMO counterparts, and an evaluation of potential environmental impact (Regulation (EU) No 503/2013). The microalgae that were employed in the Europe before May 1997 and consequently authorized as food within the EU include *A. platensis*, *C. luteoviridis*, *C. pyrenoidosa*, and *C. vulgaris* (EU, Novel Food catalogue). The diatom *Odontella aurita* received authorization in 2005 (EU, 2005), while in 2009, docosahexaenoic acid-rich oil from *Ulkenia* sp. obtained approval as a novel food ingredient (EU, 2009). Additionally, in 2014, *Tetraselmis chui* and astaxanthin from *Haematococcus pluvialis* were also sanctioned as novel food ingredients (Molfetta et al. 2022).

Downstream processing of SCP

Downstream processing of SCP refers to series of steps that are performed after the initial cultivation of microorganisms to extract and purify protein products. These steps typically include harvesting, centrifugation, cell lysis, protein purification, and drying or packaging of products. The goal of downstream processing is to increase the overall yield and purity of the protein products, making them suitable for use as food or feed ingredients. These steps are discussed briefly in the following sections and Figure 2.

Degradation of cell wall

Some of the SCPs are prepared as whole cells, while others may require disruption of cell wall to make the protein accessible. Although algae are good sources of nutrition, their human intake has certain limitations. One among those is the existence of cell wall in them. Since humans lack cellulase enzymes, they cannot degrade the cellulose constituent of the algal wall. Therefore, algal wall must be disrupted before the resultant product being consumed as human food. On the other hand, cellulose-degrading symbiotic bacteria and protozoa are present in the rumen of the cattle, and hence is easy for them to digest cellulose and utilize it as food (Anupama and Ravindra 2000). To break the cell wall, various approaches have been used, such as mechanical, chemical, enzymatic, or combination of these.

Table 7 SCP-based feed utilized for different animals.

Microorganism	Animal	Effect	References
Algae			
Schizochytrium limacinum and *Nannochloropsis salina*	Largemouth bass (*Micropterus salmoides*)	The results indicated no noteworthy disparity in weight gain, specific gain rate, viscerasomatic index, hepatosomatic index, conditioning factor, and whole-body composition.	Liao et al. (2022)
Spirulina platensis	Broiler chickens	Incorporating Spirulina at a rate of 10% in a protein-reduced diet enhanced footpad score in male broilers and elevated meat and skin pigmentation in all the birds.	Mullenix et al. (2022)
Bacteria			
Clostridium autoethanogenum	Largemouth bass (*Micropterus salmoides*)	About 150 g/kg of fish meal can be effectively replaced with *C. autoethanogenum* protein in a diet comprising 350 g/kg of fish meal without harming growth, intestinal histology, or feed utilization efficiency.	Yang et al. (2023)
Clostridium autoethanogenum	Black sea bream (*Acanthopagrus schlegelii*)	It is a safe and efficient substitute for fish meal in the diet of black sea bream, replacing up to 58.20% of it without negatively impacting growth, antioxidant activity, or activities of digestive enzymes.	Chen et al. (2020)
Methylococcus capsulatus, Bath	Pacific white shrimp (*Litopenaeus vannamei*)	The substitution of fish meal with bath protein meal (BPM) had no detrimental effects on growth performance and feed utilization of shrimps. However, elevated inclusion of BPM in the diet led to increased oxidation in hepatopancreas of shrimps. Despite this, BPM positively influenced height of mucosal folds, enhanced structure of gut microbiota, and augmented disease resistance of shrimp.	Chen et al. (2021)
Bacillus subtilis	Broiler chicken	It has notably enhanced growth performance of the broilers and influenced colonies of beneficial bacterial communities in the gut and excreta.	Ciurescu et al. (2020)
Yeast			
Wickerhamomyces anomalus and *S. cerevisiae*	Rainbow trout (*Onvorhynchus mykiss*)	These yeasts can substitute up to 40% of fish meal in the rainbow trout diets at existing inclusion levels, without compromising growth performance, nutrient digestibility, or intestinal health.	Vidakovic et al. (2020)
Cyberlindnera jadinii	Broiler chicken	Broiler chicken diets incorporates up to 10% *C. jadinii* cell protein as a substitute of soybean meal, and preserves growth performance and digestive function.	Cruz et al. (2020)
Fungi			
Aspergillus niger fermented plant protein	*Penaeus vannamei*	Utilization of a combination of fermented ingredients in the shrimp feed was more effective than using them individually, showcasing significant potential in alleviating reliance on fish meal.	Dayal et al. (2020)
Aspergillus niger	Broiler chickens	The weights of specific intestinal segments were increased, and there was a notable decrease in the red blood cell, hemoglobin, hematocrit, and blood enzyme activity as the dietary level of SCP increased.	Chiou et al. (2001)

Mechanical methods use physical forces for cell disruption. It includes bead milling, sonication, pressure homogenization, microwave treatment, etc. The utilization of glass beads is a prevalent and simple method employed to facilitate disruption of microbial cells. The selection of bead diameter is contingent upon the specific cell type (Goldberg 2015). For instance, when disrupting bacterial cells, it is recommended to use glass beads with a diameter of 0.1 mm. Different diameters are suggested for yeast or fungi (0.5–1.25 mm) and microalgae (0.3–0.4 mm) (Montalescot et al. 2015; Postma et al. 2017). Sonication is an extensively employed method for laboratory disruption. In this method, ultrasound, characterized by sound waves exceeding 15–20 kHz in frequency, exhibits the capability to induce both inactivation and, with increased acoustic power inputs, disruption of suspended microbial cells. The mechanism behind cell disruption is linked to cavitation phenomena, wherein shear stress results from viscous dissipative eddies generated by shock waves produced during the implosion of cavitation bubbles (Geciova et al. 2002). Disruption in a high-pressure homogenizer is achieved by passing a cell suspension under high pressure through an adjustable, restricted orifice discharge valve. The major parameters determining efficiency are operating pressure and number of passes through the valve, suspension temperature, and design of homogenizer valve (Gomes et al. 2020). Microwaves are electromagnetic waves with frequencies ranging from 0.3 to 300 GHz. Microwave irradiation induces cell disruption by interacting with dielectric and polar molecules present in the solution or cell suspension. This interaction results in local heating and a subsequent increase in the pressure, ultimately leading to disruption of the cells (Nagarajan et al. 2020). Jacob et al. (2019) explored different cell disruption techniques and the results revealed that the bead milling yielded highest protein content (321.56 mg/g) followed by ultrasonication (285.40 mg/g). Mechanical method is extensively employed to disrupt cells and release intracellular biomolecules due to its scalability, efficiency, and comparatively low operational cost. However, this approach has certain drawbacks, including a lack of selectivity and simultaneous release of cytoplasmic components. This may increase the number of unit operation steps required during downstream processing of the SCP (Gomes et al. 2020).

Chemical methods employed for disruption of microbial cells involve use of chemical agents to break down or permeabilize the cell membranes, thereby releasing cellular components, including proteins. Various chemicals can be employed to facilitate cell disruption or permeabilization, including isoamyl alcohol, toluene, ammonia, sodium hydroxide, benzene, ether, acetone, methanol, and hexane, as well as detergents like Triton X-100, sodium dodecyl sulfate (SDS), and N-lauroylsarcosine (sarcosyl). Alteration in the pH of the cell membrane to induce protein release can be achieved using buffers that incorporate organic solvents, alkalis, and detergents (Gomes et al. 2020; Wang et al. 2020; Oliveira et al. 2022). Chemical lysis serves as a viable pretreatment method to enhance various procedures, including ultrasonication and bead milling. Selection of the specific chemical agent, whether it be an organic solvent, detergent, or alkali, is contingent upon the type of microorganism involved. Despite being easily scalable and demanding low energy input, the application of chemical lysis is somewhat constrained due to the elevated risk of compound degradation (Gomes et al. 2020).

Additionally, the enzymatic method is utilized to degrade cell walls and release intracellular substances, such as proteins (Geciova et al. 2002; Gomes et al. 2020). For this purpose, enzymes such as lysozyme or cellulase are commonly used. For example, lysozyme targets the peptidoglycan layer of the bacterial cell walls. The cell wall weakens and eventually lysed as a result of cleaving the β-1,4-glycosidic linkages between N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) by this enzyme (Salazar 2008), whereas fungal and algal cells are susceptible to cellulase degradation due to the cellulose present in their cell walls (Geciova et al. 2002). In the yeast cells, it involves targeting the mannoprotein complex and glucan backbone of the cell wall through processes like autolysis or the action of lytic enzymes. Among the enzyme classes considered most effective for yeast cell wall lysis are zymolyase, lysozyme, glycosidase, glucanase, peptidase, and lipase (Liu et al. 2016). The enzymatic method offers specificity in disrupting cell walls without affecting intracellular components, making it suitable for extracting intact and functional proteins. However, the choice of enzyme depends on the type of microorganism and its cell wall composition. One challenge associated with enzymatic cell disruption is the high cost of enzymes. Despite these, the enzymatic method is valued for its selectivity and ability to yield high-quality proteins in SCP production processes (Gomes et al. 2020). Salazar (2008) and Bayarjargal et al. (2011) documented utilization of lytic enzymes for extracting proteins from *S. cerevisiae*. Various disruption methods have been employed under distinct mechanisms. Combining these methods synergistically enhances the disruption effect, resulting in a higher product recovery compared to employing any of these methods alone (Liu et al. 2016). The majority of these combinations involve pairing mechanical disruption with chemical or enzymatic method, as evidenced by several studies in the literature. Examples include integration of two mechanical methods (Stirk et al. 2020), enzymatic lysis with the high-pressure homogenization technique (Baldwin and Robinson 1990; Vogels and Kula 1992), enzymatic with mechanical (Alavijeh et al. 2020), or a combination of chemical and mechanical processing (Harrison et al. 2015).

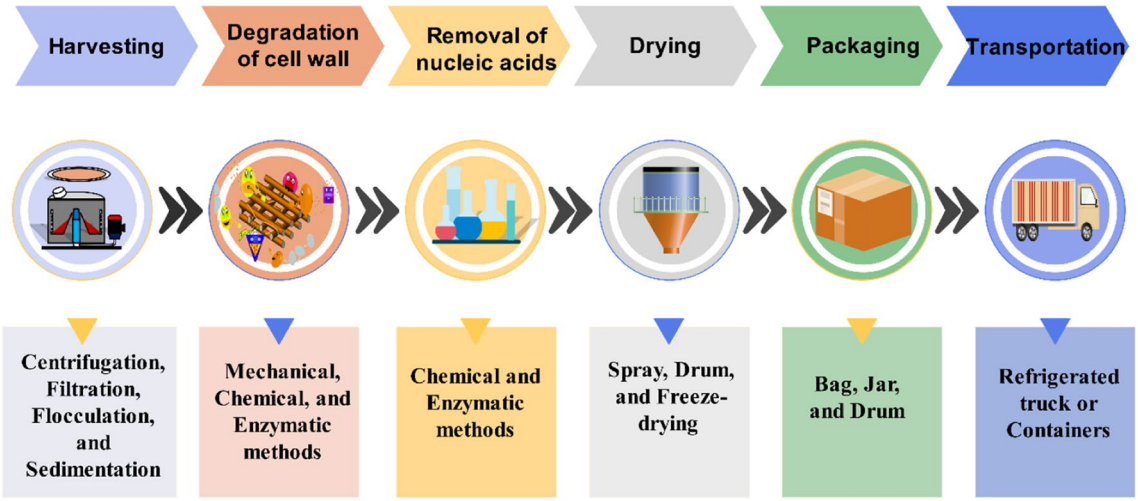

Fig. 2 Schematic representation of downstream processing of single-cell protein.

Harvesting

Harvesting of SCP refers to the process of separating the microorganisms from the growth medium after they have reached to an optimum population density (Sheth and Patel 2023). This step is critical for the downstream processing of SCP as it allows for the recovery of microorganisms, which are the source of the protein products (Patel et al. 2019). Several methods are there for harvesting SCP, including centrifugation, filtration, flocculation, and sedimentation (Cheirsilp et al. 2020; Tan et al. 2020). Among these, centrifugation is the most commonly used method, in which microbial culture is spun at high speeds to separate the cells from the growth medium (Mujahid et al. 2020). The separation process primarily depends on the size and density variation among the medium components. This technique is relatively fast, shows high harvesting efficiency, and does not require any chemicals (Majekodunmi 2015). The primary drawback of centrifugation lies in its energy-intensive nature, making it less cost-effective. Furthermore, the capital investment cost associated with centrifugation is relatively higher (Najjar and Abu-Shamleh 2020). Bacterial biomass is mainly separated and harvested by centrifugation. Patel et al. (2019) harvested *Lactobacillus* biomass grown with mixed fruit waste following centrifugation. In another study, biomass of *Rhodococcus opacus* (Mahan et al. 2018) and *Cellulomonas* sp. (Jeder et al. 1987) has been separated by centrifugation. Disc-stack, multi-chamber, solid-bowl, tubular, and hydrocyclones are most popular centrifugal devices that have been recognized as significantly effective for harvesting algal biomass (Najjar and Abu-Shamleh 2020). Milledge and Heaven (2011) utilized the method of disc-stack centrifugation to separate microalgal biomass. In centrifugation, gravity is replaced by the force causing separation by a significantly larger force; 4000-to-14,000-fold gravitational force is generally used in case of disc-stack centrifuges, which significantly reduces the separation time (Milledge and Heaven 2011).

Filtration is a mechanical or physical process designed to separate solids from gases or liquids by introducing a permeable separator, such as screens, filter cloths, and permeable membranes, that retains the solids. It helps in careful separation of cells from the culture medium (Hassanpour et al. 2020). For example, filtration was used to separate *A. oryzae* and *Rhizopus oligosporus* biomass (Jin et al. 2002). In a large-scale system, vacuum or pressure filters are adequate for recovering microbial biomass having a large size or filamentous cells (Lemos et al. 2018). Filtration is an appealing method for harvesting due to its effectiveness in recovering materials, its capacity to separate shear-sensitive species, and its capability to achieve separation without the need for added chemicals (Barros et al. 2015). Conversely, notable disadvantages include potential issues of clogging and fouling, the necessity for cleaning processes for the membranes, the associated investment costs for both membranes and pumps, and a limited understanding of the most crucial operating conditions (Muylaert et al. 2017).

Flocculation is a method in which microbial culture is treated with a chemical reagent that causes the cells to form flocs, which can then be separated by sedimentation (Chatsungnoen and Chisti 2019). The major disadvantage of this method is the addition of chemicals that further contaminates the biomass (Muylaert et al. 2017). In flotation and sedimentation, gravitational forces induce solid or liquid particles to separate from a liquid of a different density. It presents an appealing choice as it demands minimal energy and involves a relatively inexpensive infrastructure. Nevertheless, the process may be very slow, particularly if the difference in density or particle size is tiny (Milledge

and Heaven 2011). The choice of harvesting method will be based on the type of microorganism used, the growth medium, and the steps of downstream processing to be followed. The efficiency of the harvesting method has a significant impact on the overall yield and quality of the SCP and its products.

Drying

Drying is a common method of concentrating and preserving SCP, as it reduces moisture content and prevents bacterial growth. There are several methods of drying, including spray drying, freeze drying, and drum drying (Labuza et al. 1970; Hedenskog and Mogren 1973). The choice of method depends on factors such as the type of SCP, the desired final product, and the available equipment and resources.

Spray drying is the most commonly used method for drying SCP. In this process, the SCP is suspended in a liquid and then atomized into small droplets using a spray nozzle. The droplets are then exposed to hot air in a drying chamber, which evaporates the moisture and forms a dry powder (Patel et al. 2009). The main advantage of spray drying is that it can produce a dry powder with a high degree of uniformity, which is important for consistency in animal feed and other applications (Labuza et al. 1970). Additionally, it is a relatively fast and efficient method of drying, which can be beneficial for large-scale production (Patel et al. 2009). The quality of the final product can be affected by several factors such as the spray drying conditions, the type and concentration of SCP, and the properties of the liquid medium (Hedenskog and Mogren 1973). Proper control over these factors can help achieve the desired properties in the final product.

Drum drying is another method used to dry SCP. In this process, the SCP is spread in a thin layer on the surface of a heated drum, which rotates continuously. As the drum rotates, the SCP is exposed to hot air which evaporates the moisture, forming a dry film on the drum. The dry film is then scraped off from the drum and ground into a powder (Abdel-Azeem and Sheir 2020; Onyeaka et al. 2022). One of the main advantages of drum drying is that it can handle large quantities of SCP, making it more suitable for large-scale production. Additionally, it is a relatively simple and low-cost method of drying compared to other methods like spray drying (el Abbadi et al. 2021). However, the final product obtained by drum drying may not be as uniform as that obtained by spray drying, and it may also have a relatively lower protein content. Also, drum drying may not be as efficient in terms of energy consumption as spray drying (Yarkent et al. 2020).

Freeze-drying also called lyophilization is a method of drying that can be used for SCP. In this process, the SCP is frozen, and then frozen water is removed from it by sublimation, which is the process of changing from a solid to a gas state without passing through a liquid phase (Nail et al. 2002). This is done by applying a vacuum and low temperature to the frozen SCP, which causes the ice to sublimate directly into water vapor, leaving behind a dry powder (Khoshnevisan et al. 2019). Freeze-drying is considered to be one of the best methods of preserving SCP, as it can preserve the integrity of the protein and other nutrients, while also providing a long shelf life. Additionally, it can produce a dry powder with a high degree of uniformity, which is an important property of animal feed (Onyeaka et al. 2022). However, freeze-drying equipment can be expensive and requires a large space. Also, the process can take longer time than other methods such as spray drying and drum drying (To and Etzel 1997; Haque and Roos 2006). While using all of these drying methods, it is important to ensure that the SCP used for drying should be free from pathogenic microorganisms, toxins, and other contaminants. Also, the dried SCP powder should be stored properly to prevent reabsorption of moisture and to maintain quality and safety of the product (Hedenskog and Mogren 1973).

Packaging

Packaging is an important step in preservation and distribution of SCP. The type of packaging to be used depends on the specific application and desired shelf-life of the SCP.

For short-term storage, SCP can be packaged in airtight containers, such as bags or jars, and stored at a cool temperature to prevent reabsorption of moisture and bacterial growth (Almasi et al. 2021), while, for long-term storage, vacuum packaging or modified atmosphere packaging (MAP) can be used to extend the shelf life of the SCP (Rahman and Al-Farsi 2020). Vacuum packaging involves removal of air from the package before sealing it, which can help to prevent oxidation and growth of microorganisms (Inmanee et al. 2019; Li et al. 2019), while MAP involves adjusting the composition of air inside the package to create an environment that is inhospitable to microorganisms, such as increase in the level of CO_2 (Tarlak et al. 2020).

For commercial distribution, SCP can be packaged in larger bags or containers, such as drums or sacks, with appropriate labeling and usage instructions. It is important that the packaging is tamper-evident and able to protect the product from damage during transportation (Kuswandi 2020). It is also important to keep in mind that the packaging should be of food-grade and comply with the regulations and safety standards of the country. If the SCP is intended for animal feed, it is essential to check the regulations and packaging requirements set by the concerned authorities (Kuswandi 2020).

Transportation

Transportation is an important step in the distribution of SCP. The method of transportation to be opted depends on the specific application and desired shelf-life of the SCP. For short-distance transportation, SCP can be transported in refrigerated trucks or containers. This will help to preserve the integrity of the protein and other nutrients, while also preventing bacterial growth and spoilage, while, for long-distance transport, it is recommended to use refrigerated containers and maintain a cool temperature throughout the journey to preserve the quality and safety of the product (Hadi and Brightwell 2021). If the SCP is intended for animal feed, it is essential to check the guidelines and safety standards approved by the concerned authorities regarding the transportation of animal feed products (Chowdhury and Morey 2019).

When transporting SCP, it is important to consider the potential effects of temperature fluctuations, vibration, and exposure to light and moisture. These factors can affect the quality and safety of the SCP, so it is important to take appropriate measures to protect the product during transportation (Liu et al. 2020a; Mohammadian et al. 2020). It is also important to ensure that the SCP is properly packaged and labeled, and that the packaging can protect the product from possible damage during transportation (Daniloski et al. 2021). To ensure that the SCP reaches the destination in good condition, it is recommended to monitor the temperature and humidity constantly during transportation and to document the entire process for quality and traceability purposes (Mohammadian et al. 2020).

Techno-economical aspects

Production of SCP on a large scale not only deal with food crisis and water shortage concerns that are affecting a significant percentage of the world today but also promises to lower down the cost of agricultural and industrial wastewater treatment. The cost of SCP production mainly depends on the fermenters, substrates, operational, and capital expenses. Cost of the substrate is a major factor in SCP production. Simplification of production of raw materials and purification can save expenses. The site, method of raw material production, capacity of plant, yield of substrate, and product are key factors that influences cost of the substrate (Srividya et al. 2013). The second most crucial cost component is energy required for cooling, sterilization, compress air, and drying. Sites providing inexpensive electrical, thermal, process-derived, or fossil energy are preferred. In addition, capital costs are defined by the cost of the processing equipment, capacity of the plant, and other conditions. For instance, when lignocellulosic waste is used for manufacturing SCP, only wheat straw is produced at a pace of 865 million tonnes per year (Bajpai 2017). Voutilainen et al. (2021) estimated that the requirement of manpower was two laborers per process section in continuous operations and three laborers in fed-batch process in three shifts, and the manpower fee was considered total wages of 76,141.66 US$ including expenses of all the employees (Pihlajaniemi et al. 2020). Reactors, tanks, filtration systems, centrifuges, dryers, compressors, agitators, cooling, and wastewater systems are needed for SCP production. To the estimated costs of purchased equipment, an additional 20% was added for other equipment, such as pumps and conveyors. Working capital was assumed to be 5% of fixed capital investment (Voutilainen et al. 2021).

In an economic analysis conducted by Voutilainen et al. (2021), optimal hydrolysis conditions were applied, revealing minimum protein selling prices (MPSPs) for Pekilo, *Fusarium*, *Torula*, and recombinant protein as 5634 (4713–6495), 7150 (6072–8122), 7982 (6820–9121), and 9834 (8687–10,972) US$/ton, respectively. These MPSPs fall within the range of commercial food protein products when compared to plant-based protein products (average 8100 US $/ton) and egg and milk protein ingredients (average 11,500 US$/ton). It is worthy to note that these MPSPs represent wholesale protein prices, excluding final product formulation and considerations for food safety measures. Comparing minimum selling prices across similar processes is challenging due to the limited available studies to our knowledge. Upcraft et al. (2020) examined the economics of the Quorn™ process, estimating a minimum selling price for *Fusarium* paste as 6250–7470 US$/ton (54,350–64,960 US$/ton protein), notably higher than the values reported by Voutilainen et al. (2021). However, Quorn™ utilized over 10-fold purchase costs for the fermenters and separation equipment, along with higher capital costs for RNA reduction equipment (Upcraft et al. 2020). Pihlajaniemi et al. (2020) explored the production of feed Pekilo SCP from grass silage fiber and suggested a slightly over protein price: 2200 US$/ton. The RNA reduction process increases costs, and the development of food products necessitates suitable texture, potentially requiring texturizing operations comparable to the Quorn™ process. Comparing the product's value to commercially available proteins is intricate due to the lack of public information on prices for precisely similar proteins, and predicting future price development is challenging.

Recent advances and emerging technologies

Advancements in genome sequencing, genetic engineering, and multi-omics analysis are empowering microbial engineering for enhanced production of SCP (Maruyama 2021). The field encompasses various tools, including genetic components such as promoters, ribosomal-binding

sites, transcription factors, synthetic biosensors, and pathways of genes/enzymes (Balagurunathan et al. 2022; Gao et al. 2023a). One such example includes implementation of a successful breeding strategy to enhance the methylotrophic performance of *P. pastoris*. The global metabolism of *P. pastoris* was effectively reprogrammed to enhance robustness during SCP overproduction, presenting a novel approach for constructing versatile cell factories in *P. pastoris* (Gao et al. 2023a). Synthetic biology tools exhibit significant potential in improving conversion efficiency and generating intermediate feedstocks like C5/C6 sugars, acetate, and methane through processes such as carbon fixation and bio-saccharification (Bourgade et al. 2021; Alloul et al. 2022). Rational engineering approaches enhances growth, substrate utilization, protein production, and strain tolerance, potentially achieving high biomass-based SCP yields across diverse microbial hosts. Employing transcription factors, transporters, and metabolic pathways through a rational engineering approach can achieve desired phenotypes, such as enhancing cell growth and improving substrate utilization (Hara et al. 2017; Gupta et al. 2020). Various methodologies, including adaptive lab evolution (ALE), chemical mutagenesis, and genome engineering can be utilized for enhancing cell growth and biomass accumulation (Pham et al. 2017; Adiego-Perez et al. 2019; Bennett et al. 2021). Meng et al. (2023) employed ALE to address the challenges of low methanol utilization efficiency and intolerance to higher temperatures (33°C) in *P. pastoris*. This approach resulted in reduced carbon loss due to the diminished detoxification of intracellular formaldehyde through the dissimilation pathway. Microbial consortia, employing synergistic growth, have been involved in improving biomass accumulation for SCP (Vethathirri et al. 2021; Hu et al. 2022). These consortia can be tailored to regulate genetic factors and pathways for detoxification, harnessing nutrients, and improving biomass production (Szepe et al. 2021; Alloul et al. 2022; Balagurunathan et al. 2022). The commercial success of genetically modified SCP products, such as KnipBio Meal in the aquaculture industry, underscores the efficacy of these approaches (Balagurunathan et al. 2022).

One approach to sourcing feedstocks involves utilization of by-products and waste materials of the agri-food industries (Matassa et al. 2016a). Some of the companies are exploring innovative methods to produce proteins, such as extracting carbon from the air (Sillman et al. 2019; Marcellin et al. 2022), and even utilizing plastic waste as a substrate (Schaerer et al. 2023). Waste materials can be gasified into CO_2, CO, or CH_4 gases, and their release into the atmosphere can significantly impact climate change (Kumar and Jujjavarapu 2023). However, these gases can be repurposed as carbon feedstocks for SCP production. The use of gasified waste materials as carbon sources for SCP production plays a substantial role in fostering the transition to a circular economy (Marcellin et al. 2022). Conversely, exploring the nitrogen-fixing potential from side streams of the Haber-Bosch process through hydrogen-oxidizing bacteria has been investigated for the production of edible protein (Hu et al. 2020).

The utilization of bioprocess design has proven success in the expansion of scale-up, cost reduction, and enhancement of various biotechnological processes, establishing its significance in microbial protein production (Bajić et al. 2022). A substantial focus is directed towards the commercialization of industrial manufacturing, recognized as a pivotal step for numerous companies engaged in alternative protein production. Furthermore, the exploration and establishment of platforms for downstream processing, continuous bioreactor operation, and virtual platforms stand out as key factors and opportunities within the bioprocess design (Jones et al. 2020). These endeavors are poised to significantly contribute to the increase of microbial proteins.

Commercial applications of SCP

The SCP is used for the enormous production of microorganisms for ingestion by either humans or animals. Due to its high protein content, synthesis and use of microbial protein have drawn attention as a potential replacement of proteins from agricultural sources. Because SCP contains many components in addition to proteins, such as lipids and vitamins, the utilization of biomass as a nutritional supplement has recently gained more consideration than its usage as a straight forward protein source. Possible commercial applications of SCPs are depicted in Figure 3. The below sections discuss some of the critical commercial applications of SCPs.

As human food

Because of their sustainability and effectiveness, SCPs are usually considered prospective contenders of protein and nutritional supplies in the future ahead. Depending on social, climatic, and economic factors, SCP (microalgae, fungi, and bacteria) has a significant history of global usage. It is employed in many food products to increase active components or nutrients, including proteins, trace elements, and fibers. However, their storage stability and quality, protection from pathogens or spoiling organisms, and sensorial properties must be considered when used as food. Microbes are widely cultivated to produce SCP for incorporation into food items as an inexpensive and sustainable protein replacement to fish or soy meals and to alleviate the protein shortage (Kumar et al. 2022). The acceptance of a species as feed or food depends on its

growth rate, substrate, pathogenicity, and the presence of related toxins. This limits the use of only a few microbes to manufacture SCPs for human food (Verstraete et al. 2016; Pihlajaniemi et al. 2020). However, given the existing amount of food generation, this additional requirement cannot be supplied with current agricultural practices. Proteins are essential for metabolic activities and serves as nitrogen source for animals and humans to form their functional and structural components needed for survival. PCM primarily affects children, resulting in poor mental development and weak immunity. The amino acid content of protein shows its nutritional value. The most common are EAAs, which animals and humans cannot synthesize.

Tempeh is a traditional Indonesian cuisine, particularly from the Islands of Java and Bali. This item is produced by fermenting dehulled, boiled soybeans with *Rhizopus* spp. particularly *R. oryzae*, *R. oligosporus*, and *R. stolonifera* (Ahnan-Winarno et al. 2021). In line, Kinema is a fermented product of soy milk and is popular in the cuisine of the Eastern Himalayas. The fermentation species, particularly *B. subtilis*, provide distinctive alkalinity and stickiness to this product. It is a good source of protein, vitamin B-complex, and minerals such as Ca, Cu, Fe, K, Mg, Mn, P, and Zn. Process of fermentation promotes conversion of complex proteins into the amino acids that are readily digested (Kumar et al. 2022). On the other hand, SCP derived from *S. cerevisiae* possesses significant functional properties, including high water and oil absorption capacity, low bulk density, and high foaming and emulsifying ability, that allows it to be used as a food additive in baked products, sauces, and desserts (Razzaq et al. 2020). Similarly, Solein®, a bacteria-based SCP marketed by Solar Foods Company, exhibited high oil and water binding capabilities, foaming as well as emulsifying capacity and therefore could be potentially used as a fundamental component in a variety of food items, including baked products, pasta, yogurt, and also microbial-based meat products (Molfetta et al. 2022).

Despite the high protein content, dried microalgae have not yet attained significance as a food or food substitute. Limitations for incorporating algal material into food products are the powder-like consistency, dark green color, and subtle fishy odor of the biomass in dried form. Different methods, including heating, baking, and mixing, have been used in a series of tests to adapt or combine algal material with recognized food products. For instance, attempts have been made to incorporate algae into bread or noodle recipes. However, only a few quantities could be put into bread, as its appearance, dough consistency, and taste become unpleasant, and the noodles look like an unappetizing brownish colored (Becker 2007).

As space food

The SCP may find its usage in space missions and natural disasters that devastate agriculture practices on the Earth, such as sudden changes in climate or super volcanic eruptions. Similar approaches might be used to feed refugees to recolonize the Earth, which could occur in space, underground, or under the sea. In either scenario, hydrogen-oxidizing bacteria must be combined with other food materials to complete a diet. The SCPs from electroactive bacteria, non-biologically synthesized food, photosynthetically generated food using artificial light, greenhouse (space only), or packed food can be a good choice in space or for refugees (Alvarado et al. 2021). Astronauts also use spirulina as food in space (Kumar et al. 2022), which is marketed in various forms, including powder, tablets, and capsules (Al-Hadithi et al. 2018).

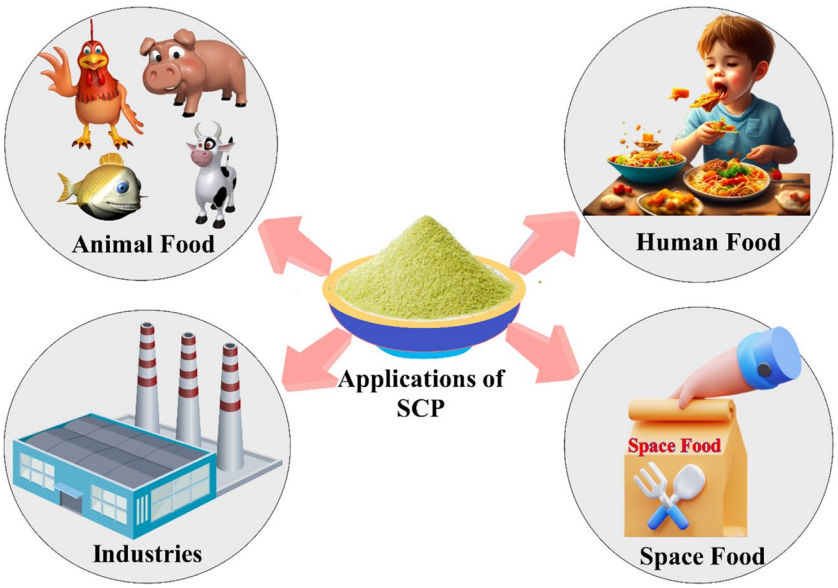

Fig. 3 Commercial applications of single-cell protein.

As animal feed

Various nutritional and toxicological analyses have proven that the algal biomass has a good potential as a feed supplement or alternative to traditional protein sources, i.e., soybean meal, rice bran, fish meal, etc. The inclusion of algae into poultry diets might provide the commercial use of algae in animal nutrition. Another growing market is use of microalgae in aquaculture (Becker 2007). The SCPs have a high nutritive value and may be applied as a protein source in aquafeed in place of costly conventional components (Bhusare et al. 2022). It was observed that the substitution of soybean meal by SCP did not pose any adverse effect over growth, feed consumption, nutrient absorption, or immunity of fish (Hardy et al. 2018). Additionally, use of SCP as fish meals decreased feed expenditure without affecting shrimp growth (Hamidoghli et al. 2019). Because of their chemical constitution and availability, marine species of microalga are most frequently employed as aquaculture feed. Along with high protein content, microalgae are also rich in astaxanthin, which may be added with feed to enhance the color of shrimps and raise their antioxidant capacity and resilience to stress during harvesting and transport (Teimouri et al. 2019). As a replacement for fish meal, the SCP derived from *Clostridium autoethanogenum*, methanotrophic bacteria, and *C. vulgaris* showed considerable potential (Li et al. 2022a). Likewise, strains DSM 1069 and PD630 of *R. opacus* have been used to produce SCP for aquafarming or animal feeds (Mahan et al. 2018). Similarly, *S. cerevisiae* was grown on multi-food waste to produce animal feed (Tropea et al. 2022).

In production of bioplastics

Protein-based bioplastics can be produced from microbial biomass, avoiding the requirement of chemicals for the extraction of microbial polymers, such as PHB, using natural bio-based plasticizers, such as glycerol (Areniello et al. 2023). The production of plastic films and other plastic items from SCP for various purposes like packaging will be an environmentally acceptable alternative to petroleum-based plastics and a material that does not conflict with food production. The SCP produced out of potato starch waste was a feasible alternative to conventional plant or animal proteins used in the manufacturing of films for packaging. Bioplastics derived from microbial protein have relatively good mechanical strengths and biodegradability in addition to the added benefit of preventing the release of microplastics into the environment (Singha et al. 2021). Protein-based biopolymers can be used for food-packaging purposes due to their high biodegradability, functional characteristics, and probable edibility (Garavand et al. 2017).

In agriculture

The utilization of SCP is not limited to its potential as a protein source for animal and human consumption; instead, it can also be used as a slow-releasing organic fertilizer in the agriculture practices (Areniello et al. 2023). It is generated from microorganisms that are cultivated over waste and remnants containing essential elements such as N, P, and K in organic forms that are absorbed and concentrated by growing microbes (Kantachote et al. 2016). The organic forms of these essential nutrients in SCP-based fertilizers provide bio-stimulating and growth-enhancing/nutritional properties to the microbial biomass. The slow release and absorption of these nutrients by plants promotes their growth and overall health (Kantachote et al. 2016; Spanoghe et al. 2020). Furthermore, SCP-based fertilizers offer numerous advantages over conventional chemical fertilizers, including sustainability and reduced impact on soil degradation and nutrient depletion (Pikaar et al. 2018).

SCP-based fertilizers have also been shown to promote soil health by supporting microbial activity and improving soil structure. The use of SCP as an organic fertilizer can be a promising approach to promote sustainable agriculture and eliminate negative effects of traditional farming practices on the environment (Areniello et al. 2023). However, further research is required to optimize SCP-based fertilizer production and its widespread practical implementation in the agriculture.

Others

The SCPs may also be employed in the technological area, such as in the paper and leather industries, and as foam-stabilizing agents. In the paper industries, it can be used as an additive to increase the strength and quality of the products. Addition of SCP into the pulp can enhance the mechanical and physical properties of the paper, resulting in better quality, higher production efficiency, and less environmental pollution (Ali et al. 2017). In the leather industries, it can be used as a substitute of expensive protein sources such as soybean meal and fish meal, which are commonly used in tanning processes. The use of SCP as a protein source in the leather industries can reduce the overall cost of the process and decrease the environmental impact associated with traditional protein sources (Kumar et al. 2022).

The SCPs from different microbial sources can contain high levels of hydrophilic and hydrophobic proteins, which can act as excellent foam-stabilizing agents (Ritala et al. 2017). In the food industry, it can be used as a foaming agent for the production of cakes, meringues, and other bakery products. The proteins of SCP can help in creating stable foams that can increase the volume and texture of these

products, and make them more attractive to the consumers (Mensah and Twumasi 2017). The SCP can also be used in the production of detergents, where it can act as a foam stabilizer to enhance the cleaning power of the detergents. In addition, it can also be used in the cosmetic industries as a foam stabilizer for shampoos, soaps, and other personal care products (Murali Sankar et al. 2023). Furthermore, it can also be used in the petroleum industries to enhance recovery of oil. The proteins in SCP can help stabilize the foam used to inject gas into the reservoir, which can reduce the surface tension of the oil and increase the amount of oil that can be extracted (Ritala et al. 2017).

Current challenges and future perspectives

Production of SCP, despite its potential benefits, faces several challenges that need to be addressed for widespread adoption of this important nutritional supplement. A notable nutritional limitation lies in the elevated presence of nucleic acids. Additionally, concerns arise regarding unfavorable odors and textures that may not align with the human palate. Certain microorganisms, including yeast, fungal, and algal clades, are characterized by thick cell walls. While these walls often contribute significantly to dietary fiber, they can pose challenges for some of the SCPs as discussed above. However, these drawbacks could be mitigated through approaches such as selective breeding, genetic engineering, or formulating mixtures and co-cultures to create novel and appealing flavors (Steensels et al. 2014).

Ensuring the safety of food is an imperative requirement, and this consideration extends to each species involved. Rigorous examinations should be conducted on key target species, validating their food safety for both SCP production and consumption. Ensuring attention is crucial in addressing potential contamination and toxins generate during the production process. Additionally, challenges such as scalability, economic viability, and ecological sustainability need to be overcome. The application of advanced novel tools in these domains can play a pivotal role in augmenting and expediting the advancement of microbial-based foods, thereby overcoming existing constraints. The widespread adoption of SCP and genetically engineered microorganisms in the food industry may probably encounter the obstacles related to consumer acceptance (Onwezen et al. 2021). The regulatory landscape surrounding genetic modification is strict, and varies across the countries. However, with the growing awareness of the need to enhance the ecological sustainability of diets, there is a possibility that this attitude may evolve. To encourage consumption, it becomes imperative to consider the preparation methods and cultural context associated with microbial foods. Education and strategic marketing efforts can play a vital role in addressing unfamiliarity and the lack of consumption experience, thereby paving the way for greater consumer acceptance.

Considering the above challenges, proper processing of SCPs is required before their use as food. Production of SCP can turn out to be a global market for the supply of quality dietary proteins to different organisms. However, there is a need to develop improved techniques for bulk production of SCP, which will be fast, reliable, and cost-effective to meet the current demands. Further, attempts to increase the acceptability of SCPs as food should be performed. Advanced techniques like genetic engineering should be employed for the mass production of protein-containing microbes. Extensive research and development would probably open new avenues for the proper utilization of microbes as SCPs or as dietary supplements in developing nations, particularly.

Conclusion

The increasing interest from researchers and businesses world towards SCP production have been arise recently due to the escalating global protein demand. SCP, derived from renewable feedstocks, holds promise in addressing both waste management and protein shortages, contributing to socio-economic and environmental sustainability. By aligning with circular bio-economy principles, SCP can diversify into animal feed, food, and packaging materials, expanding its market impact. The challenge lies in selecting highly efficient microbial cells for enhanced production of SCP, leveraging their value-added properties shaped by cultivation, processing conditions, and production optimization for the increased biomass yield and, consequently, SCP output. With an appealing nutritional profile, SCP emerges as a viable alternative to traditional protein sources like soymeal and fishmeal. However, effective commercialization necessitates enhancing consumer acceptance of this alternative protein source.

Acknowledgements The authors are thankful to the School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India, for providing the necessary facilities to prepare the manuscript and for permission to publish it.

Author contribution The first author Sharda Devi Rajput has contributed conceptualization; roles/writing—original draft; writing—review and editing. The second author Neha Pandey contributed writing—review and editing. The third author Keshavkant Sahu (corresponding author) contributed supervision; writing—review and editing; and software. All the authors read and approved the final manuscript.

Funding The authors also are thankful to the Department of Biotechnology, Ministry of Science & Technology, Government of India, for the financial aid to carry out the Ph.D. work under the DBT-JRF program.

Data availability Not applicable.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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