

Review Article

# A Review on the Importance of Producing Single-Cell Protein (SCP) from Agricultural By-products and Waste

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## Abstract

The production of Single-Cell Protein (SCP) from agricultural by-products and waste has emerged as a sustainable and cost-effective solution for addressing global protein demand in human and animal nutrition. Agricultural residues such as stalks, fruit peels, and seed remnants serve as low-cost substrates for microbial fermentation, facilitating SCP synthesis. Various microorganisms, including bacteria, fungi, yeasts, and algae, are utilized for their ability to produce high-quality protein with balanced amino acid profiles, potentially replacing traditional plant- and animal-derived proteins. This review explores SCP production processes, microbial sources, and optimal processing techniques, emphasizing their role in sustainable protein supply and environmental impact mitigation. The selection of appropriate microorganisms and fermentation methods significantly influences SCP yield and nutritional composition. SCP production from renewable biomass not only addresses food security challenges but also reduces agricultural waste and greenhouse gas emissions, supporting circular bioeconomy initiatives. Furthermore, SCP application extends beyond nutrition to industrial sectors such as food processing, bio-based materials, and wastewater treatment. Despite its advantages, SCP production faces challenges such as high nucleic acid content, potential microbial toxins, and process scalability. Continued advancements in bioprocess optimization, metabolic engineering, and strain selection are crucial for enhancing SCP efficiency and market viability. This review highlights SCP's potential as a transformative protein source, offering an environmentally responsible and economically viable alternative to conventional protein production systems.

## Keywords

Agricultural Waste, Environmental Sustainability, Microorganisms, Protein Sources, Single-cell Protein

## 1. Introduction

With the rapid growth of the global population, increasing demand for food, especially protein, is inevitable. The production of bulky volumes of agricultural and livestock products marks one of the major concerns of modern societies. Demand for animal protein is forecasted to reach over 400

million tons for meat and over 800 million tons for dairy products by 2050. However, since the feed used is very inefficiently converted to meat or dairy product, this is impossible to meet by increased production. Accordingly, such issues as excessive freshwater usage, greenhouse gas emissions re-

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sulting from livestock and poultry production, and the limited availability of arable land will balance the large-scale food requirements of human societies. The structure of proteins includes all essential amino acids, which provide nitrogen for building new functional proteins in humans and animals. The amino acids, namely leucine, valine, isoleucine, phenylalanine, methionine, tryptophan, lysine, and threonine, and also for an infant, arginine, cannot be synthesized in adequate amounts by the living organisms and must be provided through an adequate diet correspondingly [1]. Hence, alternative products and new ways of production are an urgent need. Lately, much research interest has been directed at the use of alternative protein sources to replace animal proteins by extensive studies of plant-based products, insects, and microorganisms. One of them is single-cell protein utilization. Single-cell protein, also known as microbial protein, is defined as the dried cells of microorganisms like fungi, bacteria, yeasts, and algae resulting from the fermentation process. These chemicals are non-toxic and act as supplements for human beings and animals due to their high production of protein from renewable and inexpensive raw materials. Moreover, they have a high nutritional value in view of their content of other compounds such as fats, vitamins, mineral salts, and non-protein nitrogen (NPN) compounds like amino acids. SCPs have various industrial applications, including their use as foaming agents, in paper and leather treatment, and in packaging materials [2, 3]. Hence, single-cell proteins become very good replacements for different protein sources because they have all the advantages of having no requirement of arable land, specific climatic conditions, and excessive fresh water. They make use of a variety of fermentation substrates which are low priced with high yields that enable fast microbial cell growth. This also contributes to decreasing the amount of greenhouse gas emissions emitted. Although food processing with SCPs currently holds a small share in the market, its value is expected to exceed \$18.5 billion by 2030 [2, 4].

## 2. SCP Producers

Even though the proteins obtained from microbes are designated as single-cell proteins, some microorganisms used in SCP production are multicellular. The examples of multicellular organisms involved in SCP production are filamentous algae and filamentous yeasts [1]. The protein and amino acid profile decide the value of biomass originated from microbes. However, presence of lipids and varieties of vitamins creates added value of biomass. However, the volume, type, and quality of protein produced are a function of the microorganism type and fermentation process applied. The preferred microorganisms for making SCP are those that have high growth rates and the ability for cultivation in quantity. They must also be non-toxic for human and animal consumption and compatible with a wide range of substrates. Although a wide variety of microorganisms can produce SCP, numerous

studies have shown that fungi and yeasts are ideal candidates for producing SCP, single-cell oils, and other high-value-added compounds. Additionally, using mixed strains instead of pure strains can increase the amount of SCP produced [2, 5].

### 2.1. Bacteria

They can grow within a wide temperature range of 15 to 45°C in environments with suitable pH levels, ranging from 6 to 8, depending on the culture conditions and strain, with growth cycles ranging from 20 to 120 minutes. They can utilize a broad spectrum of substrates, such as carbohydrates (e.g., starch and sugars), CO<sub>2</sub>, methane, and hydrogen, for SCP production. However, due to their widespread pathogenicity, only a limited subset of bacteria can be safely used for SCP production [3, 5, 6]. Under optimal conditions, many types of bacteria used to make SCP grow rapidly and can consist of high protein content-50-80% of dry weight-providing even essential amino acids such as methionine with a content of about 3%, while tryptophan is considerably lower. These proteins are produced in higher quantities compared to those from algae and fungi. Moreover, SCP produced from bacteria includes lipids and B-complex vitamins, which increase the nutritional value of SCP [1, 2]. For commercial production of bacterial SCP, phototrophic and methanotrophic bacterial species are mainly employed. Hydrogen oxidizing bacteria, purple photosynthetic bacteria, and methanogens are also being employed for the production of SCP. Recently, some purple phototrophic bacterial species, including *Rhodovulum*, *Rhodobacter*, *Rhodospirillum*, and *Rhodopseudomonas* have been reported as good candidates for microbial protein production. These bacteria have also been reported to exhibit probiotic effects on aquatic organisms [2, 3, 5, 6]. Other bacteria, which are generally studied for SCP production, are methylotrophic bacteria, such as *Methylophilus methylotrophus* and *Methylococcus capsulatus*. This was the first bacteria to be grown commercially because methanol/methane is used as the carbon source. Other examples include *Brevibacterium lactofermentum*, which is dependent on sugars as a carbon source. Thus, for the above bacteria, the substrate/feedstock choice is restricted and the process, as mentioned above, has limited industrial applications. The biotechnology company, KnipBio, has genetically engineered *Methylobacterium extorquens* for its application in aquaculture feed supplements. KnipBio's food product, a genetically modified single-cell protein, has been granted GRAS status by the U.S. Food and Drug Administration (FDA) [5]. Another notable company is Calysta, a multinational biotechnology firm, which markets SCP derived from *Methylococcus capsulatus* as an aquafeed supplement under the brand name FeedKind. It has a well-balanced amino acid profile, high digestibility, and rapid absorption. The protein content of more than 80% DCW (Dry Cell Weight) has the production capacity of 20,000 tons per annum underlining its

important nutritional and industrial value [6]. The major benefits of hydrogen-oxidizing bacteria are a short production cycle, high protein content (up to 75 % of DCW), and the production of amino acids similar in composition to animal proteins. SCP produced by these bacteria also contains poly-beta-hydroxybutyrate (PHB), a probiotic with potential application as an inhibitor of pathogenic bacteria in aquaculture. Gram-negative aerobic hydrogen-oxidizing bacterial strains can accumulate substantial concentrations of protein although containing lower levels of cysteine, methionine, and isoleucine. However, they are not suitable for SCP production due to the fact that their cell walls contain Lipopolysaccharides (LPS) endotoxins. In a high dose these endotoxins, can create further complications and mortality in human and animals. LPS is structured-based toxic. For instance, lipid A from the LPS of *Rhodobacter sphaeroides* is completely non-toxic. In contrast, gram-positive bacteria are better candidates for SCP production because they do not produce endotoxins; however, the amount of tryptophan present is lower than in gram-negative bacteria [3, 5, 6].

Spirulina is one type of cyanobacteria with about 50-70% protein. However, due to relatively low levels of the amino acids methionine, lysine, and cysteine, the amino acid composition of its protein is suboptimal. However, there are other beneficial compounds, such as pigments, lipids (containing omega-3 and omega-6 fatty acids), mineral salts, and vitamins like B12, B1, B2, B3, and E, in such an organism. The most available commercial cyanobacterial products are typically produced from such species as *Arthrospira platensis* and *Arthrospira maxima*, among others. These species are commonly exploited as sources of SCP for foodstuffs and dietary supplements, for feeds in aquaculture and poultry [1, 3]. One of the disadvantages of bacterial SCP is its high nucleic acid content, primarily RNA, which exceeds the limit acceptable for human consumption. Breakdown products of purine compounds derived from RNA tend to elevate blood uric acid levels, leading to kidney stones or gout. Therefore, it is recommended that microbial SCP be fed on poultry and finfish with a short life span because bacteria do not have any problem in the urea cycle. The nucleic acid level in the human diet must be lowered chemically, which increases the price of the end product [1]. One of the developments in bacterial SCP production is the creation of different substrates, such as wastewater and effluent treatment. For example, in China, a two-stage process was used with *Aspergillus niger* to break down potato starch residues, and then *Bacillus licheniformis* was used to produce single-cell protein for animal feed and wastewater treatment. Another example is that the *B. subtilis*-assisted fermentation of soybean hulls produces supplements of animal feed for monogastric animals. *B. subtilis* is a GRAS strain and also a Gram-positive bacterium with an obligatory aerobic environment. Its rapid growth, short fermentation cycle of usually 48 hours, high protein yield at 40-60% DCW, and the ability to use different substrates, such as agricultural and industrial by-products, make it a very

efficient candidate for SCP production [1].

## 2.2. Fungi

Fungi are diverse heterotrophic eukaryotic microorganisms, including unicellular yeasts and filamentous fungi. Single-cell protein (SCP) is primarily produced on a large scale using heterotrophic yeasts and fungi [3]. Major portion of the prepared SCP used in animal feed was by-product of food and beverage as well as bio-refinery industry because fungi acted like biocatalyst and produced the initial product first. The protein-enriched residues also offer a byproduct usable for animal feed. SCP from fungi includes essential amino acids (lysine and threonine levels high, but relatively low in methionine), B vitamins (thiamine, riboflavin, biotin, niacin, pantothenic acid, pyridoxine, choline, streptogenin), folic acid, lipids, and fiber (cell wall glucan and chitin) [2, 3]. In addition, fungal SCP can be used in the enhancement of quality and functional properties for products, such as texture and foaming capacity as well as emulsification. On the other hand, several SCP generated by yeasts have higher acceptability because of their potential usages in supplement addition in the feed sector. The one advantage during SCP production by yeast is their non-toxic characteristics. In addition, yeast can work at acidic pH and generally has high lysine content. Harvesting biomass is also easier because of their size, which is much bigger than that of bacteria. Even though interest has been generated by the potential of fungi as a meat substitute, mycotoxin production from species such as *Fusarium* and *Aspergillus* should be considered in the process of cultivation [3, 6]. Various fungal species have been utilized for the production of SCP, including *Nectaromyces*, *Pichia*, *Galactomyces*, *Kluyveromyces*, *Candida*, *Saccharomyces*, *Meyerozyma*, *Rhodotorula*, *Aspergillus*, *Fusarium*, *Aureobasidium*, *Neurospora*, and *Trichoderma*. In general, the most common ones are *Fusarium venenatum*, *Aspergillus oryzae*, and *Paezilomyces varioti* [2]. For example, the fungal protein produced by *F. venenatum* can be used to replace chicken breast in chicken nugget. The *A. oryzae* also can be used to make burgers [2, 6]. Quorn™ is one of the commercial meat alternatives that was exclusively developed for human consumption by Marlow Foods in 1985. This product is prepared from the filamentous fungus *F. venenatum* and a glucose substrate derived from starch, with mycoprotein containing about 45% protein and low fat. It is used to manufacture ready-to-eat sausages, salami, and burgers [3, 6]. Furthermore, *Yarrowia lipolytica* is a yeast that has been used extensively in industrial biotechnology because of its ability to utilize different carbon sources and produce lipids [3]. With optimum cellular protein fermentation, the protein content could be as high as 30 to 50%, but the application is restricted because of poor digestibility of the cell wall and a high nucleic acid content-up to 10%. The fungal cell wall contains proteinaceous elements like glucan, which is digestible by the gastrointestinal system. However, such elements may have allergenic potential on the

digestive system and certain skin areas [2]. In Finland, animal feed SCP was produced using *Paecilomyces varioti* filamentous fungi and lignocellulosic sugars from sulfite wastewater in paper mills. Besides sugar sources, yeasts and filamentous fungi also use carbon, methanol, and alkanes as substrates [1, 3]. British Petroleum cultivated the yeasts *Candida lipolytica* and *Candida tropicalis* on C12-C20 alkanes as substrates. The result of product - Toprina - was tested for toxicity and carcinogenicity for 12 years and then marketed as a replacement for fish meal in high-protein feeds and for dried milk powder in milk replacers. However, there are no operating petrochemical SCP factories. In the United States, Ameco grew the yeast *Torula* on ethanol as substrate. The final product, Torutein, which includes about 52% protein, is marketed as a flavor enhancer and alternative to meat, egg, and milk protein in several countries such as Sweden and Canada [7]. Fungal SCP is generally produced by submerged fermentation, but there is also a strong interest in solid-state fermentation because of its advantages, like lower energy use and less generation of wastewater [3].

### 2.3. Algae

Algae are unicellular organisms that grow individually, in groups, or in chains, and they vary in cell size [3]. Algae are capable of photosynthesis and convert small molecules, such as carbon dioxide or ammonium, into valuable macromolecules like proteins. Their applications can be valorized due to their capabilities for using atmospheric nitrogen, fixing carbon dioxide as the only carbon source, fast growth rate, protein content of 40-70%, and low nucleic acid levels (4-6%) [1, 2, 5]. Besides, algae composition includes mineral salts, pigments-chlorophyll, carotenoids-vitamins A, B, C, and E [1]. Due to the fact that a number of microalgae species, among which are *Arthrospira*, *Chlorella*, *Dunaliella*, *Haematococcus* and *Schizochytrium*, have been regarded by FDA as GRAS, their possible toxicity in food formulations must be considered seriously [2]. Large-scale production of SCP from algae in the initial stages needs significant investment due to the possible utilization of closed photobioreactors, preventing the growth of toxin-producing algae. In addition, bioaccumulation of heavy metals has been found to occur in algae, and cellulosic walls in certain types of algae may be hard to digest [5]. The major portion of these microbial cultivations is performed in simple, open-pond systems that are more or less susceptible to contamination and low productivity. Another challenge is the high light requirement of algae. High light can cause the generation of reactive oxygen species that can damage cells. Besides, not all algae can grow well under continuous light or unsuitable light cycle. The special flavor of algal products also limits their applications in human consumption. However, algae are used mainly as nutritional supplements; it provides protein combined with fatty acids, carotenoids, and vitamins for livestock and aquatic animals [3].

### 2.4. General Steps of SCP Production on an Industrial Scale

No matter what the microbial strain used for SCP production is or type of substrate - general steps involve the following: a; preparation of a suitable culture media, b; fermentation, c; separation and downstream processing and d) final processing of obtained SCP into components and products [1]. Depending upon the nature of the primary carbon source, the substrate may have to undergo preliminary physical or chemical treatment. Consequently, it undergoes hydrolysis prior to its mixing with the addition of necessary nutrients such as nitrogen, phosphorus, and other supplements. Biomass after fermentation is recovered from the spent culture medium through filtration or centrifugation. This protocol generally gives high production rates and yields by processing the biomass through purification, cell disruption, washing, and extraction of proteins. Further steps may be involved, depending on the substrates used for the preparation of SCP, prior to final formulation, such as the removal of nucleic acids or degradation of cell walls. These techniques have a profound impact on the quality and quantity of proteins and other derived compounds, however. Various methods include mechanical forces-mechanical grinding, ultrasonication, and high-pressure homogenization-of hydrolytic enzymes and chemical disruption using detergents that may be utilized in disrupting cell walls for some SCPs to render the proteins more available. On the other hand, SCP derived from the algae *Euglena* lacks a cell wall but instead possesses protein pellicles-a thin membrane that makes the SCP easily digestible. Besides that, the RNA content of the biomass produced is related to growth rate, growth conditions, and carbon-to-nitrogen ratio, among other factors, while its degradation rate depends on different factors. As it was underlined, such high levels of nucleic acids in SCP are not suitable for human consumption; thus, a processing step should be foreseen for its adjustment. For this purpose, various methods have been studied, including the use of ribonuclease enzymes, heat shock, alkaline hydrolysis, sodium chloride treatment, and mineral acid-alkali treatments. In general, high temperatures, around 72–74°C, for 30–45 minutes can thermally activate ribonucleases in microorganisms with minimal loss of biomass around 30–33% [2, 5, 8].

### 2.5. SCP Production Using Agricultural Waste and Various Substrates

Due to specific limitations of different substrates, SCP has been widely used in animal feed rather than human consumption so far. Fermentation media suitable for SCP production should be nontoxic, inexpensive, renewable, available, and non-seasonal. However, industrial effluents such as paper and pulp wastewater, oils, methanol, crude glycerol, and latex waste have also been used as unconventional substrates for SCP production. Various microorganisms may produce sin-

gle-cell proteins on such cheap media like molasses, starch-containing waste, milk by-products, fish flour factory waste, fruit wastes, or whole fruits-peel and pulp-glycerol wastewater from bio-fuel production plants and plant residues husks and bran. Waste from agriculture may occur as solid wastes: bread waste, discarded food, sugar residues; semisolid ones: for instance, olive pomace; or liquid: sugar-rich wastewaters, olive mill wastewaters, and whey. They can also be hydrophilic or hydrophobic [2, 4]. Substrate pretreatment or hydrolysis, in the case of starch, polysaccharides, proteins, and fats, is generally needed before their application in fermentation media for SCP production when compared to mono and disaccharide sources. As such, pretreatment should be done to convert cellulose into glucose or lignocellulose into fermentable sugars for use as substrates. In that respect, one of the most expensive stages could be the pretreatment itself. However, a good pretreatment method can enhance the activity of hydrolytic enzymes and limit the production of inhibitory compounds [2, 4].

### 2.5.1. Dairy Waste

The high content of organic matter, lactose, minerals, whey, fatty acids, oil, nitrogen and phosphorus compounds, high COD (Chemical Oxygen Demand) and (Biochemical Oxygen Demand) BOD-all make the dairy wastes very critical. Disposal of untreated waste directly into the local environment results in acute environmental problems. Depending on the processing technology used in milk production, dairy waste can be categorized as a rich source of mono- and disaccharides, protein, or fat [4, 9]. In recent years, studies have explored the use of dairy waste as a substrate for SCP production. Since about 50% of the total milk solids are present in whey, and lactose is the major component, finding microbial strains that can utilize lactose for the production of SCP is the need of the hour. *Kluyveromyces marxianus* and *Candida krusei* co-cultured on whey substrate demonstrated 8.8% higher COD removal efficiency, an improved productivity by 33%, and an increased biomass yield by 19% over single cultures. Also, the maximum SCP yield produced was reported to be 43.4 percent [2].

### 2.5.2. Sugars

Molasses is a thick syrup, rich in carbohydrates and containing sucrose (30–35%), reducing sugars, i.e., glucose and fructose (10–25%), non-sugar constituents (2–3%), besides minerals, obtained from sugarcane or sugar beet. Molasses generally remains one of the most usable substrates for SCP production by fermentation but may be treated to remove suspended solids as well as minerals. Recently, soybean molasses also became available as a by-product of the manufacture of soy protein concentrate. Adding this molasses has already shown to increase the production rates of SCP [2, 4, 7]. This fed-batch method in order to enhance the production of SCP used a substrate with molasses and vinasse 50:50. Yeast strains tested are *Saccharomyces cerevisiae* CCMA 0186 and

CCMA 0188, *Candida parapsilosis* CCMA 0544, *C. glabrata* CCMA 0193, *Meyerozyma caribbica* CCMA 0198. Results indicate *C. parapsilosis* as the best performing yeast in producing 8.8 g/l of biomass. Another study using *Rhodospseudomonas faecalis* investigated various sugar processing wastewater environments. It was found that anaerobic pond effluent, containing more than 50% protein and rich in essential amino acids, has significant potential for SCP production [2]. Additionally, the potential of five edible filamentous fungal species, including *Aspergillus oryzae*, *Neurospora intermedia*, *Rhizopus oryzae*, *Monascus purpureus*, and *Fusarium venenatum*, was evaluated using vinasse as a substrate. High protein contents of 44.7%, 57.6%, and 50.9% were obtained for *A. oryzae*, *N. intermedia*, and *R. oryzae*, respectively, showing that biomass of the three fungal species is an excellent protein supplement in aquaculture feed [9, 10].

### 2.5.3. Starch-Based Waste

Starch is an inexpensive substrate derived from corn, rice, cereals, root crop residues, cassava, and potatoes. It requires hydrolysis to proceed, but with the help of enzymes from yeast and mold, it can be broken down into simple fermentable sugars. However, the use of mixed cultures of amylolytic microorganisms and SCP-producing microorganisms can facilitate a decrease in consumption cost because amylolytic microbes are allowed to digest starch and produce sugar needed for SCP synthesis. This method still maintains fermentation at a lower cost. A Swedish study fermented starch waste with a mixed culture of *Endomycopsis fibuligira*-an amylase producer-and *Candida utilis*-a fast-growing yeast [4, 7]. The environmental challenge is represented by the considerable amount of waste arising from potato starch processing. Due to the high water content within potato pulp, it has limited nutritional value for being utilized in animal feed. In one study, four microorganisms were cultured on a mixed substrate of potato residue and wastewater without additional nitrogen sources: *Curcubacter*, *Pseudoalteromonas*, *Pae-nibacillus*, and *Bacillus*. The resulting SCP products contained 46.09% protein [6].

### 2.5.4. Fruit Waste

Fruit processing waste composition is dependent on the fruit type and the fraction making up the major proportion of the waste. For instance, if whole fruits are contained in the wastes, they would contain a high proportion of mono- and disaccharides, whereas wastes generated from juice production, which are made up mainly of outer peels, skins, and seeds, can be regarded as a source of structural polysaccharides. In general, fruit and vegetable wastes are suitable as a carbon source for SCP production due to their high monomeric sugar content, such as glucose and fructose, biodegradable nature, and low lignin content. Wastes of orange peels, pineapple peels, potato peels, banana peels, and carrot pulp contain high amounts of sugars, ranging from 17.54% to 83%, which enhances SCP yield. As these sources require less

energy-intensive pretreatment than those rich in lignin, they reduce the processing costs by as high as 30-50% [4, 11]. Five fungal strains, namely *Trichoderma reesei* ATCC 13631, *Fusarium venenatum* ATCC 20334, *Thermomyces lanuginosus* ATCC 34626, *Aspergillus oryzae* ATCC 14895 and *Fusarium graminearum* ATCC 20333, were compared in a research on date waste collected from the date syrup industry under optimized production conditions. *A. oryzae* was best among these five strains since it produced the greatest amount of protein. This fungus produced an essential amino acid ratio to total protein of 46%, hence good nutritional value [11, 12]. In another study, wastes from various fruits were evaluated as a substrate for SCP production using *S. cerevisiae*. The results indicated that the maximum yield of SCP and biomass production was 48.3% and 0.4 grams, respectively, when pineapple waste was used as a fermentation feedstock. In another study, the RSM (Response Surface Methodology) was applied for the optimization of conditions to enhance SCP production from banana peels by *A. niger* through submerged fermentation. The highest biomass and SCP yield obtained were 24.7 g/L and 61.2% (w/w), respectively [2]. In one such study, banana peels were used as a substrate for SCP production by *Saccharomyces cerevisiae* Y1536 and *Rhizopus oryzae* FNCC 6157 in production utilized as fish feed [7]. Cucumber peels and orange peels were also tested as substrates for SCP production from *Saccharomyces cerevisiae* via submerged fermentation. It yielded that cucumber peels produced a higher yield in protein content, 53.4% from 100 grams substrate, as compared to that of orange peels [7].

### 2.5.5. Bakery Industry Waste

Utilizing bread waste as a carbon source and the fungus *Rhizopus delemar*, one study was able to obtain a protein content that ranged from 27% to 36% [11].

### 2.5.6. By-products of Livestock and Fisheries

The problem of poultry waste disposal has constantly existed, and the direct application without the removal of pathogens could be dangerous for public health. Compared with other animal wastes, poultry wastes have a higher content of micro- and macro-elements and also a high amount of organic compounds; hence, SCP production seems to be a very promising method for processing such wastes. Poultry waste is generally rich in nitrogenous compounds and fiber, for which proper pretreatment is indicated to remove potential pathogens and hydrolyze the polysaccharides. Microorganisms such as *Candida*, *Saccharomyces*, and *Rhodotorula* spp. could be employed for the conversion of nitrogen-rich material into SCP [4]. Horns, feathers, nails, and hair are some of the wastes generated in great amounts from slaughterhouses that contain a good amount of fiber and protein sources. These can be processed into protein or amino acid concentrates by using appropriate proteolytic microorganisms. For example, ram horns containing cysteine and other amino acids can be

used as a substrate to give high protein concentration in biomass. In a batch system at 30 °C, with *Bacillus cereus* NRRL B-3711, *Bacillus subtilis* NRRL NRS-744, and *Escherichia coli* in ram horns, the biomass arising from the process exhibited a high total protein content with essential amino acids good for ruminant feed. One study produced SCP from fish-processing wastewater with the help of *Lactobacillus acidophilus* and *Aspergillus niger*. The biomass yield of the biomass in this work obtained from *L. acidophilus* was 7.29 g/L, and for *A. niger* was 5.20 g/L. Other waste reviewed for raw materials included shrimp shell waste and undigested poultry litter in a different study; the results were indicative that SCP products can act as supplements in animal feeds [6].

### 2.5.7. Plant-Based Waste

Plant-based wastes, including wheat bran, barley bran, and other cereal residues, are composed of cellulose, hemicellulose, and lignin. These chemicals require a pretreatment or enzymatic hydrolysis process to make the monosaccharides available for microbial consumption. In one study, *Candida utilis* and *Rhizopus oligosporus* were applied on wheat bran. The maximum SCP yield, after optimization of the fermentation parameters, reached 41%. In another, the residues of rye straw, rye bran, and oat bran were used as substrates with the yeast *Y. lipolytica*. The maximum biomass produced from oat bran contained 30.5–44.5% protein (dry weight) [2, 13]. The impact of composite kinds of waste as substrates for the production of SCP has also been investigated. In one study, co-fermentation of whey wastewater with vinasse was conducted by using the filamentous fungus *Neurospora intermedia*. The obtained yield was 45% SCP. The amino acid content was comparable to commercially available protein feed sources for aquafeed formulation. In another study, the microorganisms selected were *S. cerevisiae*, *K. marxianus*, and kefir, grown in a mixed substrate of orange peel, potato peel, molasses, whey, spent brewery grains, and spent malt roots under Solid-State Fermentation (SSF). Among the analyses performed, the fermented substrate with *K. marxianus* yielded the highest protein content value (59.2% w/w) and, moreover, an elevated fat concentration, making this suitable for animal feed enrichment [2, 14].

### 2.5.8. Agricultural Waste Rich in Structural Polysaccharides

One of the most available wastes is lignocellulosic agricultural waste. It consists of 30-56% cellulose, 3-30% lignin, 10-24% hemicellulose, and 3-7.2% protein. However, due to its very low digestibility and low content of protein, its usage in animal feed is limited. Besides, the use of these wastes as a substrate for SCP production is much more complex than that of starch and simple sugars. The necessity of mechanical, chemical, or biochemical pretreatment significantly inflates the costs of SCP production [4].

### 3. Conclusions

Single-cell protein production by microorganisms is a novel approach to present sources of protein in an efficient and cost-effective manner. At present, microbial proteins contribute very little to human nutrition, but this increasing demand in human food generally for proteins has led to increased research interests in the production of SCPs. Besides, SCPs can also replace some of the conventional protein feeds like fishmeal and soybean meal for animals. However, one of the industrial challenges to the production of SCP is obtaining a sustainable, protein-rich, renewable source. In order to help reduce costs, different kinds of agriculture and industry wastes like molasses, whey and agricultural and food residues are being used as low-priced renewable substrates for the production of SCP. This will address the environmental problems brought about by the accumulation of waste. By valorizing renewable raw materials, food waste can be managed, and the issue of protein shortages in society can be alleviated. Selecting suitable and efficient microbial strains is a key challenge in enhancing SCP production, as it depends on cultivation and processing conditions. Moreover, optimizing culture processes, such as employing mixed culture media, can significantly improve SCP production efficiency. The suitability of microorganisms, especially fungi and yeasts, for SCP production has been considered due to their most rapid growth without toxicity to humans and animals. Although SCP production through yeast utilization has been performed over a very long period, among various microorganisms, methane-oxidizing bacteria are considered the most advanced and prepared bacteria in SCP production. A high growth rate or the ability to use substrates such as CO<sub>2</sub> or methane leads to processes far more efficient or sustainable than traditional agriculture. However, in the SCP production industry, issues such as high nucleic acid percentage compared to animal and plant protein sources, presence of anti-nutritional factors, toxic substances in carbon substrates like heavy metals, persistent organic pollutants, and pathogenic microorganisms have to be considered and have to be carefully evaluated.

### Abbreviations

SCP	Single-Cell Protein
NPN	Non-protein nitrogen
COD	Chemical Oxygen Demand
BOD	Biochemical Oxygen Demand
GRAS	Generally Recognized as Safe
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
DCW	Dry Cell Weight
RSM	Response Surface Methodology
SSF	Solid-State Fermentation
FDA	Food and Drug Administration
PHB	Poly-beta-hydroxybutyrate

LPS      Lipopolysaccharides

### Author Contributions

**Abbas Abedfar:** Conceptualization, Supervision

**Fatemeh Abbaszadeh:** Conceptualization, Supervision

**Fatemeh Mardiha:** Investigation, Methodology, Writing—original draft

### Conflicts of Interest

The authors declare no conflicts of interest.

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