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Published in:
Food Research International

Link to article, DOI:
10.1016/j.foodres.2023.112596

Publication date:
2023

Document Version
Peer reviewed version

Citation (APA):
Cardoso Alves, S., Díaz-Ruiz, E., Lisboa, B., Sharma, M., Mussatto, S. I., Kumar Thakur, V., Kalaskar, D. M., Gupta, V. K., & Chandel, A. K. (2023). Microbial meat: A sustainable vegan protein source produced from agri-waste to feed the world. *Food Research International*, *166*, Article 112596. https://doi.org/10.1016/j.foodres.2023.112596

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PII: S0963-9969(23)00141-2
DOI: https://doi.org/10.1016/j.foodres.2023.112596
Reference: FRIN 112596

To appear in: *Food Research International*

Received Date: 6 November 2022
Revised Date: 27 January 2023
Accepted Date: 14 February 2023

Please cite this article as: Cardoso Alves, S., Díaz-Ruiz, E., Lisboa, B., Sharma, M., Mussatto, S.I., Kumar Thakur, V., Kalaskar, D.M., Gupta, V.K., Chandel, A.K., Microbial meat: A sustainable vegan protein source produced from agri-waste to feed the world, *Food Research International* (2023), doi: https://doi.org/10.1016/j.foodres.2023.112596

Microbial meat: A sustainable vegan protein source produced from agri-waste to feed the world

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Abstract

In the modern world, animal and plant protein may not meet the sustainability criteria due to their high need for arable land and potable water consumption, among other practices. Considering the growing population and food shortage, finding alternative protein sources for human consumption is an urgent issue that needs to be solved, especially in developing countries.

In this context, microbial bioconversion of valuable materials in nutritious microbial cells represent a sustainable alternative to the food chain. Microbial protein, also known as single-cell protein (SCP), consist of algae biomass, fungi or bacteria that are currently used as food source for both humans and animals. Besides contributing as a sustainable source of protein to feed the world, producing SCP, is important to reduce waste disposal problems and production costs meeting the sustainable development goals. However, for microbial protein as feed or food to become an important and sustainable alternative, addressing the challenges of raising awareness and achieving wider public regulatory acceptance is real and must be addressed with care and convenience. In this work, we critically reviewed the potential technologies for microbial protein production, its benefits, safety, and limitations associated with its uses, and perspectives for broader large-scale implementation. We argue that the information documented in this manuscript will assist in developing microbial meat as a major protein source for the vegan world.

Keywords: *Single-cell protein, Microbial meat, Protein production, Sustainability*

1. Introduction

The world population, which currently surpassed 8.0 billion inhabitants, is growing exponentially and is expected to reach around 9.2 billion people by 2040. This rapid population growth has led to global food insecurity. Furthermore, the COVID-19 pandemic has increased the risk of food insecurity and global hunger. According to the United Nations, hunger is about to reach millions of families worldwide due to factors such as geo-political conflicts, population rise, climate extremes, and the COVID-19. With this scenario, the agricultural sector needs a tremendous transformation to satisfy the growing global demand for food (UN, 2019; FAO, 2021; GLOBAL TRENDS, 2021).

The expansion of food production and the intensification of agriculture have caused a high cost to the environment, contributing 31% of global anthropogenic GHG emissions and being responsible for strong changes in the composition and biodiversity of natural ecosystems, such as soil erosion, acid rain, eutrophication, and climate change. Thus, international organizations, industries, governments and society have been called upon to provide generalized responses to prevent the global food crisis (Hashempour-Baltork et al., 2020; Kusmayadi et al., 2021; Tubiello et al., 2022). Nearly a billion people worldwide cannot afford food that contains enough protein and calories required for their health. The lack of necessary protein sources causes serious health problems such as muscle weakness, defective immune system, and growth deficiency (Berners-Lee et al., 2018). On the other hand, high consumption of meat products can cause health problems and seriously affect the environment/climate. Therefore, it is crucial for the food businesses to provide viable alternatives to animal proteins, particularly those derived from meat, into the market that are less expensive and consume less natural resources. (Bonny et al., 2015; Hartmann & Siegrist, 2017; Prosekov & Ivanova, 2018).

Converting waste into valuable food or feed for humans and animals is not only an environmentally friendly activity but also a healthy business work (Jurasz et al., 2018; Sharif et al., 2021). Currently, large part of waste pollutes the environment or is processed into low value-added products such as biofuels and biogas. However, high-quality products such as single cell oil, single cell proteins, chemicals, and enzymes, among others, can also be obtained from wastes, and different production methods are being developed for that.

Microbial meat is one of the most relevant high-quality diet products that can be obtained from agri-waste resources (El-Bakry et al., 2015; Finco et al., 2017; van der Spiegel et al., 2013). SCP is a protein of microbial origin produced from a pure or mixed culture of bacteria, fungi, yeasts or microalgae (Hashempour-Baltork et al., 2020). SCPs are dry cells that can be used as protein supplements or as protein-rich ingredients in human and animal diets, providing interesting benefits from a nutritional point of view (Anupama & Ravindra, 2000; Geada et al., 2021). Moreover, from an environmental perspective, SCP does not require a large area of land or large reservoirs of water for its production, making it an excellent alternative to vegetable protein sources. Its production also does not emit greenhouse gases into the environment as animal protein sources do. Furthermore, the production of SCP is independent of seasonal and climatic variations and can be carried out throughout the year (Mekonnen & Hoekstra, 2014; Miller et al., 2019; Sharif et al., 2021). Cultured meat is quite different from microbial protein as it is produced by cultivating animal cells in reactors and considered as pure animial meat (Choudharay et al., 2020). While microbial protein seems a viable protein source for vegan population, cultured meat is considered an ideal protein for non-vegeterians.

The selection of cheap and suitable substrates or biodegradable agro-industrial by-products as a source of nutrients for microorganisms to grow and produce proteins is of fundamental importance to allow an incredible growth of the microorganism at a reduced production cost (Pogaku et al., 2009; Ravindra et al., 2009). In this sense, apple pomace, yam skins, potato skins, citrus pulp, pineapple residues, and papaya residues are some of the substrates used as a nutrition source in microbial cultivation (Diwan et al., 2018; Spalvins et al., 2019).

Microorganisms (algae and molds, 2-6h; bacteria and yeasts, 0.33-2h) generate protein more efficiently than any animal or plant (1-2 years and a few months, respectively). In this way, the production of protein biomass has several advantages in relation to livestock and conventional crops. Furthermore, the microorganisms have a high protein rate based on dry mass (30-80%, depending on the applied microorganism) and the protein has good nutritional value. In addition, a wide variety of raw materials can be used as a substrate in the SCP, including low-value agro-industrial waste and by-products. Relatively small land areas can be used to carry out continuous fermentation processes to cultivate microbial proteins in large quantities. The generation of SCP does not depend on seasonal, weather and weather variations. In addition, microorganisms are more easily genetically modified than plants and animals. In addition, the SCP presents the essential amino acid requirements for human nutrition (Octasylva & Rurianto, 2020; Anupong et al., 2022).

Single-cell proteins will contribute to the greater popularization and wider availability of protein sources in foods. The greatest growth and demand for vegan meat was found in Europe. The growth rate of meat analogues is projected at 7.1% in 2025, growing sharply to 73% by 2050. European countries (51.5%), North America (26.8%), Asia-Pacific (11.8%), Latin America (6.3%), and the Middle East and Africa (3.6%) have the highest share of the global market for plant-based meat analogues (Kumar et al., 2022; Sheth & Patel, 2023).

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2. Single Cell Protein (SCP)

Microbial protein is referred to as single-cell protein, although some of the producing microorganisms are multicellular, such as filamentous fungi or filamentous algae. In 1968, the term SCP was introduced for the first time, when scientists gathered to know the most appropriate terminologies in common practice, i.e. microbial protein at the Massachusetts Institute of Technology, United States (Matassa et al., 2020; Shharif et al., 2021). Microorganisms help with protein deficiencies when used to increase the amount of protein and improve the quality of fermented feeds (Bratosin et al., 2021).

The increase in the global search for protein will certainly make SCP more and more interesting, although protein of microbial origin has a low proportion of current human nutrition. The high speed of growth or the ability to apply substrates such as CO2 and methane, as carbon sources, makes the processes more efficient and sustainable compared to those employed in traditional agriculture (Balagurunathan et al., 2022; Yang et al., 2022).

Currently, SCP can be produced by a limited number of microbial species, especially when human demand is taken into account. The diversity of SCP sources applied in animal feed is greater than that certified for human consumption and is expanding (Thiviva et al., 2022). According to what will be brought forward, products derived from fungi, algae and bacteria are under development or being used. Typically, production processes proceed first with the preparation of the nutrient medium, then with the cultivation, then with the separation and concentration of the SCP, in certain cases drying, and finally the final processing of the SCP into ingredients and products (Jones et al., 2020; Nyyssölä et al., 2022).

High food grade substrates are generally used to produce SCP for human consumption. However, there is belief in the development of processes to produce SCP from cheap waste from the food and beverage processing industries, as well as from agricultural and forestry sources. The SCP is composed of a high protein content, which varies between 60 to 82% based on dry matter, in addition, carbohydrates, nucleic acids, vitamins, minerals and fats are also part of its composition. Another benefit related to SCP is that it is rich in several essential amino acids, such as methionine, lysine, which are not present in adequate proportions in most animal and plant sources (Al-Mudhafr, 2019; Zha et al., 2021; Khan et al., 2022).

2. Microorganisms as a protein source

Different microorganisms, including microalgae, fungi, yeasts and bacteria, can be used as single cell proteins for food and feed applications due to their protein-rich composition. **Table 1** summarizes some examples of microorganisms used as SCP and their protein content. More details on their relevance as a source of protein and their production and utilization are discussed in the following sections.

Table 1

2.1. Microalgae

The consumption of algae dates thousands of years across different cultures. However, new developments are still needed today to boost the use of microalgae as a mainstream food option. Development of improved organoleptic traits, evaluation and increase of nutritional content, development of large-scale production units and also optimization of yields are some of the challenges for microalgae to be seen as a more common food source (Mourelle et al., 2017; Torres-Tiji et al., 2020).

Microalgae have several features that make them attractive for large-scale production, food, and feed applications. These features include high biomass yields per unit area, the ability to grow on non-arable land, and the possibility of using non-potable water and even salt water for its cultivation. Nevertheless, the scale up of the appropriate technologies and efficient management of precision fermentation parameters and investments are necessary to develop new microalgae-based products. First is selecting adequate species, which can be done by using bioprospecting methods and searching from established Generally Recognized as Safe (GRAS) species. The GRAS certification is needed for a new species, which is costly and time-consuming (FDA, 2017).

After the strain selection, it may be necessary to carry out genetic improvements to the strain to enhance the desired traits, such as the yield, organoleptic trait or nutritional content. Related to yield, high productivity, resistance factors and adaptation to outdoor growth are examples of characteristics to be improved. Regarding the organoleptic traits, taste, aroma, texture, palatability, color and appearance are some of the traits that can be improved. Finally, in terms of nutritional content, the protein content and amino acid profile, the lipid content and profile, and the aggregation of other nutritional molecules can be improved (Anderson et al., 2017).

Genetic improvements in microalgae can be done by random DNA alteration, UV mutagenesis, mating and genome shuffling, but these processes can be labor-intensive and time-consuming. Controlled DNA manipulation can deliver faster and more precise results using techniques like targeted mutagenesis, synthetic genetic tools and recombinant protein expression systems. Finally, to obtain a high yield during the cultivation of the final species, it is necessary to work on bioprocess development, including medium optimization, growth systems adaptation, and developing a robust and cheap downstream process (Torres-Tiji et al., 2020).

Microalgae constitute a large market today since derived products like alginates and carrageenans are widely used in several industrial sectors, but specifically related to food and feed applications, there is still no precise market defined. Algae has several components of value for human nutrition, like, omega-3 fatty acids: DHA and EPA, natural pigments (beta-carotene and astaxanthin) and glucans. Algal biomass can also be used as a nutritional complement (Gong & Bassi, 2016).

2.2. Fungi and yeast

Like algae, fungi are also not new to human diets. Mycoproteins, more specifically, were first discovered in the early 1960's (Derbyshire & Delange, 2021). Since then, many studies have been done to assess the safety and benefits of this type of proteins. When talking about fungi protein, this type of food includes the fruiting bodies of edible mushrooms, as well as several species of micro fungi such as molds and yeasts, and their derivatives. Recently, research has been focused on the production and characterization of vegetative mycelia from fungi to increase its protein content and further processing to obtain meat alternatives for human consumption (Schweiggert-Weisz et al., 2020).

There are a number of advantages of using fungi as a food source, primarily the low land requirements since they can grow in bioreactors with high metabolic rates, which avoids the extensive use of land needed for growing and feeding animals for meat. Production of mycoproteins in bioreactors is generally done based on submerged fermentation, with fungi growing in liquid media containing its nutritional requirements. Another advantage of using fungi as a food source is that their single-cell proteins can provide other nutrients to the human diet, including different B-complex vitamins (Sharif et al., 2021). However, there are still some challenges to be overcome to allow broader use of mycoprotein as a food source, mainly related to the production costs. Further research is also needed to evaluate safety issues and also to spread its benefits to the public (Schweiggert-Weisz et al., 2020). Recently, metabolic engineering technologies have been proposed to modify microorganisms to obtain, for example, an improved utilization of agro-industrial residues with simultaneous production of SCP (Hülsen et al., 2018). However, the use of genetically modified organisms (GMO) does not have public acceptance and is still a topic of discussion (Sharif et al., 2021).

Filamentous fungi are the preferred choice in SCP production at large scale. The fungus is grown in a synthetic medium and further mixed with egg albumin and other compounds to confer color and flavor, mimicking meat. In addition, using filamentous fungi has an advantage over plant-derived meat substitutes, as fungi produces filaments comparable to meat fibrils, conferring a similar meat texture to the product (Gmoser et al., 2020).

Yeasts, however, have been in the market for a long time. The production of SCP using yeasts had an expressive significance in the war times. During the First World War, Germany managed to substitute almost half of its imported protein sources by yeast. Initially, they used brewer's yeast, but it was not enough to meet the demand as a protein source. In the beginning of the Second World War, yeasts were used as a protein source in both army and civilian diets (Ugalde & Castrillo, 2002). Today, yeasts are often used as supplements in animal feed and in vegetarian diets. Fungi, including the yeasts market, is the second largest single-cell protein market after algae. Most of the SCP is still destined for the animal feed market, but human consumption has been growing in recent years.

It is worth noting that yeasts have various benefits over bacteria in their manufacturing process, for example, they are larger than bacteria (cell size), and harvesting them from culture media is easy. Yeasts also have higher lysine and malic acid contents, although their protein content is usually lower, and they also have longer doubled times compared to bacteria (Raziq, 2020).

2.3. Bacteria

Bacteria have also been used as SCP for a long time, mainly for animal feed. Single-cell protein derived from bacteria usually contains between 50 and 80% of protein (dry basis), and the amino acid content is higher or similar to the FAO recommendations. Like fungi, bacteria also have a high nucleic acid content, requiring previous processing (Strong et al., 2015). On the other hand, bacteria have some advantages regarding the production process, such as faster growth and shorter generation time when compared to fungi and yeasts. Additionally, they can grow on several types of substrates, even in gaseous ones like hydrocarbons (Anupama & Ravindra, 2000; Mussatto et al., 2021). In fact, using gases like CO_2, or diverse raw materials, mainly waste/side streams from other industries, for bacteria cultivation may be appealing from the perspectives of cost and sustainability. However, they are more difficult to harvest from the culture medium due to their smaller size, requiring multiple unit operations for their recovery. Some bacteria also have complex nutritional requirements (Nasseri et al., 2011).

For the selection of new strains for large-scale production of SCP, multiple criteria must be considered, including the complexity of nutrients requirements, fermentation performance, genetic stability during the cultivation process and growth morphology, the composition of the final product generated by each strain in terms of protein and other components, and the complexity of the downstream process required for purification (Raziq, 2020). A significant concern related to the utilization of bacteria as SCP is the possibility of producing toxins, which can be extracellular (exotoxins) or intracellular (endotoxins). Toxins may cause adverse effects in both animals and humans. Therefore, toxins' production must be carefully assessed to avoid problems with regulatory bodies (Ritala et al., 2017).

3. Composition and safety issues of SCP obtained from different microorganisms

Table 2 shows the average composition of SCP obtained from algae, fungi/yeasts and bacteria, focusing on their nutritional value. Some important parameters/limitations to be considered for the SCP application in human and animal nutrition are also presented.

The acceptance and interest of a particular species for food or feed application greatly depend on its composition, growth rate, and associated toxin production. SCP for human consumption or animal feed must be free from all kinds of pathogens, toxins, and contaminants (heavy metals or other metal compounds, hydrocarbons). In addition, they should not cause food allergies, skin reactions, gastrointestinal reactions, diarrhea, vomiting, and other diseases (Ugbogu & Ugbogu, 2016). Therefore, it is essential to use toxicological studies to evaluate the safety of any produced SCP before marketing the products.

The main anti-nutritional factor in SCP is the presence of a high concentration of nucleic acids, which is usually more abundant in microbial proteins than in other conventional protein sources. This is one of the main factors limiting the SCP application in the food sector (Nalage et al., 2015). Most nitrogen in SCP is in the form of amino acids, while the rest is in the form of nucleic acids, which is a key property of fast-growing microorganisms. High nucleic acid content is a problem because purine compounds derived from RNA break down and increase uric acid concentration in the serum, ultimately leading to kidney stones and gout formation. It has also been reported that living cells of microbes should be inactive before consumption. Using an unprocessed product before killing the active microbes increases the incidence of skin and gastrointestinal infections that can cause nausea and vomiting (Sharif et al., 2021). Anti-nutritional factors of SCP, like an elevated presence of nucleic acids, can be eliminated by applying physical and/or chemical treatments during processing (Dantas et al., 2016). Different techniques for nucleic acid reduction have been proposed to make SCP suitable for food applications. Chemical (sodium chloride, ammonium hydroxide, sodium hydroxide) and enzymatic (ribonuclease, deoxyribonuclease) treatments can be used to treat biomass, obtaining nucleic acid concentrations below 2% (w/w) (Yadav et al., 2016).

Table 2

Certain microorganisms can also produce toxic substances such as mycotoxins and endotoxins during the production of SCP (Sharif et al., 2021). In addition, some carcinogenic substances can be produced when microbes undergo mutations during the processing and formation of the final product, which can be toxic to both humans and animals. However, all these problems can be avoided by carefully selecting the microorganism and optimizing the fermentation protocol for the production of SCP. The use of an appropriate substrate is also useful to obtain SCP more beneficial to health. Recently, bacterial SCP obtained by culturing bacteria in methanol as a carbon source was evaluated for mutagenicity in five in vivo tests in various mammalian test systems, and the results showed no evidence of mutagenic activity due to the substrate utilized for cultivation (Mahan et al., 2018; Spalvins et al., 2018).

Mycotoxin-generating fungi are undesirable sources of SCP as their toxins can cause allergic reactions, carcinogenesis and even death in humans and animals. The fungus species *Aspergillus flavus*, for example, produces aflatoxins of the B1, B2, G1 and G2 types, *Penicillium citrinum* can produce citrine, trichothecenes and zearalenone, while *Fusarium* and *Claviceps* species produce ergotamine. There is epidemiological evidence linking aflatoxins to human liver cancer (Maiuolo et al., 2016). Recently, molecular biology techniques have been explored to eliminate genes linked to mycotoxin synthesis. To isolate *A. parasiticus* and *A. flavus* aflatoxin pathway clusters, the techniques of probing, cloning, expression libraries, transcript mapping, and gene disruption have been applied. As an example, the aflR regulatory gene, which controls the production of aflatoxins in *Aspergillus*, can become a target for controlling the production of mycotoxins in this species. Although research in this field is still starting (Dubey et al., 2018; Xu et al., 2021), reliable and easily applicable techniques can be expected in the near future. Some species of bacteria can also produce toxins, which limit their use as SCP. *Methylomonas methanica* and *Pseudomonas* species, for example, produce endotoxins that cause febrile reactions. However, heating can destroy these toxins (Mahan et al., 2018; Ravindra et al., 2009).

According to the composition and potential limitations (**Table 2**), algal SCP has greater safety in terms of nucleic acid content and no toxin production compared to fungi and bacteria. In this way, the order of preference for food and feed application could be proposed as algae > fungi > bacteria (Anupama & Ravindra, 2000; Nasseri et al., 2011). However, this is a very general classification criteria, and studies should be done on each microorganism of interest to elucidate its potential to be used as SCP in food and feed applications.

4. Technology for SCP production

SCP can be produced by submerged, semi-solid, or solid-state fermentation. The process for SCP production follows the steps shown in **Figure 1**. The first step consists of a screening of potential microbial strains. This step is essential for an adequate selection of microorganisms capable of producing a good amount of protein. Microbial strains can be isolated from different habitats such as water, air, soil or other biological materials. The best strain can also be optimized if necessary by mutation, selection and/or genetic protocols. The next step is the choice of raw materials to be used for the bacterium cultivation, which is necessary to obtain an appropriate composition of carbon, phosphorus, and nitrogen able to favor a high biomass formation in a short time. The most desirable carbon sources are those containing monosaccharides and disaccharides, as they are ready to use. The third step involves process engineering and process optimization. At this stage, the best growing conditions for the selected strain are determined and the metabolic pathways are elucidated (Nasseri et al., 2011; Ritala et al., 2017; Singh et al., 2019; Ukaegbu-Obi, 2016). Then, the next step is developing the technology, which consists of defining all the technical details and performance of the process to make the production robust for large-scale application. Studies of economic factors make up the next stage, where energy consumption and production costs are considered for the implementation of the large-scale production process. Such analysis can also be done in parallel or integrated with the technology development in the fourth step. Finally, attention should be given to safety and environmental protection requirements. Since the single-cell protein will be used for human or animal food, the product must have high safety, as some microorganisms can also produce toxins that cause side effects to humans as well as to the environment. In this way, the entire process must be properly monitored. Product authorizations for particular applications and legal protection of innovative processes and strains of microorganisms, namely exploration licenses, are the legal and controlled aspects that the innovation requires (Ritala et al., 2017; Singh et al., 2019; Ukaegbu-Obi, 2016).

Figure 1

Process optimization for fermentation is a very important step in the processing of single cell protein production since it should be able to result in high product yield. In this sense, an important aspect to consider is the medium's composition to be used for fermentation, which must contain all the nutrients necessary for appropriate growth of the microorganism (Bellamy, 2009; Kadim et al., 2015). For fungi cultivation, for example, the fermentation medium must include nutrients such as potassium, phosphorus, magnesium, trace elements, ammonium salts and vitamins (like biotin), which are needed to develop mycelia (Gervasi et al., 2018). In addition, the fermentation process requires a pure culture of the chosen organism, sterilization of the growth medium, and a fermenter operated under suitable conditions to favor the microbial growth (Nasseri et al., 2011). SCP can be produced by solid-state, semi-solid or submerged fermentation. From these options, the submerged system usually results in higher production efficiency (Hashempour-Baltork et al., 2020; Suman et al., 2015). At the end of the fermentation, the resulting biomass is collected by filtration or centrifugation and goes through washing and drying steps to obtain the SCP (**Figure 2**).

Figure 2

Submerged fermentations are usually more expensive, leading to higher operational costs than semi-solid or solid-state fermentations, giving lower protein yields. An alternative to reduce the costs of submerged fermentations is to use food wastes, which have low value or no value at all, to formulate the fermentation medium. Food wastes and a variety of cheap raw materials, including agricultural wastes, have already been tested as substrates for the production of SCP by different microorganisms, including orange peel, sugarcane bagasse, rice husk, wheat straw, cassava residues, sawdust, corn cobs, coconut residues, mandarin residues, beet pulp, among others (Bellamy, 2009; Diwan et al., 2018; Reihani & Khosravi-Darani, 2019; Ritala et al., 2017). Of course, the SCP production yields vary according to the substrate and microorganism used. The use of banana peel as a substrate for the production of *Aspergillus niger* biomass, for example, gave better yields (biomass yield of 2.29 and protein yield of 0.57 g/L) than crop wastes like cucumber, orange, and pineapple waste (Oshoma & Eguakun-Owie, 2018). Also, the use of pea processing by-products resulted in the production of *A. oryzae* var. *oryzae* CBS 819.72 mycobiomass with 38% more protein compared to the mycobiomass obtained from synthetic medium (Souza Filho et al., 2018). Vinasse, the final residue obtained from the production of bioethanol, can also be used for the production of SCP. *Candida parapsilosis* was successfully cultivated in a medium containing 5 g/L of peptone and 70% v/v of vinasse (dos Reis et al., 2019). The bioconversion of cheese whey is another appealing method for SCP production since lactose can be used as a carbon source by different microorganisms, including yeasts of the genus *Kluyveromyces* (Coelho Sampaio et al., 2016; Dragone et al., 2009). It is worth noting that the use of lignocellulosic materials, particularly, as substrates for the production of SCP, is hindered by the complex structure of these materials, which is mainly composed of cellulose, hemicellulose and lignin fractions. Thus, pre-treatment methods have become essential to break the resistant lignin layer, reducing the crystallinity of cellulose and increasing the availability of carbohydrates to be consumed by microorganisms (Mussatto & Dragone, 2016; Sun et al., 2015; H. Zhang et al., 2021).

Tropea et al., (2022) utilized mixed food waste (fish, pineapple, banan, apple, citrus peel etc.) as a substrate to produce SCP by using *Saccharomyces cerevisiae* and observed the that the final protein concentration reached uo to 40.19% after 120 h of fermentation. While the true protein percentage was 10.86%. SCP production from pineapple waste, studied by Aruna (2019) and Tropea et al. (2015), observed the highest crude protein yields of 13.56% and 17.2%, respectively.

In a different study, certain food wastes (banana peel, citrus peel, carrot pomace, and potato peel) were utilized for the production of SCP using yeast (isolated from durien fruit), and it was reported that the crude protein yield was increased from 14.07 % (before fermentation) to 30.82% (after fermentation) (Chun et al., 2020).

It was investigated how well some industrially important microbes (kefir, *K. marxianus*, and *S. cerevisiae*) grew on substrates obtained from several common food industry wastes (whey, molasses, brewer’s solid wastes, orange, and potato residues) during SSF (Aggelopoulos et al., 2014). Among all the three varieties, the highest protein content (38.5% w/w) of SCP was observed with *S. cerevisiae* AXAZ-1. The protein content observed in fermented biomasses of *S. cerevisiae* AXAZ-1 (38.5% w/w) and *K. marxianus* IMB3 (33.7%) were observed two times higher than their corresponding substrates before treatment (20.9-22.9%).

In summary, various materials and wastes can be used as a substrate for producing SCP. However, the material to be used for this application should meet some criteria, for example, they must be non-toxic, regenerable, abundant, and inexpensive. So, besides contributing to reducing production costs, the use of organic wastes as a carbon source for SCP production also helps with a more valuable solution for eliminating such residues (Reihani & Khosravi-Darani, 2019; Srivastava et al., 2011).

Table 3 summarizes different fermentation parameters related to the production of SCP from algae, fungi and bacteria. The growth rate, the substrate used for production (which will also impact the costs of the process), and risks of contamination are important aspects to consider for developing an SCP production technology. It is also of fundamental importance to optimize the fermentation process for each type of substrate and microorganism in order to maximize the production yield (Reihani & Khosravi-Darani, 2019; Sharif et al., 2021).

Table 3

5. Protein recovery and purification

The downstream process for protein recovery will have small variations according to the fermentation system and conditions used, microorganisms employed, and fermentation media used, among others.

5.1. Biomass recovery

Mechanical separation technologies allow the fermentation products to be recovered from the fermentation broth. In submerged fermentation, for example, both the substrate to be fermented and biomass produced are present in the liquid medium. The biomass can be harvested continuously in this system, being then filtered or centrifuged and dried to obtain the SCP. In the solid-state fermentation, biomass can be recovered by using less steps than in semi-solid-state fermentation. Because the later uses a higher amount of free water than solid-state fermentation, it requires facile processing to obtain microbial biomass. The abysmal amount of water in solid-state cultivation makes the downstream processing much more efficient, but heat dissipation from the biomass poses a critical problem. While the microbial biomass in semi-solid-state fermentation can be recovered by centrifugation, solid-state fermentation requires special scrappers or mechanical systems to remove the biomass from the fermented slurry.

Single-cell from bacteria and yeasts are usually recovered by centrifugation, while filamentous fungi are recovered by filtration (Ritala et al., 2017). Centrifugation is an energy-intensive process; however, such energy requirements can be offset by the high value of the final product (Sheng et al., 2017). Membrane technology has been considered more favorable than centrifugation for microalgae biomass collection due to its lower energy consumption, being generally more cost-effective, and offering full biomass recovery (Zhang et al., 2019).

5.2. Cell disruption

To isolate an intracellular protein, cell membrane must be disrupted to release the cell contents, which can be done by using a suitable lysis buffer to achieve high solubilization of the target protein (Hernández et al., 2018). It is possible to use SCP as a complete cell preparation; however, breaking the cell makes the protein more accessible. Several methods can be used to break the cell wall, including mechanical forces (crushing, crumbling, crushing, pressure homogenization or ultrasound), hydrolytic enzymes (endogenous or exogenous), chemical disruption with detergents, or even combinations of these methods (Nasseri et al., 2011).

5.3. Protein secretion

Several methodologies, such as precipitation, extraction, and filtration, have been developed to recover proteins from biological systems (McDonald et al., 2009). These methods have different advantages and disadvantages. Precipitation, for example, involves the adjustment of the physical properties (pH, salt and heat treatment) of the medium to improve the insolubility of proteins (Tovar Jiménez et al., 2012), being an easily scalable and low-cost method. However, thermal precipitation has the disadvantage of changing the structural characteristics of native proteins, which can affect their functional and nutritional value (Yadav et al., 2014). Protein extraction is an important biological process to recover protein from the grown microorganism with desired purity. However, the extraction of protein from filamentous fungi is a complex and cumbersome process due to the presence of a chitinous cell wall. Several processing steps include the extreme conditions (pH, temperature, pressure, and requirement of solvents, among others) required to extract the protein. However, in the case of the use of microbial proteins in food and feed applications, extraction of protein employing these techniques is not required.

Pressure-driven membrane processes (microfiltration and ultrafiltration) and direct osmosis membrane processes have also been explored for SCP collection due to their high efficiency, ease of operation and scalability (Ye et al., 2018). Microfiltration membranes are commonly used as a clarification step to ultrafiltration and nanofiltration processes, as well as for cold sterilization of liquid foods and pharmaceuticals, and can also be used to fractionate large macromolecules from smaller ones, such as casein from whey fractionation in the dairy industry. In the biotech and pharmaceutical industries, microfiltration membranes offer the safety of a physical barrier to remove bacteria and other microbes (Tijing et al., 2020). On the other hand, ultrafiltration has been more applied to eliminate organic substances from wastewater treatment and in the textile industry. In ultrafiltration, the ultrafilter is supported over a wire mesh, and the impure sample is poured over it. The impurity particles (electrolytes) pass through the ultrafilter while the larger colloidal particles are retained (Tovar Jiménez et al., 2012). This process is usually slow, but it can be accelerated by applying pressure or by using a suction pump on the filtrate side (Tijing et al., 2020). Commercial whey protein is purified through microfiltration and ultrafiltration processes, which avoid denaturation of the protein. These methods are performed at low temperatures, removing the impurities in the whey (fats and sugars) and producing a very high-quality protein.

It is worth noting that membrane contamination significantly decreases water permeability and the overall system performance. In this sense, several techniques have been reported to control/avoid membrane contamination, including feed pretreatment, system operation below the critical flow, backwash, ultrasonic cleaning, chemical cleaning, and mopping with air bubbles (Liao et al., 2018). In addition, most published studies use commercial membranes, which are also used in the food industry. However, developing a custom membrane to collect a specific type of microorganism would be very feasible considering the diversity of species that can be used for SCP production, which have different cell sizes (Lau et al., 2020). Moreover, coupling a good inlay control system with a suitable membrane could also offer substantial performance advantages (Discart et al., 2015).

5.4. Purification

Chromatographic methods, including gel permeation, hydrophobic interaction, and affinity chromatography, can be used for SCP purification. Such methods are easy to perform but are complex to optimize due to the numerous parameters that need to be considered for this process (Wingfield, 2015). For example, the choice of the column matrix, the buffer to be used, the salt, the organic solvent, the reaction temperature, and the gradient are some of the parameters to be considered for an efficient chromatography process. Chromatographic methodologies are very popular for separating and purifying whey proteins, being used even for large-scale protein separation (Bonnaillie et al., 2014).

5.5. Drying

Several techniques have been reported to dry microbial proteins and obtain powdered proteins with desirable characteristics for application on an industrial scale. The mostly used techniques are freezing-drying and spray-drying (Maltesen & van de Weert, 2008). Recently, supercritical drying has emerged as another viable alternative for obtaining powdered proteins.

These techniques are based on three physical principles: sublimation, evaporation, and precipitation (Son et al., 2020). Of course, different techniques use different stresses that can compromise the final stability of the proteins. In addition, different techniques result in protein powders with significantly different characteristics (Raziq, 2020). This fact should be considered when focusing on the desired characteristics of the final product.

6. Industrial scenario and market of SCP

Currently, consumers demand healthy food and, at the same time, are concerned about the environment. To meet a demanding and competitive market, manufacturers have continuously innovated their production process using different raw materials and developing more sustainable technologies to attract customers. Through iterative and incremental methodologies, companies are creating innovative solutions to their existing products within a constant innovation cycle to reduce toxic waste and the content of nucleic acids in their products for a better human consumption, which, in the end, signals sales growth opportunities in the food and beverage industries. Lallemand Inc., Montreal, Canada, for example, has been developing innovative microbial products through external research partnerships with important universities and their own internal projects to create new and healthier products (Hülsen et al., 2018; Matos, 2019).

The SCP production process can contribute to the environment's safety by reducing the carbon footprint and using wastes/renewable resources as carbon sources for fermentation. Due to this positive aspect, the high demand for healthy products and alternative protein sources, the SCP market is expected to expand significantly from 2020 to 2030 (Banovic et al., 2018; Hülsen et al., 2018; TMR, 2021). SCP has applications in food products as an important source of proteins and vitamins and has also been used to improve the nutritional value of products such as soups, baked goods, ready-to-serve meals, in diet recipes, among others. For animal nutrition, SCP is used for fattening calves, pigs, poultry and fish. SCP is also used to increase the nutritive value of soups, baked products and specialized diets. Besides the main applications of SCP in food and feed products, it is also applied in the leather and paper processing industries and as a foam stabilizer (Kumar et al., 2017; Zakaria et al., 2020).

The global SCP market is segmented into North America, Latin America, Western Europe, and Pacific Asia (excluding Japan, the Middle East and Africa). A recent market study indicated that the SCP market in Malaysia was at US$ 9.7 million in 2020 and is expected to reach US$ 24.5 million in 2030 at an annual growth rate (CAGR) of 9.7%. Vietnam's SCP market revenue was valued at over US$ 26.7 million in 2020 and is expected to exceed US$ 69.4 million by the end of 2030 (Khoshnevisan et al., 2020; TMR, 2021). The Asia region is also made up of other prominent countries in the Association of Southeast Asian Nations (ASEAN), such as India, China, Indonesia and Bangladesh. According to the Food and Agriculture Organization of the United States, these countries produce about 50-60% of the total aquaculture production. SCP is the most nutritious and cost-effective option for fishmeal, and global growth is expected through the expansion of the aquaculture industry (Jones et al., 2020; Matos, 2017; Ritala et al., 2017).

North America is a global leader in the global SCP market due to the region's highly developed food and feed industries. North America is favored by some prominent organizations related to the food and feed industries, which allows a greater manufacturing and development capacity for SCP production. Furthermore, the majority of individuals who adopt a high-protein diet are in North America, which makes it possible to increase the market value share of the one-time-only protein in the region (Nasseri et al., 2011; Spalvins et al., 2018). European consumers are concerned about increasing the protein content in their food products as well as in finding alternative protein sources (Banovic et al., 2018). Furthermore, the increased support in sustainability and actions against animal cruelty in the region has also contributed to a better market scenario for non-animal-based protein sources. SCP is also gaining significant attraction in Latin American countries. However, the major share of the population relies on animal protein because of the culture and availability of grazing lands, feeding crops for animals. Nevertheless, the rising awareness about high-quality protein and the right nutrition, food - changing patterns, vegan proteins, and SCP is also getting sizeable attraction.

The main players in the SCP market, their respective countries and their segments are shown in **Table 4**. These companies are focused on business growth and innovation to strengthen their positions in the global SCP market. Developing new products and strategic collaborations are other approaches that the key players are considering to gain a competitive advantage in the global SCP market. Angel Animal Nutrition, for example, launched an innovative product called GroPro, which is a yeast-derived feed ingredient composed of proteins necessary for the development of young animals. Afterwards, they launched a completely innovative semi-dry yeast in the market in the form of a tetra pack, which is easy to use and hygienic due to its reusable upper opening. This yeast product has about 20% moisture, with characteristics of dry and fresh yeast (Ritala et al., 2017; TMR, 2021).

Table 4

Besides the big companies, startups are also increasingly investing in innovation, from microalgae supplements for athletes to ice cream based on probiotic bacteria. For example, Noko Foods, a French startup recently founded in 2021, develops herbal microalgae supplements, drink shakes and food products for athletes. In addition, the startup Ninoko Labs, founded in 2020 in Germany, produces alternative proteins from fungal mycelium in order to compete with real meat in terms of cost and flavour. Another example of a biotechnology company focused on innovation for human food is Bidifice Inc., Santiago, China which develops ice cream rich in healthy probiotic bacteria to help people with chronic diseases and allergies (Bratosin et al., 2021; Sally Ho, 2021).

7. Regulatory aspects

SCP used as food or feed must be safe to produce and use. In most countries, there are regulations to certify that food or feed is safe for human consumption. Generally, these regulations differentiate between human food and animal feed, food that provides nutrition and potentially flavor and aroma, and food additives such as colorants, preservatives, or feed additives. In addition, although definitions differ among regions, international standards regulated by the Joint FAO/WHO Expert Committee on Food Additives apply to internationally traded products (Kannan et al., 2020; Ritala et al., 2017; Sharif et al., 2021).

Although the final SCP product is a protein and nutritional source, certain products may enter the market as additives, providing, for example, color rather than SCP, which restricts the extent to which they are added and their value as SCP. Therefore, the regulations differ depending on the application (Zepka et al., 2010). Also, Smedley (2013) reported similarities and differences among 7 jurisdictions (Canada, European Union, Brazil, China, Japan, United States and South Africa) in terms of regulation of authorized food ingredients, as well as the approval and management assessment process for feed components, and peculiarities between regulations in these regions. In addition, as animals are not all the same in all regions, the regulations for feeding pets differ in certain regions, requiring authorization before selling new pet foods or additives.

It is worth noting that the final SCP product must not only be nutritious but also pass all toxicity tests to be marketed as a food product. In addition, the unwanted nucleic acid content, toxins and unwanted compounds that accumulate during the strain cultivation using substrates, such as hydrocarbons and petroleum contaminated with heavy metals, need to be removed (Gervasi et al., 2018). Decontamination and purification of the final product are essential for SCP to be used as a food source for consumption.

8. Role of microbial protein in the circular economy

Recently, the concept of circular economy, which implies the transformation of wastes and industry side-streams to produce renewable energy and added-value compounds, has been strongly encouraged to design a more sustainable economy (Dragone et al., 2020; Stiles et al., 2018). Microbial protein is becoming a potential product for incorporation in a circular economy model due to its increased interest as an alternative protein source and numerous applications in food, chemicals and pharmaceuticals (Lai et al., 2019). Microbial biomass, particularly microalgae, can also be recycled and used as a biofertilizer to sustainably improve soil quality and crop nutrition (Abo et al., 2019).

In recent years, the global need to find alternative protein sources has driven the development of new SCP processes. Using readily available raw materials and waste streams as a substrate for SCP production is also a relevant driver to develop new processes. In this sense, SCP production can fortify biorefineries' economic feasibility, besides being a sustainable option for managing residual raw materials and wastes (Mahan et al., 2018). Concerns about environmental pollution have also driven the development of new SCP production processes. This can be seen especially in processes that have applied greenhouse gases as a substrate, for example, the production of SCP using CO_2 or methane as a carbon source. Although there are still important challenges to overcome for large-scale and economically feasible implementation of these new processes using gases as substrate, they have attracted great interest from a sustainability point of view (Puyol et al., 2017; Ritala et al., 2017; Ukaegbu-Obi, 2016).

Investment and profitability are key elements in estimating the economic viability of an SCP production process. For large-scale SCP production, large bioreactors are required. Thus, high oxygen transfer rates are needed to obtain a high amount of biomass during the cultivation, which may cause an increased generation of heat from microbial metabolism that will lead to the need for temperature control and reduction. In fact, operating costs, including labor, consumables and energy, represent 45-55% of SCP manufacturing costs, while raw material costs range from 35-55%. Using cheap raw materials and/or waste streams as carbon sources is a good strategy to reduce substrate costs, as long as they do not compromise the quality of the final product (Rodrigues, 2020). Finally, there is a relationship between cost and production scale. Most of the SCP processes practiced on an industrial scale were set for a continuous design, which proved to be the most profitable option (Poutanen et al., 2017; Ritala et al., 2017).

After everything that was presented and discussed in this work, the research brought here on microbial biomasses, showed that SCPs gained momentum, due to the increased demand for alternatives to proteins derived from plants and animals. In this way, certain products can become popular due to consumer acceptance and significant encouragement from government and regulatory authorities. The literature also made it possible to verify a diversity of possibilities of microorganisms capable of producing SCPs, including microalgae, fungi, bacteria and cyanobacteria.

9. Conclusions and future prospects

Microbial engineering has a relevant ability to enhance the competitiveness of the SCP product in terms of production cost, functionality and nutrition. The application of GRAS microorganisms is considered safe and is always the right choice in microbial engineering for SCP production, with the main objective of improving the production of intermediate raw materials and the accumulation of biomass. Future research and promotion of meat-optional protein sources is a major challenge. The judicious utilization of agri-residues and by-products of agriculture and food processing for the cultivation of filamentous fungi, yeasts, bacteria and microalgae, would allow in developing SCP in a sustainable manner. Furthermore, studies aiming to correlate the consumption of alternative sources of protein and gains for human health would very possibly increase consumers' attention to a more sustainable diet. In the near future, it is expected that the development of new processes for SCP production using residual raw materials, industry-side streams or even greenhouse gases (CO_2 or methane) as carbon sources will become a reality to increase the protein market without affecting the environment and potentially with a low production cost. This review can be useful for the start-ups to create new products or processes by combining fermentation technologies and alternative meat protein sources. Indeed, the sensory attributes and nutritional value of meat alternative foods can be improved by fermentation with selected microorganisms.

ACKNOWLEDGEMENTS: A. K. Chandel gratefully acknowledges the financial support from CNPq for scientific productivity program (Process number: 309214/2021-1). S. I. Mussatto acknowledges the Novo Nordisk Foundation (NNF), Denmark, grant number NNF20SA0066233. V. K. Gupta and V. K. Thakur would like to acknowledge the institutional funding sources supported by the SRUC, UK for research and development.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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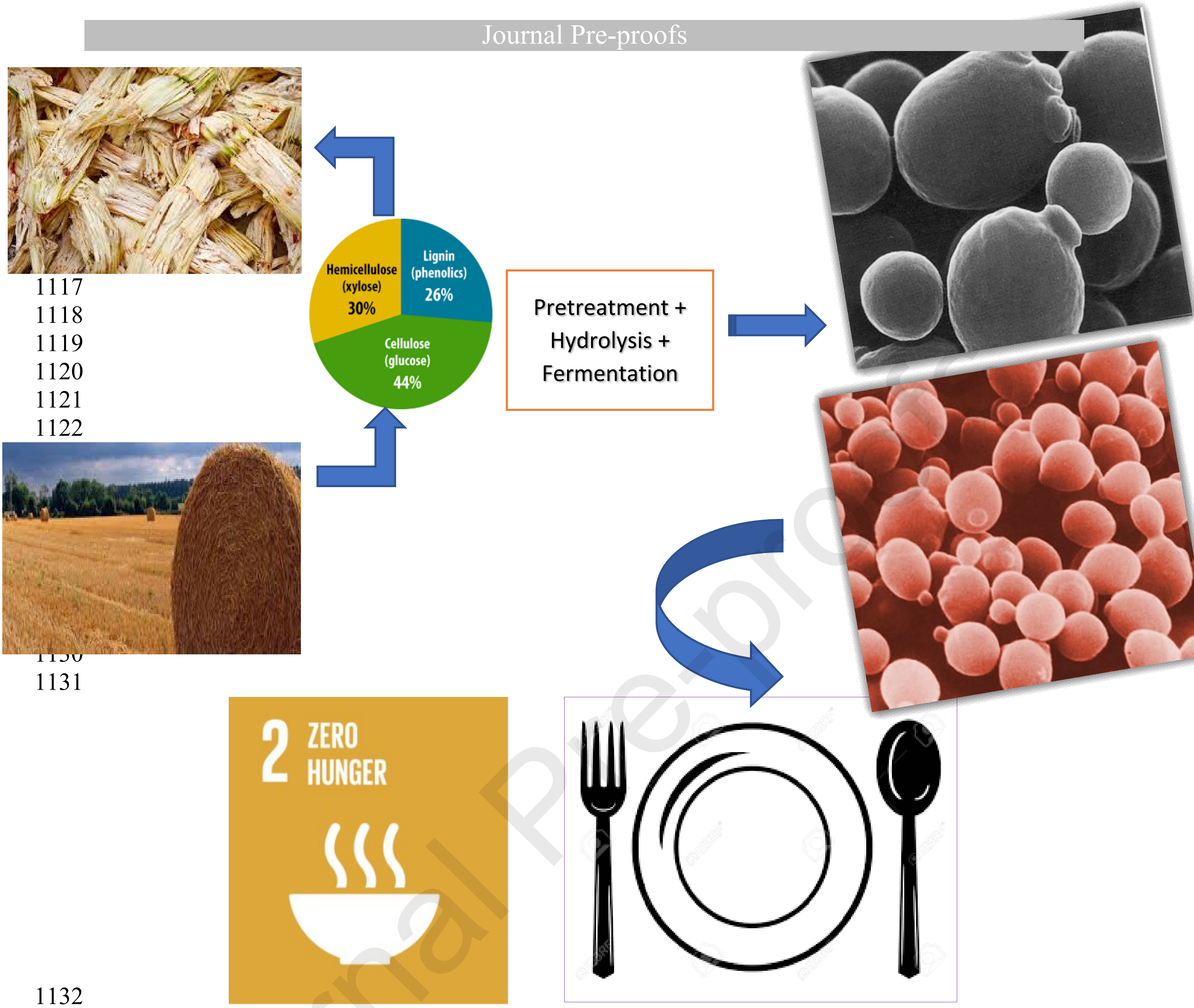

Microbial protein: a genuine source of holistic food linking with **Sustainable Development Goal of number 2-Zero Hunger** of United Nations

Highlights:

- Microbial protein as a sustainable vegan protein source for the growing population
- Agro-industrial byproducts are renewable and surplus feedstock available round the year for microbial protein production
- Presence of high content of nucleic acid and toxins is a major concern of using microbial protein as food alternative
- Microbial protein can be produced with minimum carbon footprints and low water usage.
- Continuous cultivation of microorganisms employing semisolid state fermentation seems industrially viable strategy

Figure captions

Figure 1. Flowchart of the overall process for microbial protein production.

Figure 2. Industrial production of microbial protein with a focus on the fermentation step

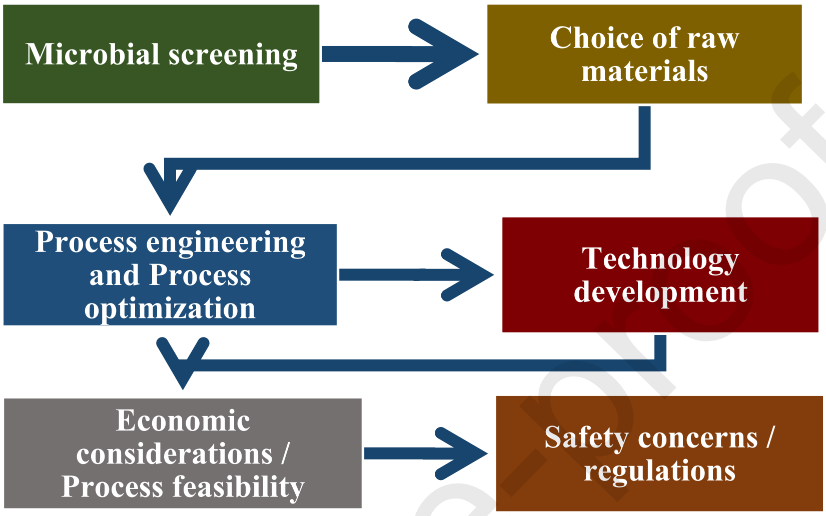
Microbial screening
Choice of raw materials
Process engineering and Process optimization
Technology development
Economic considerations / Process feasibility
Safety concerns / regulations

Figure 1


Steps of SCP production

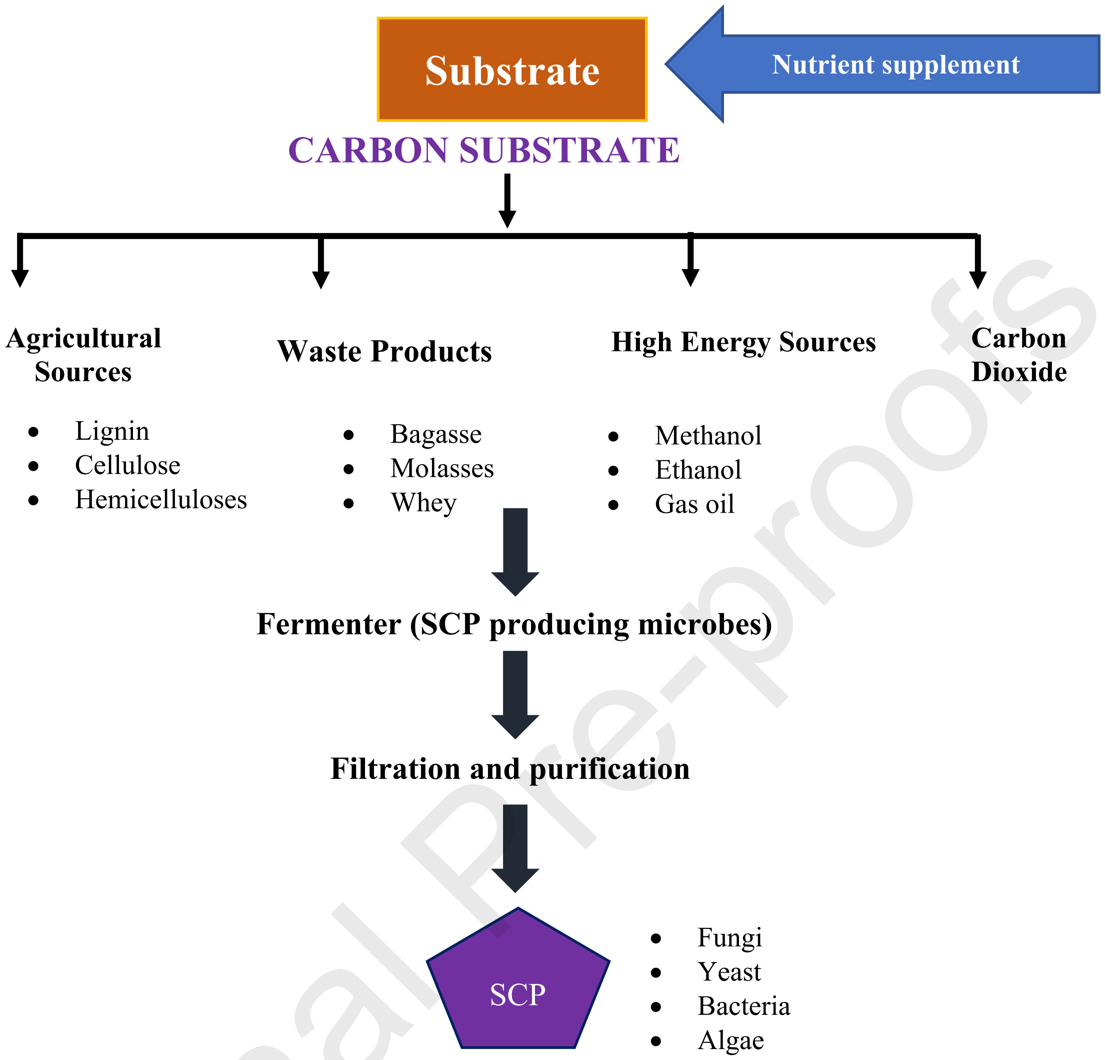
Substrate
Nutrient supplement
CARBON SUBSTRATE
Agricultural Sources
Lignin
Cellulose
Hemicelluloses
Waste Products
Bagasse
Molasses
Whey
High Energy Sources
Methanol
Ethanol
Gas oil
Carbon Dioxide
Fermenter (SCP producing microbes)
Filtration and purification
SCP
Fungi
Yeast
Bacteria
Algae

Figure 2

Table 1. Examples of microorganisms used for single cell protein production and their protein content

Microorganism		**Protein content (%)**	**Reference**
Microalgae	*Arthospira platensis (Spirulina maxima)*	60-71	De Oliveira et al., 1999
	Chlorella pyrenoidosa	45	Waghmare et al., 2016
	Chlorella sorokiana	46-65	Safafar et al., 2016
	Euglena gracilis	50-70	Rodríguez-Zavala et al., 2010
Fungi and yeasts	*Aspergillus niger*	49	Kam et al., 2012
	Candida tropicalis	56	Gao et al., 2012
	Debaryomyces hansenii	32	Duarte et al., 2007
	Kluyveromyces marxianus	59	Aggelopoulos et al., 2014
	Yarrowia lipolytica	54	Zinjarde, 2014
Bacteria	*Bacillus cereus*	68	Kurbanoglu and Algur, 2002
	Escherichia coli	66	Kurbanoglu and Algur, 2002
	Haloarcula sp. IRU1	76	Taran and Asadi, 2014
	Rhodopseudomonas palustris	55–65	Kornochalert et al., 2014

Table 2. Average composition of SCP obtained from different types of microorganisms and parameters / limitations to be considered for application in human and animal nutrition (Source: Anupma and Ravindra, 2000)

Component	**Composition (wt%)**		
	Algae	**Fungi/yeasts**	**Bacteria**
Protein	40–60	30–70	50–83
Total nitrogen (Protein + nucleic acids)	45–65	35–50	60–80
Lysine	4.6–7.0	6.5–7.8	4.3–5.8
Methionine	1.4–2.6	1.5–1.8	2.2–3.0
Fats/Lipids	5–10	5–13	8–10
Carbohydrate	9	n.a.	n.a.
Bile pigment and Chlorophyll	6	n.a.	n.a.
Nucleic acids	4–6	9.70	15–16
Mineral salts	7	6.6	8.6
Amino acids	n.a.	54	65
Ash	3	n.a.	n.a.
Moisture	6.0	4.5–6.0	2.8
Fiber	3	n.a.	n.a.

Range of values are due to the type of substrate, culture conditions and microorganism used.

n.a.: not available.

Table 3. Fermentation parameters related to SCP production by different microorganisms (Source: Anupma and Ravindra, 2000).

Parameter	**Algae**	**Bacteria**	**Fungi (Yeast)**	**Fungi (Filamentous)**
Growth rate	Low	Highest	Quite high	Lower than bacteria and yeast
Substrate	Light, carbon dioxide or inorganic samples	Wide range	Wide range except carbon dioxide	Mostly lignocellulosic
pH range	Up to 11	5–7	5–7	3–8
Cultivation	Ponds, Bioreactors	Bioreactors	Bioreactors	Bioreactors
Contamination risks	High and serious	Precautions needed	Low	Least if pH is less than 5

Table 4. Single cell protein market players and their application segments (Source: Ritala et al., 2017).

Company	**Country**	**Microorganism**	**Application segments**
Amoco (BP)	United States	*Candida utilis*	Petrochemicals
Bega Cheese Ltd	Australia	*Saccharomyces*	Human food
Blue Green Foods	---	*Aphanizomenon flos-aquae*	Human food and animal feed
Cyanotech	United States	*Spirulina platensis*	Human food
BioProcess Algae LLC	United States	*Desmodesmus* sp.	Animal feed and nutrients
Calysta	United States	*Methylococcus capsulatus*	Fish feed
Algaeon Inc.	United States	*Euglena gracillis*	Human food and fish feed
Nucelis Inc.	United States	*Yarrowia*	Human food
Unibio A/S	United Kingdom	Methanotrophic bacteria	Dietary supplement and nutrients for animal
Euglena Co. Ltd.	Japan	*Euglena*	Human food
Biomin Holding GmbH	Austria	n.a	Animal feed
Evonik Industries AG	Germany	n.a	Animal feed
BlueBioTech Int. GmbH	Germany	*Spirulina and Chlorella*	Human food
Nutreco NV	Netherlands	n.a	Animal feed
Lallemand Inc	Canada	*Saccharomyces cerevisiae and Torula*	Human food and animal feed
Marlow Foods Ltd	United Kingdom	*Fusarium venenatum*	Human food
Vagan Pharma Ltd.	China	Bacterial	Animal feed
Angel Yeast Co. Ltd	China	Yeast	Animal feed
LeSaffre	France	*Saccharomyces cerevisiae*	Human food

n. a: not available

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Conflict of Interest

- ○ All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

- ○ This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

- ○ The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

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