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# SINGLE CELL PROTEIN IN AQUACULTURE: A COMPREHENSIVE REVIEW

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## ABSTRACT

Single cell protein (SCP) represents a promising remedy for the worldwide need for protein-rich foods of high quality. SCP products consist of protein meals derived from microbial or algal biomass, offering a promising solution to meet the demand for a sustainable and renewable protein source in the field of aquaculture. In this review, we identify novel SCP strains and feedstocks, discuss new feeding trial results on significant aquaculture species, including Atlantic salmon, rainbow trout, and white leg shrimp and highlight prospective sources of SCP strains and their corresponding production procedures. Sustainable production of SCP flours can be achieved by using algae or microbial biomass, utilizing waste materials from food, agriculture and cities.The use of SCP is a prominent substitute for fish protein in aquaculture diets.Protein that has been extracted from pure or mixed cultures of microorganisms, such as fungus, bacteria, yeast, or microalgae, is referred to as SCP and can be utilized in place of more traditional protein sources.The potential of SCP as a sustainable supply of protein for the aquaculture industry is thoroughly examined in this paper. It provides important information on the nutritional value of this substance and how it affects fish development and health.



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# INTRODUCTION

The world population is continuously increasing. By 2050, mathematical models predict that it will exceed 10 billion people (Dillard, 2019). To meet the food especially protein demand of such large population, aquaculture is found to be the suitable. The global capture fisheries are declining and aquaculture is a sunrising sector with increase of 7% annually. Fish represents a significant dietary protein source for humans, providing approximately 20% of the average animal protein intake per capita to over 3.1 billion people (Abd El-Hack *et al*., 2016). To avoid any negative impact on environment with continuous production of nutritious food require sustainable food production systems Aquaculture faces challenges in feed and energy management, disease control and water pollution, making it essential to address these issues (Dabiand Dzorvakpor, 2015).SCP are prepared from bacteria, fungi, yeast and other microalgae. They are commercialised after being formulated according to their unique advantages and disadvantages. Analysis of the feed requirement of the species' is needed to formulate and enhance their diets (Boyd *et al*., 2020). Dietary protein requirement of fishes depends on their food habit.The FAO states that carnivorous species require 40-55% dietary protein, while freshwater omnivorous and herbivorous species need 30-40% (FAO, Rome, Italy, 2010). Carnivorous animals have the largest needs, often between 40 and 55% of crude protein, however some authors suggest between 35 and 60% or between 20 and 55% (Ayadi *et al*. 2012). Thisis the larger drawback as for the protein we largely depend on the wild caught small fishes. To increase the production of one the productivity of other is in stake. Therefore, alternative protein sources like plant-based ingredients, food waste, insect meals along with SCP are the best choices.As environmentally appropriate substitutes for fish protein in aquaculture diets, insect meal, plant-based components and single-cell protein (SCP) may raise feeding costs and provide a competition to the manufacture of human feed.SCP may be produced in every weather condition, takes less time to produce, and uses less area (García *et al*. 2003).The carbon-degrading microbiota was treated in a wastewater treatment facility and harvested as dried microbial biomass, known as single-cell protein (SCP).Waste streams can be utilized as a carbon source for microbes in SCP production, making it cost-effective and suitable for both high-income and low-income societies (Hülsen *et al*. 2018a). SCP, an immunostimulant and probiotic, enhances the growth, health, disease resistance and immune system of cultured organisms and serves as an alternative protein source for aquafeeds. One of the most effective ways to control disease recurrence in intensive aquaculture is to use probiotics (Ige, 2013). Lactobacillus (gram-positive bacteria) as a probiotic has proven a viable alternative to antibiotics in aquaculture disease management (Kolndadacha *et al*. 2011). Emergence of pigment cells like melanocytes from different migratory neutral cells is evolutionarily conserved (Adameyko *et al*., 2009). SCP can be photoautotrophs, chemotrophs, methylotrophs, heterotrophs or mixotrophs.

**Production of SCP:** Large-scale manufacturing SCP has advanced biotechnology in the modern era. Every germ has benefits and drawbacks of its own. Protein makes up 45–55 percent of the microbial biomass on average. Protein concentration in certain bacteria can reach 80%. In addition to protein, the biomass also includes other necessary elements, making it a perfect addition to the traditional food supply.

**Bacteria:** Their rapid growth rate and short generation time make them suitable for application in aquaculture. It can be culturedin variety of raw materials like starch sugar liquid hydrocarbon. Since methylophilus takes around two hours to generate, phototrophic and methanotrophic bacterial strains are advised for use as animal feed. Generally speaking, bacteria produce a higher-quality protein composition than fungi or yeast (Adedayo *et al*., 2011). *Brevibacterium Methylophilus*, methylitropous, *Acromobacterdelvaevate*, *Acinetobacter calcoacenticus*, *Aeromonas hydrophilla*, *Bacillus megaterium*, *Bacillus subtilis* Lactobacillus species, Cellulomonas species, *Methylomonasmethylotrophus*, *Pseudomonas fluorescens*, *Rhodopseudomonas capsulate*, Flavobacterium species and *Thermomonosporafusca* are some of the major species in use (Dhanasekaran *et al*., 2011). The disadvantages include the small size of bacterial cells and low density which affects harvesting of biomass from fermented 2 mediums, which ultimately increases the cost of operation (Production of Single Cell Protein).

**Algae**: Spirulina, the most commonly utilized type of algae, is classified as a blue-green alga (BGA) renowned for its potent antioxidant properties and its ability to stimulate a system of enzymes that scavenge free radicals. Tribal tribes worldwide have utilized Senedessmus and Chlorella biomass as a primary food source.Algae may be consumed in a variety of ways and some of its benefits include being easy to grow, a high protein content and efficient solar energy consumption. It has been thought that Spirulina algae might be used as an additional protein to protect stem/progenitor cells. Maximina Spirulina avoid the formation of fatty liver caused by carbon tetrachloride (CCl4) by reducing the likelihood that the product will include high amounts of heavy metals when algae are employed as the substrate (Raja *et al*., 2008).

**Yeast**: Due to its huge size and ability to thrive in acidic pH, it is recommended since it is easy to harvest. Yeast species that are often utilized include Saccharomyces, Pitchia, Hansenula, and Torulopsis. You may cultivate Saccharomyces cerevisiae on a variety of fruit wastes (Tanveer, 2010). Sengupta *et al*. (2006) evaluated the production of Saccharomyces cerevisiae single cell protein using cucumber and orange peels through submerged fermentation, with Rhizopus arrhizus chosen due to its nontoxic nature.

***Fungi**:* Actinomycetes and filamentous fungus at MIT's Second International Conference in 1973 generated protein from a variety of substrates. Studies into the potential application of fermented Rhizopus and Fusarium cultures as a source of protein were carried out during World War II.Both the substrate and the organisms affect mycelia yield. It is advisable to conduct toxicological assessments prior to suggesting usage as SCP (Yousuf, 2012). Fungal species are being used by SCP technology to bioconvert lignocellulosic wastes, a new development (Lenihan *et al*, 2010), *Fusarium graminearum* (Zubi, 2005), *Aspergillus fumigates*, A. niger, A.oryzae, *Cephalosporiumcichorniae*, *Penicillium cyclopium*, Rhizopuschinensis, *Scytalidumaciduphlium*, *Tricodermaviridae*, and Tricoderma alba Paecilomycesvarioti are commonly used fungi. Filamentous fungi are simple to harvest, but because of their low protein content and slower growth rate, they are not very productive (Jaganmohan *et al*., 2013).

***Potential subtrates:*** The substrate was primarily derived from poor-quality waste material and exceptionally high-quality protein.Conventional substrates used in SCP synthesis include starch, molasses, and leftover fruit and vegetable materials. Unconventional substrates include petroleum byproducts, natural gas, ethanol, methanol and lignocellulosic biomass. The primary determinants of a SCP manufacturing process's design and strategy are the substrate's availability and proximity to the production plant. The availability and affordability of molasses, as well as its composition and lack of harmful ingredients and fermentation inhibitors, dictate its usage in the manufacturing of SCP (Bekatorou *et al*., 2006).

***Cultivation Methods:*** Fermentation is the process used to produce single-cell protein. Following the completion of the technical requirements for the optimized strains' culture, all metabolic pathways and cell structures will be identified. In addition, apparatus technology and process engineering modify the process's technical performance to prepare the output for large-scale application. The material undergoes further filtration, centrifugation, and drying after considering cost and energy factors (Chandrani and Tayathilake, 2000).

**Types of fermentation**

***Submerged fermentation:*** The substrate utilized in the submerged process is constantly liquid and provides the nutrients required for continuous operation, as well as continuous harvesting of the biomass product. During production, aeration is crucial to releasing heat and maintaining device coolness. Numerous techniques are available for harvesting the microbial biomass (Kargi *et al*., 2005).

***Semisolid fermentation:*** Semisolid fermentation is more common in solid states, such as cassava waste and does not require clearing the substrate preparation (Adedayo *et al*., 2011). The U-loop fermentor is a unique type of bioreactor used to study mass and energy transit phenomena (Jorgensen, 2010).

***Solid state fermentation:*** Numerous experts talked on the different kinds of bioreactor designs, process parameters and microbes that may be used to produce a variety of value-added products, including as feeds, ethanol, organic acids, B complex vitamins, colors, flavors and SCP (Singhania *et al*., 2009). Stirring the fermentation material is how liquid state fermentation is carried out in tanks ranging in size from 1,001 to 2,500 square meters (review). It is necessary to control nutrition, pH, soluble oxygen, temperature and ionic strength in order to accurately manage the metabolites. The engineers have created other methods such as precipitation, centrifugation, flotation, coagulation and the use of semi-permeable membrane to boost the SCP production (Capalbo *et al*., 2001).

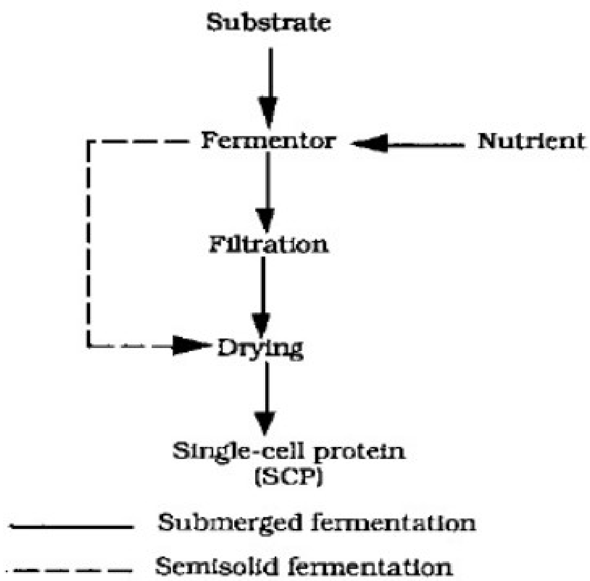


**Fig. 1. Flow chart of SCP production**

***Nutritional profile of the microorganisms:*** The microalgae, fungi and bacteria are all part of the SCP. Single-cell organisms that might be added to aqua-feeds are mostly utilized for their protein, lipids and particular nutritional qualities. Protein content in SCP resources is often greater than 300 g/kg (dry basis). Several species use it as a source of bioactive compounds, omega-3 fatty acids and protein and amino acids (Glencross *et al*., 2020). It intensifies conflict on the international stage. Due to their high carotenoid concentration, goods like Dunaliella and Haematococcus were among the first profitable products.Temperature, salinity, nitrogen supply and carbon source all had an impact on production (Pisal *et al*., 2005).

***Protein***: Depending on the source material, different single-cell proteins (SCPs) from microalgae have different amino acid compositions. Microalgae were found to be rich in leucine and lysine but deficient in histidine. Depending on the processing technique, the protein concentration of the microalgal components varied from 0% to around 60%, with an average of 34%. Higher fat levels were correlated with lower protein levels. Fungal SCP, derived from various yeast genera, showed protein levels ranging from 33% to 47%, with glutamic acid being particularly abundant. The most common amino acid was leucine, which was followed by lysine and valine. Protein levels in fungal SCP were found to be regularly between 30% and 60%, with an average of 45%. Of the various forms of SCP, bacterial SCP typically exhibited the greatest protein levels, ranging from 79% to 84%, with some products reaching as high as 80% (average–60%). There was also more non-protein nitrogen in these products. Conversely, the protein composition of biofloc resources was found to be the lowest on average (27%), with certain products having even lower protein levels than 5% (Glencross *et al*., 2020).

***Lipds:*** Single-cell proteins (SCP) exhibit low lipid content, typically below 5% (Overland *et al*., 2010). Nannochloropsis and other microalgae have eicosapentaenoic acid (EPA) concentrations that are higher than 30% of total fatty acids (TFA).However, docosahexaenoic acid (DHA) levels in species such as Schizochytrium and Crypthecodinium exceed 40% TFA. As natural sources of carotenoids and astaxanthin, modified oleaginous yeasts enriched with EPA (over 30% TFA) are used; commercial astaxanthin sources also include bacterial (Paracoccuscarotinifaciens; Panaferd-AXTM) and fungal (Phaffiarhodozyma; RedStarTM) resources. Nucleotide levels in bacterial and yeast SCP resources have been observed to reach as high as 15.9% of the biomass, making them very rich sources of these vital nutrients (Tibbetts *et al*., 2020).

***Properties***

***Digestibility:*** Protein digestibility has posed common challenges, with reported values reaching as high as 99% for most proteins and 87% for Isochrysis SCP. However, Nannochloropsis, Phaeodactylum, and Isochrysis have been associated with a negative impact on protein digestibility, where Nannochloropsis showed the most significant adverse effect. Regarding lipid digestibility, only one study on Cryptocodinium SCP reported a high lipid digestibility of 98% in O. mykiss. Unfortunately, this study did not provide information on fatty acid digestibilities. Comparatively, fungal and bacterial SCP products generally exhibit higher protein digestibility. Fungal SCP products have shown protein digestibility levels around 80%, bacterial SCP around 86%, microalgal products at 76% and spray-dried Candida product at an impressive 98%. However, Saccharomyces product recorded a lower protein digestibility at 41% (Skrede *et al*. 2011).

***Utilisation and palatability:*** The most comprehensive source of information on the use of SCP and SCO resources in aquaculture may probably be found in the "growth studies" conducted by various aquaculture species. A focus is placed here on the application of the various classes of these resources (microalgal, fungal, bacterial, and bioflocs) in each of the four major species groups: salmonids, shrimp, tilapias and marine species, in order to avoid overcomplicating the story by presenting a wide range of studies in different species (Glencross *et al*., 2020).

***Immunology:*** In Atlantic salmon fed a Schizochytrium SCP, it has favorable immunological benefits including as goblet cell proliferation, mucus formation, microbiota diversity and stimulated nitric oxide synthase activity (Lyons *et al*., 2016).The diet of gilthead seabream (S. aurata) contains navigula SCP, which stimulates a number of innate immunological and inflammatory markers.There have also been reports on the impact of Methylococcus bacterial SCP on the decrease of intestinal inflammation in Atlantic salmon (Vasanth *et al*., 2015). There have also been reports on the impact of Methylococcus bacterial SCP on the decrease of intestinal inflammation in Atlantic salmon. The infectious myonecrosis virus fueled a microbial enhancement in haemocyte count, bacteriolytic activity and antibacterial activity in Pacific white shrimp (Zhao *et al*., 2012).

***Functionality of feed:*** Various studies have explored the effects of feed processing on different single-cell proteins (SCPs). In combining high-lipid Schizochytrium SCP to extruded feeds, pellets with less hardness and durability are produced by lowering viscosity, melt temperatures and specific mechanical energy. It is demonstrated that the optimal incorporation rate is 132 g/kg. Bacterial SCPs diminish pellet life but increase bulk density and hardness. Research on the impact of fungal SCP or bioflocs on extrusion is rare. Spirulina demonstrates quick release within 10 minutes. The processing of single-cell ingredients commonly involves a drying step for inclusion in feeds, impacting nutrient digestibility and Maillard reaction product content.

***Product quality influence:*** The use of Schizochytrium single-cell protein (SCP) in feeds to raise the amounts of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) in the meat of target species like Atlantic salmon has been investigated by researchers (Katerina *et al*., 2016; Popovich *et al*., 2019).The flesh's sensory qualities were unaffected, even though eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels were enhanced.Other aspects of flesh quality, such improved pigmentation, less gapping and the lack of melanin spots, improved as a result of the higher EPA and DHA content. Additionally, studies using Spirulina in the feeds of Nile tilapia and rainbow trout demonstrated improved suitability for sashimi and enhanced pigmentation with reduced malonaldehyde levels, indicating improved antioxidant status (Teimori *et al*., 2013).

***Evaluation of SCP on aquatic species:*** Water, ash components, proteins, lipids, carbohydrates and other substances are all included in single-cell proteins (SCP). Studies have explored the use of different SCP sources in aquaculture diets. Saccharomyces cerevisiae proved less effective, while alternatives like K. marxianus and C. utilis were used successfully in shrimp diets. Various S. cerevisiae products replaced fishmeal or soybean meal in shrimp diets without affecting growth. Methanotroph-based SCP, such as bacterial protein meal, showed positive results in Atlantic salmon, although nutrient digestibility decreased. SCP from sources like *Corynebacterium ammoniagenes* and *Methylobacteriumextorquens* positively impacted growth in fish and shrimp. Biofloc meal and *Spirulina pacifica* were used in shrimp diets with success. Although microalgae biomass in fish food somewhat decreased digestibility, feed intake rose in response. In tilapia production, effective alternative diets have been used, such as the "green water meal" based on microalgae. However, in response to graded amounts of dietary microalgae biomass, a modest loss in dry matter, protein and energy digestibility was noted; this can be made up for by adding more feed (Moomaw, W.R *et al*., 2017). *Spirulina pacifica* has been shown in another study to have the potential to increase body weight when compared to a baseline diet when used as a protein supplement. Diets containing 5% S. pacifica had the greatest outcomes in terms of food intake, weight increase and protein efficiency index. The so-called "green water meal" is one of the other effective alternative diets. The basic idea of this diet is the utilization of microalgae that have developed in the green water used to produce tilapia (Oreochromis spp.) (Hamilton *et al*., 2020).

***Economics and environment impact:*** Single-cell protein, or SCP, is used to aqua feeds with the twin goals of controlling residues and offering a sustainable supply of protein. Sustainable development aims at circular carbon and nutrient management for environmental and food safety. SCP production is seen as a more advantageous strategy than other technologies, like anaerobic digestion, in reducing food waste and managing harmful manmade waste streams.To safely generate SCP utilizing waste materials, fermentation techniques—aerobic or anaerobic/aerobicare used. This ensures the neutralization of hazardous compounds and lowers environmental contamination. Currently, global SCP production stands at an estimated 606 million tons annually, surpassing soybean meal protein production threefold. Studies show that although the environmental effect of SCP manufacturing is lower than that of soybean meal, greater thermal and electrical energy requirements limit the technology's economic feasibility.Evaluating the economic and environmental feasibility of different production systems becomes crucial for sustainable aqua feed practices.

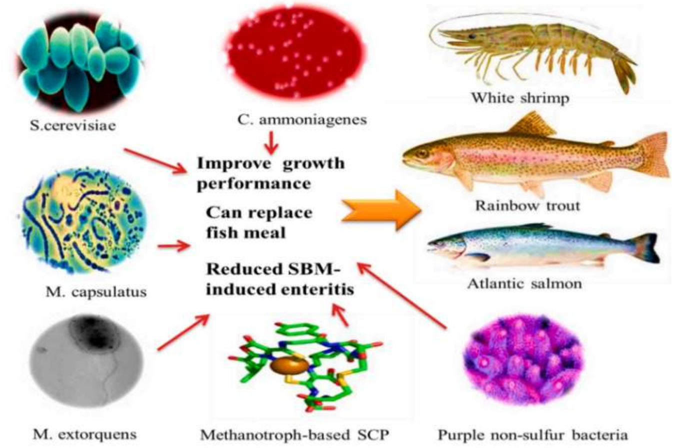


**Fig. 2. Effect of using some sources of SCP in fish feeding**

***Challenges and future perspective:*** Single-Cell Protein (SCP) is becoming increasingly popular due to its minimal land area requirement for growth and its role in waste recycling (Hojaosadati *et al*., 2000). SCP serves as an alternative substrate, addressing pollution issues associated with improper disposal.The activation of endogenous nucleases during microbial biomass production, thermal shock, sodium hydroxide treatment and sodium chloride treatment are some of the chemical treatments that can be used to reduce the high nucleic acid content in SCP, which may cause elevated serum uric acid levels and kidney stone formation. However, certain challenges impact the acceptability of SCP in global food consumption.Concerns include the possibility of skin and gastrointestinal responses leading to nausea and vomiting, the existence of indigestible cell walls in yeast and algae, unwanted colors and flavors and the requirement to destroy cells before to intake in order to prevent reactions (Adedayo *et al*., 2011). SCP also poses challenges in being indigestible for herbivore animals. High ribonucleic acid concentration, production-related contamination hazards and challenges with cell recovery are among of the factors that further reduce the attractiveness of SCP as a food source (Bankra *et al*., 2009). Antinutritional factors and imbalanced amino acid proportions, especially lysine and methionine, also contribute to lower palatability compared to fishmeal for aquatic animals.Furthermore, some of the bacteria employed to produce SCP might produce harmful compounds including cyanotoxins and mycotoxins, which can cause dyspepsia. Careful optimization of fermentation protocols and the selection of appropriate microorganisms and substrates can address these limitations, making SCP usage beneficial.Biofloc formulations, characterized by heterogeneous aggregates of suspended particles and microorganisms, offer benefits such as maintaining good water quality, reducing feed conversion ratios and costs, providing biosecurity, and sequestering greenhouse gases (Dantas *et al*., 2016). Furthermore, SCP can capture ambient $CO_2$ and produce renewable power while producing a protein output that is many times greater than soybean cultivation on the same amount of land. Raising the molecular concentration in SCP may provide chances for less expensive co-products that are high in protein, assisting in the decrease of total production costs. The production of SCP requires continual efforts to develop ways to cut costs while maintaining quality and boosting productivity because to the input costs of carbon and nitrogen supplies (Soto-Sierra *et al*., 2018).

# CONCLUSION

Single-Cell Protein (SCP) is a very attractive dietary supplement that contains protein, carbs, lipids, water and vital components such as potassium and phosphorus. One of its key advantages is its independence from seasonal or climatic variations. SCP provides an answer to the issue of food shortage because of its high protein content and wide range of amino acids, especially for populations that are expanding quickly, especially in developing nations. To further enhance its production, there is a need to explore alternative substrates and methods that can minimize current limitations.Among the systems that might be useful to meet the growing need for SCP is aquaculture. But attaining sustainability calls on the use of creative management strategies, producing SCP using renewable resources and promoting the widespread use of this important nutrient supplement. The effects of SCP include enhanced innate immunity, improved growth and survival performance, altered gut microbiome and reinforced stress tolerance. There is hope for the broad use of SCP products because of ongoing research to find new strains, a variety of substrate types, innovative procedures and successful experiments in different fish species. Therefore, SCP presents itself as a promising protein source for both animals and plants in a financially feasible way, as it has the ability to be generated year-round from a variety of inexpensive substrates with limited adverse effects.

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