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#### Abstract

Single-cell protein (SCP) holds immense promise as a sustainable and nutrient-rich source of protein for both human and animal consumption. Derived from microorganisms such as algae, fungi, yeast, and bacteria, SCP offers high protein content and a favorable amino acid profile essential for health. Algal SCPs like spirulina and Chlorella provide not only protein but also essential fats, vitamins, minerals, and antioxidants, while fungal SCPs such as mycoprotein offer beneficial dietary fiber and vitamins. Bacterial SCPs, like those

#### Introduction

Humans and Animals rely on protein for nitrogen and essential amino acids necessary for building structural and functional proteins crucial for survival, including enzymes and hormones. Proteins can also serve as an energy source under extreme conditions. The nutritional quality of a protein is determined by its amino acid composition, with several amino acids being essential and needing to be obtained through diet. Animal and dairy productions have been increasing to meet growing demands, with a focus on healthier foods with optimal from Methylophilus methylotrophus, are rich in protein and lipids. SCP production involves processing(such as cell various wall degradation and reduce nucleic acid content) to enhance protein accessibility and making them digestible. more readily With ongoing advancements in biotechnology and genetic engineering, SCP production is poised to address global protein needs sustainably while minimizing environmental impact.

**Keywords:** Single cell protein, amino acid, protein, human, animal

amino acid profiles and sustainable production methods. Additionally, protein can be sourced from microbes and algae with high protein content and a balanced amino acid profile, known as single-cell protein (SCP) Figure 1 (Ritala *et al.* 2017). SCP production involves steps like preparing nutrient media, cultivation, separation, and final processing. Efforts are underway to develop SCP production methods using inexpensive waste materials from various industries.



## Agriculture



**Figure 1** Health benefits of Single Cell Protein (SCPs) **SCP production systems, substrates used and processes** 

Algae, filamentous fungi, yeast, and bacteria are viable sources of single-cell protein (SCP). Future advancements may include deriving dietary protein from proteins secreted by engineered microbial cells, such as milk or egg white proteins. Additionally, protein production from animal and plant cell cultures, which are not microbes but not classified as animals or plants either, holds promise for future dietary protein sources.

#### **SCP from algae**

SCP derived from algae is characterized by its high protein content, typically ranging from 60% to 70% (Table 1). Additionally, algae offer essential fats, vitamins (A, B, C, and E), mineral salts, and chlorophyll, with relatively low nucleic acid content (3-8%) (Zimberoff, 2017). Algae-based products, such as spirulina, Dunaliella Chlorella. and salina. are increasingly utilized in various forms, including supplements and food ingredients like pastas and snacks. Companies like Euglena, Algaeon, and TerraVia are actively commercializing algae-derived products, primarily for their nutritional value, with a focus on beta-glucan content and whole-cell products. While algae typically rely on CO<sub>2</sub> and light for growth, some products are produced through traditional fermentation methods. Outdoor algae production in open ponds is common but faces challenges like contamination and weather variability. Indoor photo bioreactors are emerging as a solution to ensure consistent algae supply, especially for aquaculture, where algae serve as a source of omega fatty acids, carotenoids, and protein for animal nutrition.

#### **SCP from fungi**

Commercially available products from fungi like Saccharomyces, Fusarium, and Torulopsis typically contain 30–50% protein (Table 1) (Anupama and Ravindra, 2000). These fungal SCPs boast a favorable amino acid profile, with high threonine and lysine content but relatively low methionine content, albeit still meeting FAO/WHO recommendations. Some fungal products, such as Marmite<sup>®</sup>, exhibit even lower methionine content. Fungal SCPs also provide B-complex vitamins and dietary fiber from glucans in their cell walls. Mycoprotein from *Fusarium venenatum*, utilized in Quorn<sup>TM</sup> products, has shown potential health benefits,





including reducing LDL cholesterol and favourably affecting blood glucose and insulin levels. Although fungi have moderate nucleic acid content (7-10%), it requires processing to reduce levels suitable for human consumption. Quorn<sup>TM</sup> brand, known for The its mycoprotein-based meat substitutes, has been extensively marketed for human nutrition and was acquired by Monde Nissin Corp in 2015. Table 1 Algae, fungi and bacteria producing SCPs, their protein content on specific substrates.

Brewer's yeast extracts, like Marmite<sup>®</sup>, Vegemite<sup>®</sup>, and Torula, provide protein and essential B vitamins, while Torula, rich in glutamic acid, has been used as a flavour enhancer. Additionally, a process called "Pekilo" uses filamentous fungus to produce SCP from sugars found in paper mill effluents, demonstrating potential for sustainable animal feed production.

Algae	Protein content	Fungi	Substrate	Protein content	Bacteria	Substrate	Protein content
	(%)			(%)			(%)
Aphanizomeno	60–75	Aspergillus	Rice bran	10	Bacillus	Ram horn	68
n flos-aquae		flavus			cereus		
Aphanothece	42	Aspergillus	Potato	38-	Bacillus	Potato	46
microscopica		niger	starch processing waste Waste	50	pumilis	starch processing waste	
			liquor				
Arthrospira maxima (Spirulina maxima)	60–71	Aspergillus oryzae	Rice bran (deoiled)	24	Bacillus subtilis	Ram horn	71
Arthospira	46–63	Candida	Cheese	48	Corynobacteri	Glucose +	61
platensis		crusei	whey		ит	fructose	
(Spirulina					ammoniagenes		
platensis)							
Chlorella	45	Candida	Molasses	56	Escherichia	Ram horn	66
pyrenoidosa		tropicalis	Bagasse	31	coli		
Chlorella	46–65	Penicillium	Rice bran	10	Methylococcus	Methane	67–73
sorokiana		citrinum			capsulatus,	(Natural	
					Ralstonia sp.	gas)	
Chlorella spp.	62–68	Saccharomyce s cerevisiae	Orange pulp, molasses, brewer's spent grain	24	Rhizospheric diazotrophs (whole microbial community)	Brewery waste water	>55
Chlorella	42–55	Trichoderma	Cheese	34	Rhodopseudo	Latex	55-65
vulgaris		harzianum	whey filtrate		monas palustris	rubber sheet wastewater	
Euglena gracilis	50–70	Trichoderma virideae	Citrus pulp	32	Cupriavidus necator	Synthetic growth medium	40-46
Scenesdesmus obliquus	30–50	Kluyveromyces marxianus	Cheese whey	43	Methylomonas sp.	Methane salt broth	69
Quorn <sup>TM</sup> and yeast spreads such as Marmite <sup>®</sup> lignocellulosic sugars. Beyond these carbon							

are manufactured using glucose derived from starch, while the Pekilo process employs sources, alkanes and methanol have also been utilized for single-cell protein (SCP) production



by yeast and filamentous fungi. Methylotrophic yeasts like *Komagataella pastoris* (formerly known as *Pichia pastoris*) are capable of producing biomass and protein from methanol. **SCP from bacteria** 

#### Bacterial single-cell protein (SCP) typically boasts a protein content ranging from 50% to 80% on a dry weight basis, with essential amino acid levels meeting or exceeding FAO recommendations (Table 1). Some bacterial SCPs exhibit higher methionine content, up to 3.0%, compared to algal or fungal SCPs. However, bacterial SCPs, similar to fungi, have high nucleic acid content (8–12%), particularly RNA, requires processing before use as food or feed (Nasseri et al. 2011). Alongside protein and nucleic acid, bacterial SCPs provide lipids and B-group vitamins. Imperial Chemical Industries developed Pruteen, an SCP for animal feed obtained from methanol using *Methylophilus* methylotrophus bacteria, containing up to 70% protein and utilized in pig feed. Although, due to competition from cheaper animal feeds in the late 1970s, production ceased. Methane is now emerging substrate of interest for SCP production, with products like FeedKind® and UniProtein® finding application in feed for animals.

#### **SCP key processing**

The processing requirements for single-cell protein (SCP) vary depending on the substrate material and the intended application in feed or food. In the following section, we examine the key processing steps essential for SCP production.

#### **Degradation of Cell Wall in SCP Products**

Some single-cell proteins (SCP) are utilized as whole cell preparations, while others undergo cell wall breakdown to enhance protein accessibility (Trevelyan, 1976). SCP like Quorn<sup>TM</sup> can be consumed without cell wall degradation, allowing chitin and glucan from fungal cell walls to contribute dietary fiber. Euglena-derived SCP, owing to proteinaceous pellicles instead of cell walls, does not require disruption, making them more readily digestible. Various methods. including mechanical forces (crushing, grinding, etc.), hydrolytic enzymes, and chemical detergents, are used for cell wall disruption. However, this process may impact the quality and quantity of SCP components. Products such as Marmite® and Vegemite® are cell extracts obtained by heating cells to 45–50°C, allowing intracellular enzymes to partially hydrolyze the cell wall and reduce proteins to smaller peptides.

# Removal of Nucleic Acids from SCP Products

Algae are typically low in nucleic acid content, but rapidly proliferating bacterial and fungal species exhibit high RNA content. RNA levels and degradation are influenced by growth conditions, growth rate, and carbon-nitrogen ratio. High nucleic acid amounting SCP used for human intake poses health risks due to increased uric acid concentrations, leading to conditions like gout and kidney stones. Methods to decrease RNA content include utilizing endogenous RNA-degrading enzymes (ribonucleases) activated through heat treatment or adding ribonucleases directly during SCP production. Improved methods involve higher temperatures for shorter durations to minimize biomass loss. However, increased temperatures require additional energy input.

#### Safety regulations of SCPs

Like any feed or food product, single-cell protein (SCP) must adhere to safety regulations during production and use. Regulatory frameworks in most regions ensure the safety of feed and food for consumption. These regulations typically differentiate between food for humans, feed for animals, and various additives such as preservatives or colorants. International standards overseen bv organizations like the Joint FAO/WHO Expert Committee Food Additives on govern internationally traded products. Regulations vary based on the intended purpose of the SCP, whether as food, feed, or additives. Some products may enter the market as additives rather than SCP, limiting their potential usage and value as a protein source.

Key concerns during SCP production include managing RNA content, microbial toxins, potential allergic reactions, and contaminants from feedstock such as heavy metals. Technologies have been evolved to reduce RNA content to safe levels. Selecting



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production organisms and optimizing process conditions mitigate toxin production, though fungi producing mycotoxins are unsuitable SCP sources due to associated health risks ranging from allergies to carcinogenesis. Bacteria, like Pseudomonas spp. and *Methylomonas methanica*, may also produce toxins, limiting their use. Utilizing diverse waste materials for SCP production offers cost and sustainability

Future Prospects for User-Genetically Modified Organisms (GMOs) in SCP Production

The use of genetically modified organisms in food and feed faces limited public acceptance despite greater in Europe, acceptance elsewhere. Genetic modification offers numerous advantages for SCP production. For instance, DuPont has engineered yeast to produce long-chain omega-3 fatty acids, essential for human health. Genome sequencing and genetic engineering enable the disruption of genes involved in toxin production, enhancing SCP safety. Compared to traditional genetic modification mutagenesis, offers quicker and more precise gene disruption. Emerging technologies like Clustered Regularly Interspaced Short Palindromic References

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benefits, but safety concerns necessitate careful consideration of feedstock origins. Products like Quorn<sup>TM</sup> ensure safety by utilizing chemically defined mediums and adhering to Good Laboratory Practice (GLP) standards. Public perception and acceptance of wastederived foods are crucial factors when integrating SCPs into human diets.

Repeats (CRISPR) facilitate specific genome editing without introducing new DNA. Genetic modifications can also enhance the nutraceutical value of SCP biomass by optimizing amino acid composition or increasing vitamin and fatty acid content. Tailor-made SCPs with personalized nutritional profiles are possible through genetic engineering. Additionally, genetic modifications can improve protein harvesting methods, such as enhancing flocculation or designing cells with enzymes that degrade cell walls upon specific stimuli, ensuring proteins are released without cell walls.

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