



A REVIEW OF MICROBIAL PROTEIN PRODUCTION: PROSPECTS AND CHALLENGES



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Abstract: The increase in protein malnutrition due to low quality proteins in food supply, especially in developing countries, has stimulated the search for new and alternative sources of protein, both in human foods and animal feeds. The production of microbial protein or single cell protein (SCP) is revolutionizing protein farming and is, indeed, a key step in reducing the shortage of protein supply. These nonconventional protein sources are products of biotechnological processing of agricultural, industrial and forestry wastes. Single cell proteins or microbial proteins are dehydrated microbial cell culture or purified protein derived from a microbial cell culture, such as bacteria, yeast, algae or filamentous fungi, with potential to be a source of animal or human protein supplement. This type of protein has been cultivated by culturing appropriate microbes on different substrates like starch, corn cob, whey, wheat, starch hydrolysates, hydrocarbon, alcohols, molasses and sugarcane bagasse. The manufacturing of SCP as an alternative source of protein has considerable benefits over conventional sources because of its decreased production time, lower land requirement and ability to be produced in all kind of climates. In spite of the obvious advantages of SCP viz., its nutritive value in terms of protein, vitamins and lipid content, it is accepted with some measure of uneasiness and its chances of substituting conventional protein are still slim; its major disadvantage being its high nucleic acid content and low digestibility. This paper reviews the production of single cell protein, its benefits, safety, acceptability, cost and the limitations associated with their uses, as it portends great promise as an alternative source of proteins.

Keywords: Microbial protein, production, prospects, challenges

Microbial protein/single cell protein

Single cell proteins or microbial proteins are dehydrated microbial cell culture or purified protein derived from a microbial cell culture, such as bacteria, yeast, algae or filamentous fungi, with potential to be a source of animal or human protein supplement. Microbial protein or single cell protein comes to market as dehydrated, purified microbes derived mostly from unmixed cultures that are used as a source of protein for human and animal feed because of their high protein concentration, low fat content and high vitamin content especially B vitamins (Kurbanoglu, 2001; Garcia-Garibay *et al.*, 2003., Yalain *et al.*, 2008).

The development of this source of protein production was initiated during World War I and World War II as a substitute for the shortage of conventional food protein using *Saccharomyces cerevisiae* on molasses and *Candida utilis* on sulphite liquor from waste papers (Nasseri *et al.*, 2011). In 1967 SCP was produced industrially on a large scale and currently lots of varieties of microorganisms and many substrates have been used in the production of this type of protein. These microorganisms include; algae such, as *Chlorella* spp and *Spirulina* spp that photosynthesise carbon dioxide, yeasts such, as *Candida utilis* and *Candida lipolytica* which grow on ethanol, filamentous fungi like *Chaetomium cellulolicum* and *Fusarium graminearum* which grow on cellulose waste and starch respectively, and also bacteria such as *Brevibacterium* and *Methylophilus methylotropous* that uses hydrocarbon and methanol respectively as substrates (Nasseri *et al.*, 2011). The fast growth rate bacteria and very high protein value has distinguished them as a potential source of SCP compared to other microbes (Anupama, 2000; Tuse, 1984). These microbes ferment large amounts of waste which serves as their substrate and produce protein (Nasseri *et al.*, 2011). It is more advantageous to use fungi and bacteria in the cultivation of SCP when grown on cheap waste materials.

Production of Single Cell Proteins

The photosynthetic and non-photosynthetic microbes are used in the production of single cell protein grown on raw materials such as hydrocarbon, agricultural or industrial waste products (Litchfield, 1998).

Photosynthetic production of SCP

Algae are the major photosynthetic organisms that are used in the production of single cell protein. They use carbon dioxide as their substrate which is the cheapest, most inexpensive and most abundant source of carbon for microbial growth. Atmospheric carbon dioxide can be supplemented by combustion of gases, carbonate and bio-carbonate to enhance algae growth (Litchfield, 1998, Garcia-Garibay *et al.*, 2003). The sources of nitrogen used for these microbes are nitrates, nitrites, ammonia or urea, even though cyanobacteria are capable of fixing atmospheric nitrogen. They also need phosphorus and sulphur, which are incorporated in organic form, and small quantities of micronutrients such as Iron and chlorine (Litchfield, 1980).

Mass culture open systems and photo-bioreactors are used for cultivation of algae for the production of single cell protein. Mass culture open systems which are the production of algae outdoor use lakes and ponds as cultivation site; good environmental sanitation is maintained to avoid contamination and other factors that will lower the quality of the products. This method has been used to produce *Spirulina* and *Chlorella* (Litchfield, 1980).

Photo-bioreactors are a closed system and either use outdoor or indoor systems where a single species is cultivated. Indoor system is preferable because it allows better prevention of contaminants than outdoor culture system leading to greater yield and high quality of the product (Ugwu *et al.*, 2008). This method can be used to cultivate *Chlorella*, *Spirulina* and *Scenedesmus*

Harvesting of the microbes (microbial harvest) is extensive work especially when cultivated in large area such as lakes or when low production of the microalgal biomass occurs. Species like *Spirulina* and *Coclastrium* can be scooped or removed from the top of water, but in most situations harvest involves filtration using cloths or screens before centrifugation. It is then sun-dried, spray-dried or drum-dried and then packaged and sold as a nutritional supplement in the category of functional foods (Garcia-Garibay *et al.*, 2003).

Non-photosynthetic microorganism for production of SCP

Non photosynthetic organisms that are used in the production of single cell protein include bacteria and yeast. These microbes have gained wide acceptability in the production of single protein because of their notable use in bread baking, milk fermentation in cheese production and in the alcoholic beverage industry (Garcia-Garibay *et al.*, 2003).

Use of bacteria for SCP production

Bacteria were found to be more effective in the production of SCP because of their short doubling time as compared to those of yeast, but have been largely discontinued because of their toxicity and low consumer acceptability (Anupama, 2000). The carbon source of bacteria species used in the production of SCP is mainly hydrocarbon, ethanol, methanol, carbohydrate, cellulose, starch and simple sugars. Methanol is the preferred substrate as a carbon source for the bacteria because it is soluble in water, non-explosive, free of impurities and easy to remove from the microbial product (Moulin *et al.*, 1983; Ivarson and Morita, 1982). The bacterial organisms used are *Methylomonas methanolica*, *Methylophilus methylotrophus* and *Pseudomonas* spp (Singh, 1998; Anupama, 2000).

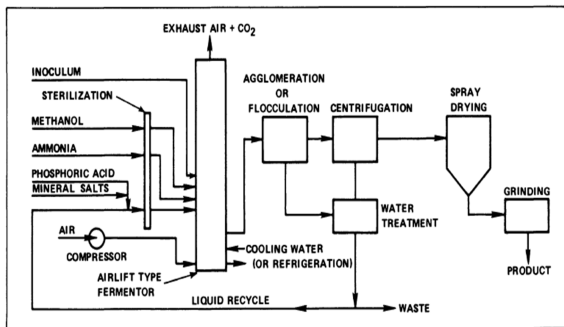


Fig. 1: Production of bacteria from methanol substrate (Litchfield, 1980)

Fig. 1 shows the production of bacteria cells using methanol substrate. All the nutrients are sterilised and bacterial cell produced in the airlift-type fermenter. The cells are concentrated by flocculation to remove water. The cells are then centrifuged; spray dried, grind and the product packaged.

Use of yeast for SCP production

The most frequent and commonly used microorganism in single cell protein is yeasts, such as *Saccharomyces cerevisiae*, *Kluyveromyces maxianus* and *Candida utilis* because of their good acceptability by the consumers. *C. utilis* grows on sulphite liquor substrate, while *K. maxianus* grows on milk whey substrate. These substrates are cheap carbon and energy source, produced from waste

product in the paper industry and they contain large amount of organic carbon compounds (Batt and Sinskey, 1987).

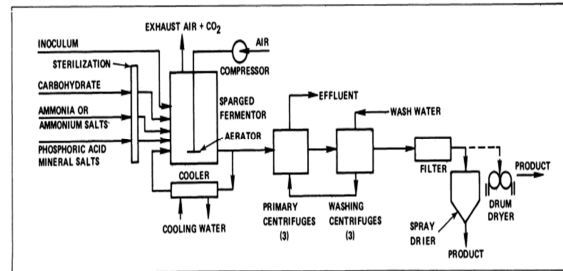


Fig 2: Production of yeast from carbohydrate substrate (Litchfield, 1980)

Fig. 2 shows the production of yeast from carbohydrate. All the nutrients used are processed under a sterilized condition and yeast cell produced in sparged fermentor. The yeast cells produced are centrifuged, filtered, spray dried or drum dried and products packaged.

Removal of Nucleic acid content and cell wall of SCP

The production of single cell protein from bacteria for human consumption requires, reducing the nucleic acid content of the cell and removal of the cell wall. The reason for reducing nucleic acid content is because high content of the nucleic acid can lead to formation of uric acid which causes gout whereas the cell wall has very poor digestibility in humans.

Nucleic acid removal in SCP

This involves both the use of chemicals; such as alcohol, acid, alkali, and enzymatic methods which includes the use of nucleases and endogenous ribonucleases which hydrolyses the ribonucleic acid and reduces nucleic acid levels (Kunhi and Rao, 1995).

Removal of cell wall

Mechanical and non mechanical methods are used in removal of the cell wall. The mechanical methods involves the wet milling, sonification, high pressure homogenization, decomposition crushing and grinding (Middleberg, 1995), while non mechanical methods are the use of chemical treatment using acid, base or detergents. Enzymatic methods such as autolysis and physical treatment such as freeze-thaw, heating and drying are used (Baldwin and Ribinson, 1994; Benaiges *et al.*, 1989). These processes result in production of large amounts of single cell protein from the microbial cell and organelles (Nasseri *et al.*, 2011).

Table 1: Comparison of SCP production from different microorganisms

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 lignocellulosics
pH range	Upto 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
S-containing amino acids	Low	Deficient	Deficient	Low
Nucleic acid removal	-	Required	Required	Required
Toxin	-	Endotoxins from gram-negative bacteria	-	Mycotoxins in many species

Sources: Singh (1998)

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Table 1 shows how SCP can be produced from different microorganisms using the same parameters. It also indicates the risk of contamination, in algae and bacteria; deficient of sulphur containing amino acids in bacteria and yeast, and requirement of removal of nucleic acid content from bacteria, yeast and fungi.

Nutritional value of single cell protein

It has been observed that single cell protein contains a very high protein content that compared favourably with conventional protein sources, like soybean and fish meal. Bacteria single cell protein contents range from 50-100%, yeast from 45-70% and algae from 45-75%, these protein sources are higher in protein content as compared with soybean 40%. They are also very acceptable when compared with vegetable protein contents (Garcia-Garibay *et al.*, 2003). Generally single cell proteins from all sources are very limited in sulphur-containing amino acids such as methionine and cysteine but when supplemented with these amino acids their protein quality accelerates and get close to that of casein. The vitamin contents of single cell protein such as thiamine, riboflavin, pyridoxine, folic acid, niacin and carotenes are higher in some microbial proteins compared to some vegetable foodstuffs (Frazier and Westhoff, 1990).

Table 2: Comparison of composition of SCP from algae, fungi and bacteria

Component	Percentage composition of weight		
	Algae	Fungi	Bacteria
True proteins	40-60 ^a	30-70 ^a	50-83 ^a
Total nitrogen (Protein + nucleic acids)	4.5-6.5 ^a	3.5-5.0 ^a	6.0-8.0 ^a
Lysine	4.6-7.0 ^a	6.5-7.8 ^a	4.3-5.3 ^a
Methionine	1.4-2.6 ^a	1.5-1.8 ^a	2.2-3.0 ^a
Fats/Lipids	5-10 ^a	5-13 ^a	8-10 ^a
Carbohydrate	9	NA	NA
Bile pigment and Chlorophyll	6	NA	NA
Nucleic acids	4-6 ^a	9.70	15-16 ^a
Mineral salts	7	6.6	8.6
Amino acids	NA	54	65
Ash	3	NA	NA
Moisture	6.0	4.5-6.0 ^a	2.8
Fiber	3	NA	NA

^a The yield varies with the type of substrate used, the specific organism used and the culture conditions maintained.

NA- Not available; Source: Anupama (2000)

Table 2 shows percentage weight composition of SCP proteins produced from algae, fungi and bacteria. Bacteria have the highest true protein content followed by fungi and algae. There is reasonable percentage content of Chlorophyll, fiber and ash in algae but these components are not present both fungi and bacteria.

Advantages of Single Cell Protein

Single cell proteins are used in food industries such as baking bread, preparation of cookies, noodles, meat product and baby meal as a protein supplements (Garcia-Garibay *et al.*, 2003). For the following advantages Listed below;

- i) It has very high protein contents, vitamins especially B-complex vitamins (yeast), amino acids and low fat content.
- ii) They can be modified genetically to produce amino acid of specific interest.
- iii) There are no constraints in the production as they can be produce throughout the year since growth is independent of climatic and seasonal changes.
- iv) They use waste materials (El-Nanwwi and El-Kder, 1996), as their substrate in producing this protein thereby helping to reduce pollution by recycling waste materials.
- v) They grow faster producing large amounts of SCP in small portions of land within short period of time (Anupama, 2000; Litchfield, 1980).

Safety and acceptability of single cell protein

Single cell protein for human consumption or animal feed must be free from all kinds of pathogens, toxins, contaminants from heavy metals or other metal compounds, hydrocarbons and free from the risk of causing food allergies or cancer. It has been observed that most foreign proteins are not suitable for human consumption because of their ability to cause allergens, gastro-enteric disturbance, diarrhoea and vomiting (Anupama, 2000). The high content of nucleic acid in bacterial cells can also cause urinary disease such as kidney stone formation or gout and should be reduced to the minimal level before consumption. Therefore from this viewpoint it is very essential to use toxicological studies to evaluate the safety of any produced single cell protein before marketing the products (Litchfield, 1980). *Spirulina* and *Chlorella* however, are widely acceptable and sold for human consumption and are produced in countries like Japan, Israel, Thailand and United States (Trehan, 1993). The acceptability is very low especially the bacteria SCP because of a generally thinking of the masses that bacteria are harmful and can cause diseases.

Economics and market of single cell proteins

Microbial proteins or single cell proteins are developed and produced with the sole aim of reducing world hunger and protein malnutrition (Khan and Dahot, 2010). For this reason it must compete with conventional protein sources with the factors such as cost of production including; energy used in production, investment, operational unit cost safety of using waste materials as the source of its substrate, acceptability by the populace and its market price (Garcia-Garibay *et al.*, 2003). The major market for single cell protein is its use for animal feeds. Although it competes poorly with soya protein as soya is 50% lower in price as compared with the price for SCP. It is anticipated that with efficient, improved fermentation and downstream processes there will be reduction in the elevated price of SCP to minimal level (Litchfield, 1991).

Disadvantages of Single Cell Protein/Limitations

Bacteria, Algae, Fungi and Yeast

Bacteria

It has been observed experimentally that bacterial SCP has elevated nucleic acid content (Anupama, 2000). The high content of the nucleic acid can lead to formation of uric acid when metabolised which can accumulate in the body due to a lack of Uricase in humans, the enzyme used to metabolise it. This can lead to severe diseases such as gout.

There are also very high risks of contamination of the product during production with heavy metals, or methanol which can lead to disorders in body function and may also cause cancer.

Bacterial toxins can be hazardous to humans when consumed, causing fever, lesions, vomiting and may also lead to paralysis.

Bacterial proteins are also very poor in sulphur containing amino acids, methionine and cysteine which are essential amino acids in human body.

Bacterial sizes are very small and they also have low density, this makes their harvest expensive (Anupama, 2000).

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Algae

It has very low density.

It contains cell wall which has very poor digestibility in humans.

There is high risk of contamination during production (Garcia-Garibay, 2003).

Fungi and Yeast

They have the potential of containing mycotoxins and neurotoxin which are capable of causing allergy, and adverse nervous reactions.

They have very low content of methionine and cysteine.

There is tendency of having high contaminants.

They also have high nucleic acid content (Anupama, 2000).

Conclusion

Single cell protein, or microbial protein, is a potential source of protein for human food as it gives promising fate as an alternative source of protein. The use of microorganisms in the cultivation of this protein have many advantages over the conventional protein including, their short doubling time, easy cultivation, utilization of many cheap/widely available substrates as energy sources, small land mass for propagation as well as being able to adapt to climatic changes. Although SCP can be used as a source of protein, it is not without challenges which currently prevent it from competing with conventional proteins. These challenges include its high nucleic acid content when produced with bacteria, possibility of causing diseases, poor digestibility, and high cost of production due to substrate cost, utilities, capital loads and product-specific variables. These problems can be minimised by improving appropriate fed-batch fermentation and developing cheaper down-stream processes, using genetically engineered microorganisms with improved substrate utilization. Also the use of efficient toxicological tests will enhance and improve its acceptability, making its price affordable by the consumer when compared with conventional proteins.

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