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MICROORGANISMS: A MARVELOUS SOURCE OF SINGLE CELL PROTEINS

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ABSTRACT

The increasing global population living below the poverty line is driving the scientific community to search for non-conventional protein sources that can replace conventional expensive ones. Microbial proteins, or single-cell protein (SCP), represent a potential future nutrient source for human food and animal feed. These microbial proteins can be grown rapidly on substrates with minimum dependence on soil, water and climate conditions. They can be produced from algae, fungi and bacteria the chief sources of SCP. It is convenient to use microorganisms for production of SCP as they grow rapidly and have high protein content. Industrially, they can be produced from algal biomass, yeast, fungi. There are several other ways of getting SCP as well. Despite numerous advantages of SCP, they have disadvantages and toxic effects too, especially related to mycotoxins and bacterial toxins.

Keywords: Non conventional, single cell protein (SCP), microbial protein, disadvantages, toxins

INTRODUCTION

"The food supply increases in arithmetic ratio but the population of animals increases in geometric ratio," was very well stated by Thomas Robert Malthus, in The Principles of Population. The scarcity of protein-rich food and the existence of millions of poor people around the world have forced mankind to search for alternative protein sources that can replace conventional but expensive, soy meal or fishmeal. Hence, the focus has shifted towards relatively cheaper sources such as single-cell proteins (SCP) **(Anupama and Ravindra, 2000)**. SCP are mixed proteins extracted from pure or mixed cultures of algae, yeasts, fungi, and bacteria, etc. which are grown on agricultural wastes. SCP are used as a substitute for protein-rich foods, in human and animal feeds.

This mini review describes the sources, properties and the production of SCP from microorganisms. This mini review will also outline the toxic effects of SCP.

HISTORY

The production of single-cell protein is not a recent development. Since 2500 B.C. a number of microorganisms have been used as a part of the diet in the form of fermented food (*Sacharomyces* sp.) **(Frey, 1930)**. Cultured dairy products contain 10^7 to 10^{10} lactic acid bacteria per gram of product. During the first century B.C., edible mushrooms were extensively consumed in Rome. In the 16^{th} century blue green algae (*Spirulina*) was consumed as a major source of protein **(Clement, 1968)**. The mass production of micro-organisms as a direct source of microbial protein was realized during World War-I in Germany, and consequently, modern baker's yeast (*Sacharomyces cerevisiae*) was produced. In the 1960s, researchers at British Petroleum developed a "proteins-from-oil process", a technology for producing microbial protein using yeast fed with waxy n-paraffin, a byproduct of oil refineries. The method had a capacity to produce 10,000 tons of microbial protein per annum **(Carter, 1981)**. The term "microbial protein" was replaced by a new term "Single Cell Protein" (SCP) in 1966 by Carol L. Wilson **(Robert, 1989)**.

PROPERTIES OF SCP

Single-cell organisms comprise proteins, fats, carbohydrates, ash ingredients, water and other elements such as phosphorus and potassium. The composition depends upon the organism and the substrate on which it grows. There are some typical compositions which are compared with soya meal and fish meal **(Algur and Kalp, 1991)**. If SCP is to be used successfully, there are five main properties that must be satisfied. It must be safe to eat. It must have a high nutritional value, particularly of amino acid **(Bhalla *et al.*, 2007)**. It must be acceptable to the general public. It must have the functionality, i.e. characteristics, which are found in common staple foods. It must be economically viable to produce.

MAIN CRITERIA TO BE SCP

In order to produce the single-cell protein, some key factors must be keep in mind so that a good amount of pure, stable, and highly nutritious SCP can be produced **(Singh, 1998)**. The key criteria used in selecting suitable SCP include that any microorganism which is used as SCP must be easy to modify genetically so that any desired improvement can be made. Protein content should be 45-85% of the dry cell weight. The microorganism must have a short life cycle so that a large amount of SCP can be produced in a short time. The microorganism must be pH and temperature tolerant. It must be easy to grow in a number of cheap substrates, especially waste products, so that a cost-effective SCP can be produced. Microorganisms should be genetically stable so that the strain with optimal biochemical and physiological characteristics can be maintained easily. The microorganism must have high specific growth rate, productivity and yields on a given substrate. It must be resistant to change in environmental conditions so that small variations in the environmental conditions do not decrease the production of SCP. Aeration requirements and foaming characteristics should be well studied before the production of SCP at industrial scale. Protein, RNA and nutritional composition of the product should adhered to recommended parameters. Structural properties of the final product should be well suited for consumption **(Scrimshaw and Dillen, 1977)**.

SOURCES

It is convenient to use microorganisms for production of SCP as they have rapid growth and high protein content. The main sources are:

Bacteria as SCP

Purple photosynthetic bacteria e.g. *Rhodopseudomonas capsulate*, *R. palustris,* are frequently among the predominant populations of microorganisms in ponds, ditches and other water sources, polluted by sewage or other types of organic matter **(Pfenning, 1967)**. Previous investigators, including Hirayama (1968) **(Hirayama, 1968)** and Shipman *et al.* (1975) **(Shipman *et al.*, 1975)**, have suggested the cultivation of photosynthetic bacteria in municipal and agricultural wastes and attempts have been made to grow photosynthetic bacteria on effluents of biogas plants **(Hirayama, 1968; Shipman *et al.*, 1975; Vrati and Verma, 1983)**. Studies have shown that the biomass of photosynthetic bacteria is not only rich in high-quality protein but also contains significantly large amounts of carotenoid pigments, biological cofactors and vitamins **(Vrati, 1984).**

Fungi as SCP

Many fungal species are good sources of protein, e.g. *Candida*, *Hansenula*, *Saccharomyces* **(Anupama and Ravindra, 2000)**. The most widespread and commonly used substrates for SCP production have been those where the carbon and energy source is derived from carbohydrates. This is due to the fact that their building blocks (mono and disaccharides) are natural microbial substrates and that carbohydrates are a renewable resource which is widely distributed. Molasses is a by-product of the sugar manufacturing process **(Oura, 1983)**. Besides its high sugar content, molasses contains minerals, organic compounds and vitamins which are valuable nutrients in the fermentation processes. In fact, about 9% of the dry matter in yeast grown on molasses has been estimated to originate from substances other than sucrose **(Olbrich, 1973)**. Nevertheless, biomass production from molasses requires supplementation with a suitable nitrogen source, as well as phosphorus. The traditional nitrogen sources used are ammonia or ammonium salts and phosphorus can be added in the form of salts **(Ugalde and Castrillo, 2002)**.

Algae as SCP

Algae are a source of high value biological molecules such as proteins, polyunsaturated fatty acids, and pigments. For protein production, there are three species most commonly used with a higher commercial value: *Chlorella*, *Spirulina* (Arthrospira) and *Dunaliella*, with 55%, 65% and 57% protein content, respectively.

Agricultural facilities and agro-industries have encountered a serious problem due to the co-products and by-products generated in their operation. The recovery of these products to produce single-cell protein (SCP) from algae may pose a solution to this problem, because the composition of agricultural by-products is similar to the ideal medium for the growth of microorganisms, e.g. the vinasse from ethanol distillation (from beet and cane molasses fermentation) is a brown liquid, which contains mostly organic matter and a high amount of inorganic salts. Its composition is similar to that of the culture medium for *Spirulina*, hence the interest in using this corrected medium for the production of this algae **(Benito *et al.*, 2009)**.

PRODUCTION PROCESS

Currently, various governments, institutions and companies are spending a huge amount of money and time on the development of a cost-effective process for the production of SCP at an industrial level **(Nasseri *et al*., 2011)**. The production of SCP by mass culture of microorganisms is in its infancy. Single-cell proteins develop when microbes ferment waste materials (including wood, straw, cannery and food processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta).The problem with extracting single-cell proteins from the wastes is the dilution and cost **(Vrati., 1984)**. They are found in very low concentrations, usually less than 5%. Engineers have developed ways to increase the concentrations including centrifugation, flotation, precipitation, coagulation and filtration, or the use of semi-permeable membranes (**Ivarson and Morita, 1982)**. There is a variety of SCP which can be produced. Some of them are as follows:

SCP production from algal biomass

Algae grow auto-trophically and synthesize their food by taking energy from sunlight or artificial light, carbon from carbon dioxide, and nutrients from carbohydrates present in the growth medium **(Oswald, 1988)**.

Chlorella strains are being used for a variety of applications in biotechnology. Due to their high protein content, they serve to improve protein intake and can be used as feed for production of animal protein. *Spirulina* is another algal SCP which is extensively grown in a many developed countries, such as Japan, the United States, and European Countries. This alga is sold in the market under brand name *Spirulina Powder* **(Ciferri, 1983)**. Mass cultivation of *Spirulina* has several advantages over other alga, such as *Chlorella* and *Scenedemus,* etc. because it is easy to grow, less prone to contamination, easy to harvest and easy to digest **(Becker and Venkataraman, 1984)**.

SCP production from yeast

During World War I, rapid development took place in the biotechnological applications of *S.cervisiae,* as far as culture development, process optimization and scale up of products were concerned. World production of yeast biomass is on the order of 0.4 million metric tons per annum, including 0.2 million tones of Baker's yeast alone **(Pirt and Kurowski, 1970)**. Yeasts synthesize amino acids from inorganic acids and sulphur supplemented in the form of salts. They get carbon and energy from organic wastes **(Bennett and Keller, 1997)**. Maul *et al.* (1970) showed the process of getting SCP, with the help of flour milling and baking RHM Mycoprotein process with the help of *Fusarium graminearum* **(Maul *et al*., 1970)**.

Production of SCP from fungi

Mushrooms are the higher fungi, belonging to the classes *Ascomycetes* (*Morchella,* Tuber, etc.) and *Basidiomycetes* (*Agaricus, Auricularia, Tremella,* etc.). They are characterized by having a heterotrophic mode of nutrition **(Riviere, 1977)**. They are rich in protein and constitute a valuable source of supplementary food. The great value in promoting the cultivation of mushroom lies in their ability to grow on cheap domestic, industrial and agricultural waste that is rich in nitrogen and carbon compounds **(Chang and Hayes, 1978)**.

OTHER MAJOR POTENTIAL PRODUCTION SOURCES

SCP from Kefir microflora

Kefir is a mixed culture that ferments lactose. In Eastern-European countries, kefir is majorly known for the production of a fermented beverage, which they get by inoculating milk with kefir grains. The kefir grain is a symbiotic association of microorganisms belonging to a diverse spectrum of species and genera including lactic acid bacteria (*Lactobacilli*, *Lactococci*, and *Leuconostoc*), yeasts (*Kluyveromyces*, *Candida*, *Saccharomyces* and *Pichia*) and sometimes acetic acid bacteria (*Acetobacter*). This micro flora is embedded in a resilient polysaccharide matrix (kefiran) composed of equal amounts of glucose and galactose **(Paraskevopoulou *et al.*, 2003, Luis *et al.*, 1993, Rea *et al.*, 1996).**

SCP from sewage sludge

The single-cell protein in activated sludge from biological wastewater treatment represents the only source of essentially unutilized protein large enough to alleviate the worldwide shortfall. However, economical processes for extracting it on an adequate scale need to be developed. Thermal treatment has inherent advantages of low treatment costs and production of no additional waste streams. Digestion in sealed tubes at 150-155°C and ambient pressure for 20 min resulted in optimal extraction of high molecular weight protein. You can extract 20-60% of protein from sewage sludge **(Shier and Purwono, 1994).**

SCP from Rice polishing with the help of *Candida utilis*

Candida utilis (A fungus) has been frequently used in biomass production because of its ability to utilize a variety of carbon sources and to support a high protein yield. It has been used for production of several industrial products both for human and animal consumption **(Otero *et al.*, 1998, Zayed and Mostafa, 1992, Rajoka *et al.*, 2006)**. SCP from rice polishing contains fairly good quality protein which is rich in all essential amino acids such as lysine, leucine, and isoleucine. Rice polishing can be used for the bulk production of SCP economically for fortification of livestock or poultry feed **(Rajoka *et al.*, 2006).**

SCP from poultry processing waste

Poultry manure can be upgraded by controlled fermentation to a high protein material which may have potential value in the feeding of monogastric animals and birds. When the SCP of aerobic fermented manure was analyzed, it was found that the protein content was 40% and of amino acids, lysine made up 3.4%, methionine 1-1.16% and cytosine 0.16% **(EL-Boushy and Poel, 2000)**.

In the processing of poultry for the production of prepared foods such as soups and frozen dinners, wastewater is generated which contains significant quantities of fats, protein and starchy materials. These materials must be removed from the wastewater before it is discharged. Typical recovery consists of removing the solids through a combination of dissolved air flotation and filtration. After the removal of these solids, it can be processed and then used as an animal feed **(Najafpour *et al.*, 1994)**.

SCP from cereal by-product

Production of SCP from cereal by-products depends to a large extent on the cellulase enzymes of the fungus *Aspergillus niger* and *Aspergillus terreus* **(Sternberg, 1976)**. Wheat bran is the best cereal by-product for SCP production and compared favorably with glucose as a carbon source for SCP production by *A. niger*. Milling cereal wastes to fine particles tended to enhance the production of SCP. Ammonium chloride is the best nitrogen source. A concentration of 1.5 and 2.0% of wheat bran in the growth media were recommended for the production of SCP by *A. terreus and A. niger,* respectively **(Gibriel *et al.*, 1981)**.

SCP from marine yeast

High protein content, high carbohydrate content, and good amino acid composition characterizes the marine yeasts. All the marine yeast strains lack the

20:5n-3 and 22:6n-3 fatty acids, making them unsuitable as a complete diet for larval rising **(Brown *et al.*, 1996)**. It was found that the marine yeast *Cryptococcus aureus* G7a, which can grow on a wide range of carbon sources and secrete a large amount of inulinase into the medium, can be used as a candidate of single-cell protein because 10.1 g of cell dry weight per litre of medium and 53.0 g of crude protein per 100 g of cell dry weight (5.4 g/L of medium) have been achieved **(Zhang *et al.*, 2009, Gao *et al.*, 2007, Sheng *et al.*, 2007).**

SCP from petroleum products

Bamberg (2000), Dieter *et al.* (1972), and Michel *et al.* (1987) demonstrated the process of getting SCP from petroleum using *Candida* species **(Bamberg, 2000, Dieter *et al.*, 1972, Michel *et al.*, 1987).**
One recent study was undertaken in the University of Technology, Baghdad. The study was based on producing SCP from a petroleum fraction such as ethanol, kerosene, or gas oil, by growing two types of microorganisms, viz. *Candida sp.* and *Bacillus subtilis,* on these materials as an energy and carbon source. The maximum protein content was 61.25%, but the sustainability of getting SCP from petroleum product is still uncertain **(Abduljabbar *et al.*, 2009)**.

NUTRITIVE VALUE

The nutritive value of SCP from any source is based on its composition **(Anupama and Ravindra, 2000)**. Algae are rich in protein, fats and vitamins A, B, C, D and E. Vitamin B is found in *Ulva*, *Enteromorpho*, *Laminaria, Alaria valida* and *Porphyra*. Vitamin C is present in *Ulva*, *Enteromorpho*, and *Alaria valida*. Algae also contain 40-60% protein, 7% mineral salts, chlorophyll, bile pigments, fiber, and have very low nucleic acid content (4-6%) **(Brock, 1989)**. The milk-yielding capacity of cattle is reportedly enhanced when Pelvetia is an ingredient in cow feed **(Vashista, 1989)**.
Fungi have a low nucleic acid content (9.7%). Yeast contains thiamine, riboflavin, biotin, niacin, pantothenic acid, pyridoxine, choline, streptogenin, glutathione, folic acid and p-amino benzoic acid **(Ugalde and Castrillo, 2002)**. Fungi contain 30-70% proteins (depending upon the types of substrates used, the specific organism used). If the nucleic acid content in fungi is controlled, then it is more nutritious than algae **(Anupama and Ravindra, 2000)**.
Bacterial SCP is high in protein and essential amino acids. The crude protein content is around 80% of the total dry weight. The nucleic acid content, like RNA, on a dry weight basis is very high and is reported to be 15-16%. Bacterial SCP is rich in methionine, around 2.2-3.0%, which is comparatively higher than that of algae (1.4-2.6%) and fungi (2.5-1.8%) **(Brock, 1989)**.

LIMITATIONS FOR USE OF SCP FROM VARIOUS SOURCES

There are various limitations on SCP which hinder it from being used for human consumption, in spite of the fact that it is a good nutritional source. In algae, the most important limitation is the presence of the algal cell wall. Humans lack the cellulase enzyme and hence they cannot digest the cellulose component of the algal wall. In order to be used as food for humans the algal walls must be digested before the final product is eaten **(Anupama and Ravindra, 2000)**. Various methods, such as treatment with lytic enzymes, high pressure homogenization, sonification etc., can be used for breaking the algal cell wall **(Nasseri *et al.*, 2011)**. Mycotoxins in certain fungal species, especially *Aspergillus parasiticus* and *A. flavus* are known to produce allergic reactions, diseases and liver cancer in humans as well as animals. Hence, it is a condition that mycotoxins are eliminated before fungal SCP is consumed **(Anupama and Ravindra, 2000)**. Use of bacterial SCP is limited due to its high cost. Harvesting protein from bacteria is costly due to the smaller cell size **(Trehan, 1993)**.

PRESENCE OF TOXIC COMPOUNDS IN SCP

Nucleic acids

Intake of a diet high in nucleic acid leads to the production of uric acid from nucleic acid degradation. Uric acid accumulates in the body due to a lack of the uricase enzyme in humans. Hence, nucleic acids in different SCPs should be reduced to acceptable limits if they are to be used as food. Bacterial SCP products may have nucleic acids as high as 16% of dry weight. Human consumption greater than 2 g nucleic acid equivalent per day may lead to kidney stone formation and gout **(Calloway, 1974)**.

Toxins

If Toxins are present in SCP, then they can act as contaminants. Possible toxins include secondary metabolites produced by certain fungi **(Bennett and Keller, 1997)** and bacteria **(Blancou *et al.*, 1978)** during growth. The types of algae that could be used for SCP production generally do not produce harmful toxins **(Anupama and Ravindra, 2003)**. The toxicity of the SCP product must be assessed before marketing.

a) Mycotoxins

Aflatoxins are the most important and best searched of the mycotoxins. The major ones include aflatoxins of type B1, B2, G1 and G2 from *Aspergillus flavus*, citrinin from *Penicillium citrinum*, and trichothecenes and zearalanone from *Fusarium* species **(Bennett and Keller, 1997)**. There is strong epidemiological evidence linking aflatoxins to human liver cancer **(Eaton and Groopman, 1994)**. Research in the removal of mycotoxins from SCP has focused chiefly on aflatoxins. Among the many methods tested, ammoniation is the most successful. It can reduce aflatoxin concentration by 99% **(Park and Liang, 1993)**.

b) Bacterial toxins

Bacteria produce either endo or exotoxins. Exotoxins are secreted by gram-positive bacteria. They do not produce fever in the host but cause various kinds of symptoms and lesions. The toxins are fatal for laboratory animals at nanogram levels **(Anupama and Ravindra, 2003)**. Endotoxins are an integral part of the cell walls of gram-negative bacteria and are liberated upon lysis. They are lipopolysaccharides, and the Lipid A portion is responsible for the toxicity. Generally they produce fever in the host and are fatal for laboratory animals at slightly higher doses than exotoxins **(Powar and Daginawala, 1995)**. Exotoxins can be removed from various processes. They are sensitive to temperatures above 60°C. Formaldehyde can convert exotoxins into non-toxic compounds **(Powar and Daginawala, 1995).**

CONCLUSION

Scarcity is the overwhelming reason why new protein sources need to be sought, as we cannot continue to rely on the inefficient production of animal proteins and plant related proteins. SCPs are a new high quality protein sources, suitable for human and animal consumption. SCP products should be popularize because in spite of hard core research, SCPs are still not popular on ethical grounds. Consumer resistance (at least in developed country markets) is very hard to overcome.
Sustainability is another big problem in SCP production that needs to be overcome. SCP production from waste products would need to be nutrient rich, e.g. adequate supply of nitrogen and phosphorus. There is a looming world-wide phosphate shortage and so supplying phosphates to the system would not be a sustainable proposition. The shortage of nutrients, especially phosphorus introduces the problem of nutrient recycling, which also needs to be overcome. Is it possible to recycle 100% of the system's phosphorus (or other nutrient) needs? In my point of view, top-ups would be needed, which would decrease the cost effectiveness of SCP.
Even so, SCPs look like they will play a key role in the future of nutrition, if we can popularize the products and assuage fears about quality control.

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