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Review Article

Production of single-cell protein from different substrates

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ABSTRACT

Single-cell proteins (SCPs) are dried cells of bacteria, algae, yeast, and fungi, which are rich in proteins and could be derived from waste organic matters (substrates) using microorganisms and also used as dietary supplements. The increasing number of population of the world is suffering from shortage of protein so that exposed to related deficiency diseases. The main points in the production of SCP are preference of cost-efficient substrates and non-toxic or non-pathogenic microorganisms. Therefore, this paper critically reviews on the available literature on SCP productions from different substrates.

Keywords: Bacteria, fermentation, single-cell protein, substrate

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INTRODUCTION

The development of single-cell protein (SCP) production contributed to the world in providing protein-rich food replacement. The increasing world population has been facing challenges in deficiency of protein due to food shortage. For this reason, in 1996, new sources mainly bacteria, yeast, fungi, and algae were used to produce protein biomass named SCP. The production involved large number of various kinds of substrates and different microorganisms.

The term SCP was coined in 1966 by Carol L. Wilson. SCP is dried cells of microorganism, which are used as protein supplements in human foods or animal feeds. Besides high protein content (about 60–82% of dry cell weight), SCP also contains fats, carbohydrate, nucleic acids, vitamins, and minerals. Another advantage with SCP is that it rich in certain essential amino acids likes lysine and methionine which are limiting in most plant and animal foods. Microorganism such as bacteria, yeast, fungi, and algae utilize inexpensive feedstock and waste to produce biomass, protein concentrate or amino acids. Conventional substrates such as starch, molasses, fruit, and vegetable wastes have been used for SCP production, as well as unconventional ones such as petroleum by-products, natural gas, ethanol, and methanol and lignocellulose biomass. The protein obtained from microbial source is designated as SCP.

Many investigations were carried out using cellulose and hemicellulose waste as a suitable substrate for increasing SCP production. Several raw materials have been considered as substrate (carbon and energy sources) for SCP production.[1] Further in many cases, these raw materials have been hydrolyzed by physical, chemical, and enzymatic methods before use. Various hydrocarbon, nitrogenous compounds, polysaccharides, and agricultural wastes[2,3] from plants and fibrous proteins such as horn, feather, nail, and hair from animals have thus been used for the production of SCP.[4] Another investigation clearly revealed that for *Saccharomyces cerevisiae*, banana skin was the best substrate followed by that of rind of pomegranate, apple waste, mango waste, and sweet orange peel. Various forms of organic waste such as cellulose hemicelluloses, hydrocarbon, and different types of agricultural waste were used in the production of SCP.[5]

Research on SCP technology started a century ago when Arora *et al*. found out the high value of surplus brewer's yeast as a feeding supplement for animals. During the World War, first, SCP technology proved to be more than useful as Germany used it to replace more than half of its imported protein sources by yeast. A method named Zulaufverfahren in which sugar solution was fed to an aerated suspension of yeast invented instead of adding yeast to dilute sugar solution. Moreover, *Candida arborea* and *Candida utilis* were used during the

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Second World War and about 60% of the country replaced the food input.[6]

In the 1960s, researchers at British Petroleum developed a technology called proteins from oil process for producing SCP by yeast fed by waxy n paraffin, a product produced by oil refineries.[7] Initial research work was done by Alfred Champagnat and BP s Lavera, oil refinery in France; a small pilot plant there started operations in March in 1963, and the same construction of the second pilot plant, at Grangemouth oil refinery in Britain was authorized.[8] In microbial protein production, several natural products have been tested. The use of natural cheap substrates and waste industrial products for cultivating microorganisms appear to be general trend in studies of applied nature.[9,10] For the same purposes Haider and EL Hassy,[11] tested date extract supplemented with nitrogen source as a suitable substrate whereas, cashew and apple juice was used by Osho.[10]

In the future, SCP production will be heavily dependent on reducing production costs and improving quality by fermentation, downstream processing, and improvement in the producer organisms as a result of conventional applied genetics together with recombinant DNA technology.[12] SCPs have application in animal nutrition as: Fattening calves, poultry, pigs, and fish breeding in the foodstuffs area as: Aroma carriers, vitamin carrier, emulsifying aids and to improve the nutritive value of baked products, in soups, in ready-to-serve meals, in diet recipes, and in the technical field as: Paper processing, leather processing, and as foam stabilizers.

PRODUCTION OF SCP

SCP can be produced by utilizing different substrates such as agricultural waste (wood cuttings, crumb, corncobs, and various others). Other potential substrates for SCP include bagasse, citrus wastes, sulfite waste liquor, molasses, animal manure, whey, starch, and sewage.

Production of SCP from different substrates and microorganisms has the following important steps:

- Physical and/or chemical pretreatments may be required based on the type of substrate used
- Apart from the carbon source, nitrogen, phosphorus, and some other nutrients are also required to maintain microorganism optimal growth
- The components of medium may be sterilized by heating or filtration and equipment used in fermentation may be heat sterilized to prevent contamination
- The selected microorganism is inoculated in the pure state
- SCP procedures are extremely aerobic (excluding those exploiting algae). For this reason, sufficient aeration must be supplied. In addition, cooling is also essential as significant heat is produced
- Cooling is necessary as considerable heat is generated. Processing of the biomass for enhancing its usefulness and/or storability is also needed.

Roth[13] has described that the production of microbial proteins seems to be for better as compared to protein problems of conventional crops used as food and feed.

- Rapid succession of generation (algae, 2–6 h; yeast, 1–3 h; bacteria, 0.5–2 h)
- Easily modifiable genetically (e.g., for composition of amino acids)
- High protein content of 43–85% in the dry mass
- Broad spectrum of original raw material used for microbial cultivation, including waste products
- Production in continuous cultures, consistent quality not dependent on climate in determinable amount, low land requirements, and ecologically beneficial.

The choice of microorganism depends on numerous criteria such as the growth of microorganisms should be fast, and a broader range of materials may be considered as suitable substrates. The other criteria may be nutritional (energy value, protein content, and amino acid balance), and technical (type of culture, type of separation, and nutritional requirements). The desired microorganisms should be cultured on the medium under sterile conditions. Organisms to be cultured must have the following properties which are:

- Should be non-pathogenic to plants, human, and animals
- Usable as food and feed
- Should have good nutritional values
- Not contain toxic compounds and
- Production cost should be near to the ground.

PRODUCTION OF SCP FROM YEAST

Yeasts are the first microorganisms to be recognized scientifically,[14] the best studied and generally best accepted by consumers. During World War I, Germany produced torula yeast (*C. utilis*) and consumed it in making sausages and soups. In vegetarian's diet, food flavoring is usually because of the yeasts. For this purpose, torula yeast has been commercially utilized.

Yeast produces amino acids from inorganic acids and sulfur supplemented in the salts. They obtain carbon and energy from the organic wastes, molasses, starchy materials, milk whey, fruit pulp, wood pulp, and sulfite liquor.

Advantages of Yeast in SCP Production

- Harvesting is easy due to their larger size than bacteria (larger than bacteria)
- Malic acid percentage is higher
- Lysine content is higher
- It has the ability to grow and maintain at acidic pH
- It has been traditionally used since a long time ago.

Disadvantages of Yeast in SCP Production

- It has a slower growth rate than bacterial cells
- It contain lesser protein content (45–65%) as compared to bacteria
- Its methionine content is lower than bacteria. Hence, addition of methionine in the final product is required to increase its value thus adding another step during processing.[15]

PRODUCTION OF SCP FROM ALGAE

Since ancient times, Spirulina was cultivated by people near Lake Chad in Africa and the Aztecs near Texcoco in Mexico. They used it as food after drying it. Spirulina is the most widely used algae so much that even astronauts take it to space during their space travel. Similarly, biomass obtained from *Chlorella* and *Scenedesmus* has been harvested and used as source of food by tribal communities in certain parts of the world. Alga is used as a food in many different ways, and its advantages include simple cultivation, effective utilization of solar energy, faster growth, and high protein content. The algae Spirulina has been considered for use as a supplementary protein.[16] It is a blue-green algae having strong antioxidant activity and provokes a free radical scavenging enzyme system.

A diet enriched with Spirulina and other nutraceuticals may help protect the stem/progenitor cells. Spirulina maxima prevent fatty liver development induced by carbon tetrachloride (CCl4). It is concluded that the use of Spirulina should be encouraged in patients suffering from malnutrition, immune suppression, hepatic, neural compromise, etc., although further investigations on the antiviral effects of this alga and its clinical implications are strongly needed. SCP production by five strains of *Chlorella* species (M109, M121, M122, M138, and M150), isolated from different habitats, and was studied under the influence of eight environmental factors.[17]

Advantages of algae in SCP production

- It utilizes the solar energy very effectively
- Its cultivation and production are simple and easy
- Growth is faster with high nutrient especially protein content.[18]

PRODUCTION OF SCP FROM FUNGI

In Europe, people made an effort to produce *Fusarium* and *Rhizopus* culture for protein supplement[19] around the Second World War. Moreover, inoculum of *Aspergillus oryzae* or *Rhizopus arrhizus* was chosen because of their harmless nature.[20] Saprophytic fungi grow up on complex organic substrates and convert them into simple forms. As a result of growth, high amount of fungal biomass is produced. Fungal biomass production may vary widely depending on type of fungi and substrates on which it is grown. Chahal[21] demonstrated that the rising status of mycoprotein may be due to:

- Some of the filamentous fungi grow fast similar to the single-celled organism
- The end product of filamentous fungi is fibrous in nature and can easily be transformed into a variety of textured foods
- The retention time of filamentous fungi in the digestive system is higher
- The protein component of molds ranges up to 35–50% with relatively less nucleic acid
- Protein digestibility and its net utilization without any pre-treatment is higher
- The protein production rate from filamentous fungi is cost effective
- The filamentous fungi have better penetrating ability into insoluble substrates, and therefore, they are more appropriate for solid-state fermentation of lignocelluloses
- Some of the filamentous fungi possess a faint mushroom such as flavor and aroma which may be more acceptable as a new source of protein supplement
- The cultivated biomass of molds can be used as such without any further processing due to carbohydrates, lipids, minerals, vitamins, and proteins
- Nucleic acid components of fungal protein are lower in comparison with yeast and bacteria.

For the use of filamentous fungal species for SCP production, the substrates are: Glucose, galactose, cellulose, semi cellulose, maltose, lactose, starch, sulfite waste liquor, and pentose.

PRODUCTION OF SCP FROM BACTERIA

Bacteria have short generation time, the cell mass of bacteria multiply within 20 min–2 h, and they can grow rapidly, due to these characteristics bacteria are suitable in the production of SCP. They also have the ability to grow on different types of raw material ranging from liquid hydrocarbons such as fractions of petroleum and methane to gases and carbohydrates such as sugars and starches[22] to wastes of organic nitrogen and petrochemicals which include nitrogen, ethanol, and methanol. It is also suggested to add mineral nutrient supplements that help the bacterial culture to fulfill deficiency of nutrients which is required in sufficient concentration for the growth in natural water. Potential phototrophic bacterial strains are recommended for SCP production. Some researchers also suggest use of methanotrophs and other bacterial species for SCP production. The methylophilus generation time almost 2 h is useful for animal feed, but generally they can produce favorable composition of protein than fungi or yeast. Therefore, animal feed can produce a large quantity of SCP using bacteria such as *Brevibacterium*,[5] *Acinetobacter calcoaceticus*, *Methylophilus methylotrophus*, *Bacillus megaterium*, *Acromobacter delvaevate*, *Bacillus subtilis*,[23] *Aeromonas hydrophila*, *Cellulomonas* species, *Methylomonas methylotrophu*s, *Lactobacillus* species,[24] *Thermomonospora fusca*, *Flavobacterium* species, *Pseudomonas fluorescens*, and *Rhodopseudomonas capsulate*.[25]

The properties that enable bacteria appropriate for this purpose consist of:

- Fast growth rate of bacterial cells
- Bacteria have small generation times as most of them are able to increase their cell mass two-fold within about 20 min–2 h
- They have the capability of growing on various raw materials, ranging from carbohydrates such as starch and other sugars to petrochemicals such as methanol and ethanol, to liquid and gaseous hydrocarbons such as methane and petroleum
- Nitrogen sources suitable for the growth of bacteria include nitrates, ammonia, urea, ammonium salts, and the organic form of nitrogen in waste materials
- Bacterial culture medium for bacterial growth must contain a mineral nutrient supplement to supply nutrients that are not there within natural waters having concentrations enough to maintain bacterial growth.

PRODUCTION OF SCP BY FERMENTATION

The production of SCP takes place in a fermentation process.[26] Process development begins with microbial screening, in which suitable production strains are obtained from samples of soil, water, air, or from swabs of inorganic or biological materials and are subsequently optimized by selection, mutation, or other genetic methods.

SCP can be produced by fermentation processes, namely:

Submerged Fermentation

Submerged fermentation contains the liquid form substrate is used, which provides all the nutrients required by the microorganism for growth. Operational conditions are applied continuously during fermentation process, and the product is harvested after regular intervals. The harvested biomass is filtered and centrifuged. SCPs are then obtained after the process of drying. Submerged fermentation is operated at a high cost and has more capital investment.[1]

Semisolid Fermentation

The substrates preparations in solid-state fermentation are less clear and are used more in the solid form rather than liquid. The process of cultivation is carried out by stirring of multiphase. The oxygen is transferred to the microorganisms in the form of bubbles through liquid phase. This liquid phase also regulates the temperature of the process. A special bioreactor called u-loop fermenter is used in solid-state fermentation. The process is carried out by sterilization of the fermenter and growth medium, growth medium with suitable carbon source, production of specific microorganisms, harvesting of product biomass, its processing, and purification.[1]

Solid-state Fermentation

Solid-state fermentation is being extensively used for the production of SCPs, enzymes, organic acids, pigments, and flavor. Solid-state fermentation is carried out in solid substrates with no free water and does not require pre-arrangement of preparation of growth media. Fungi show good growth in low water activity and yield high product biomass as compared to submerged fermentation. Solid-state fermentation involves efficient utilization of waste, which acts as solid substrate and produces commercially viable cells. The process mainly involves seeding of the rice or bran substrate with microbial cells. Then, the substrate is left for several days in the form of flat beds. Then, harvesting of cells, further processing and finally drying of the cells is carried out.[1]

NUTRITIONAL VALUE OF SCP

SCP from yeast and fungi has 50% to 55% protein, it has high protein carbohydrates ratio. It contains more lysine less amount of methionine and cysteine. It also has good balance of amino acids, and it has high B-Complex vitamins and more suitable as poultry feed. Some yeast strains with probiotic properties such as *S. cerevisiae* and *Debaryomyces hansenii* improve larval survival either by colonizing gut of fish larvae, which triggers the early maturation of the pancreas.

SCPs produced using bacteria contain more than 80% protein although they have small amount of sulfur-containing amino acids and high in nucleic acid content.[27]

The idea that the SCP could help the less developed countries in future food shortages was gaining research interest among scientists in universities and industry. For future success of SCP, first, food technology problems have to be solved to make it similar to familiar foods and second, the production should compare favorably with other protein sources.

LIMITATIONS OF SCP

The high concentration of nucleic acids which is 6–10% which elevates serum uric acid levels and becomes a cause of kidney stone formation.[28] About 70–80% of total nitrogen is present in amino acids, while rest occurs in nucleic acids, and this concentration of nucleic acids is higher than conventional protein, which is characteristic of all fast-growing organisms.[29]

Another problem is the presence of cell wall which is nondigestible. In case of algae and yeast, there may be unacceptable color and flavors, cells of organisms must be killed before consumption, there is chance of skin reaction from taking foreign proteins, and gastrointestinal reactions may occur resulting in nausea and vomiting.[5] SCP production has also gained less importance because of lack of acceptability of SCP as a nutrient supplement among people.

CONCLUSION

SCP might be considered as a helpful food supplement within tropical region countries in which conventional food items have high carbohydrate content and low protein content. This unending deficiency of protein directs to mental and physical weakening and damage. Having a role of feed supplementation, SCP has not been an achievement due to expenses considerations. Feedstock, predominantly those linked to the oil production, normally has amplified in cost, while others that came through competitive protein sources, for example, fishmeal and soybean meal, have reduced in cost.

SCPs can combat the problems of protein malnutrition by providing an alternative protein source. SCPs are still not used on global scale as it contains certain toxic elements and contaminants which are reduced to minimum level by applying a number of techniques. A SCP contains proteins, amino acids, fats, lipids, and nucleic acids. The final packaging of the SCPs must provide complete microbiological information of species, strain contained. Complete analysis and tests of the SCP products produced for the consumption of the human must be performed. Future prospects of SCPs involve the utilization of genetically improved species for high yield and production.

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