# Microbes as Food for Future

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***Abstract -*** Microbial protein is very important and alternative source of high-quality protein able to replace animal protein like fishmeal in livestock nutrition and aquaculture. Microbial protein is a type of single-celled protein used for food, which is typically made up of fungi, bacteria or algae. [8]

Since ancient times, a number of microbes have been used as a part of diet cell over the world. In the last 60's 'single cell protein' was used as substitute for microbial protein. Moreover, as we find that the proteins quality in food supply is low, especially in developing countries, these increases in protein malnutrition and has given importance for the search of a new and alternative source of protein both in human foods and animal feeds. Due to population pressure, there may be lack of animal or vegetable proteins to fulfill requirements in future, especially in developing countries like India. So, in order to meet the protein deficiency SCB can substitute entirely or moderately the valuable amount of conventional protein feed. And for these technologies should be employed in agriculture and food waste products and this would play a vital role for the production of SCB. The key step to reduce the shortage of protein supply is taken by the production of microbial protein or single celled protein (SCP). There are many advantages for using microbial proteins as food over conventional proteins. Microbial proteins are rich in vitamins, carotenes and carbohydrates. Moreover, the microbial proteins can be produced under normal conditions. So shortage of land or natural calamities such as draught or flood cannot be a bottleneck in SCP production. Therefore, as we know that SCP is very important to fulfil the future global requirement, it is important and needed to develop clean and green technology for its production. Some of its disadvantages are that it has high nucleic acid content and low digestibility.

But then also it is very much necessary to implement and use technology to produce SCP to meet the protein deficiency in future. This paper reviews the production of single cell protein, its benefits, safety acceptability, cost and the limitations accompanied with their uses as it portends great promise as an alternative source of proteins.

***Keywords: Microbial protein, livestock nutrition, aquaculture, malnutrition, conventional proteins***

## I. INTRODUCTION

Single cell proteins or microbial proteins refer to the crude, a refined or edible unicellular proteins extract from pure or mixed cultures of algae, yeast, fungi or bacteria may be used as an ingredient or a substitute for proteins rich foods & is suitable for human consumption or as animal feeds. There may not be enough animals or vegetables proteins to fulfill the requirements of population pressure in near future, especially in several developing countries. There, in the light of protein deficiency, microorganisms offer viable alternative of proteins supplements. SCP can substitute entirely or moderately the valuable amount of conventional protein feed. The use of microbial proteins as food has several advantages over conventional proteins. Microbial proteins are healthy source of vitamins, carotene, and carbohydrates. This type of proteins has been cultivated by culturing appropriate microbes on different substrates like starch, corn cob, whey, wheat, starch hydrolysates, hydrocarbon, molasses, and sugarcane bagasse.

Bacteria: Methylophilusmethylotrophus, *Brevibacterium sp*. Etc. Algae: *Chlorella, Scenedesmus* etc. Yeasts :*Saccharomyces cerevisiae, Candida utilis*etc

## II. Microbial Protein (MP)

The dried cells of microorganisms such as algae, bacteria, actinomycetes and fungi used as food and feed are collectively known as “microbial protein” (MP). This term “microbial protein” (MP) indicates the microbial biomass used as a source of food or feed. It contains huge amount of protein, all the essential amino acids, vitamins, minerals and various other nutritionally valuable substances. MP can be produced in closed and intensive system called “bioreactor”, which differ in structure and functioning according to whether the organism is a phototroph or a chemotroph, an autotroph or a heterotroph. Biomass production in a bioreactor is much more effective than plant cultivation in an open field or animal husbandry, owing to the stability of growth parameters, effective utilization of nutrients, which can be supplied to exactly match demand, low water and land footprint, no pesticide use (Pikaarst al.,2018, Tredici,2018). MP production does not require cultivable land and does not directly compete with crop-based food commodities and can be situated in industrial or metropolitan areas. In about thirty-five years from now urban areas will host more than two-thirds of the world population (FAO,2017). Millions of people living gathered in a confined urban location disposing their wastes are feeding. These will be one of the main challenges of the next decades. By using MP production and vertical farming, we can recover most of nutrients and energy embedded in solid urban waste.

In the last 60s the term ‘microbial protein ‘ was substituted with the “single cell protein “ (SCP). It refers to the “ source of mixed protein extracted from pure and mixed culture of bacteria , fungi, algae and yeast”. SCP is a term which means that microbial cells are grown and harvested to accomplish the food requirement of animals or human due to its high protein

content. It can also be branded as novel petro crop and mini food. It was introduced by Prof. Scrimshaw of M.I.T. (Massachusets Institute of Technology) to give a better image than microbial or bacterial protein (Adedayo et al.,2011). SCP was coined for the first time by Carroll L. Wilson in 1966 , a professor from Messachusetts Institute of Technology (Pvt University in Cambridge). He represents the microbial cell (primary ) grown in mass culture and harvested for use as protein source in food and animal feed. In 1950,British Petroleum initiated to produce SCP on commercial basis. The first commercially produced SCP -pruteen[3]from bacteria Methylophilusmethylotrophus and was used as animal feed additive. Another commercially produced SCP rich blue-green biscuits is Tecuitlatal[4]. It was used in Mexico.

Microorganisms have capability to produce highly nutritive proteineous food from low quality organic material and this has been exploited by industry. Roth (1982) has described that 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, 1-3h; bacteria, 0.5-2 h)

• Easily modifiable genetically (e.g., for composition of amino acids).

• High protein content of 43-85 percent in the dry mass.

• Broad spectrum of original raw material used for the microbial cultivation including waste products.

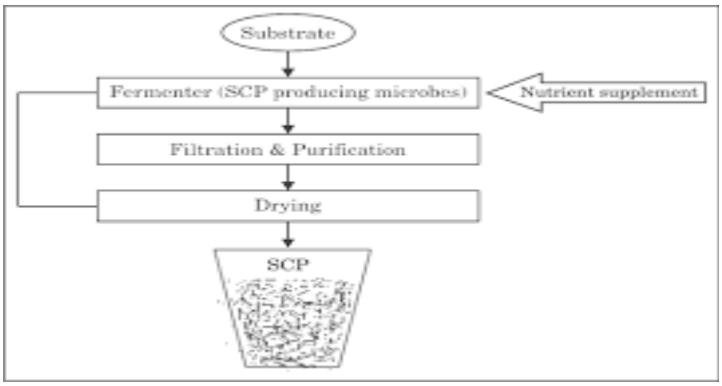
• Production in continuous cultures, consistent quality not dependent on climate in determinable amount, low land requirements, ecologically beneficial.

## III.SCP PRODUCTION:

**Contribution** of large-scale development of SCP processes has influenced greatly to the advancement of present day biotechnology. The field of microbiology, biochemistry, genetics, chemical and process engineering, food technology, agriculture, animal nutrition, ecology, toxicology, medicine and veterinary science and economics are involved with research and development of SCP processes. By development of SCP processes, many related new technologies in waste water treatment, alcohol production, enzyme technology and nutritional science also improves. The basic steps are involved for the SCP production. The basic steps are :

1. Raw materials /Substrates
2. Selection of suitable strain
3. Fermentation.
4. Harvesting and Post-harvest treatment

5. SCP processing for food




**A. RAW MATERIALS /SUBSTRARE** :SCP can be produced from high energy sources (alkanes, methane, methanol and ethano), wastes(molasses, whey, animal manures,and straw),wood,co2,sewage etc. Several microorganisms, Bactria , yeasts, fungi, algae and actinomycetes utilize this wide range of substrate.Bacteria and yeasts are mostly employed for production from high energy sources.

**a) FROM HIGH ENERGY SOURCES** : There are a large number of energy-rich carbon compounds and their derivatives which serve as raw materials( or substrate) for SCP production. These high energy sources are alkanes, methane, methanol and ethanol.

**ALKANES:** It has been observed that when cells are grown on a medium of alkanes enriched with lipids, the diffusion of alkanes into the cells is enhanced. Certain yeasts have been successfully used for producing SCP from alkanes e.g. Saccharomycopsislipolytica, Candida oleophila etc. They utilize alkanes(aliphatic hydrocarbons) for their growth and SCP production and secrete emulsifying substances during fermentation which increase the solubility of alkanes. These substrates are transported by passive diffusion into the cell. Several oil companies have developed fermentation systems, employing petroleum products for large scale manufacture of SCP by yeasts. For this purpose, two types of petroleum products are basically used.(1) Gas oil or diesel oil which contains 10-25% of long chain alkanes,(2) gas oil which contain short chain alkanes. The major disadvantages of alkanes as a substrate are formation of carcinogens along with SCP .

**METHANE :**Methans is cheap, abundant .It contains the most highly reduced form of carbon and produced during anaerobic digestion. It is a constituent of North Sea Gas. Certain bacteria such as Methylococcuscapsulatus, Methylomonasmethanica, and Methylovibriosoehngenii can utilize methane for SCP production. These bacteria contain enzyme ‘methane monooxygenase’[5] which oxidize methane to methanol. This methanol can be converted to formaldehyde and then to formic acid.

**METHANOL :** Methanol as a substrate is very good and advantageous than alkane and methane. It is used as a carbon source for SCP. It is easily soluble in aqueous stage at all concentrations and no residue of it remains in the harvested biomass. It produced from natural gas , coal, oil and methane.Methanol is fully soluble in water. Many species of bacteria (Methylobacter ,Arthobacter, Bacillus, Pseudomonas, Vibrio); yeasts ( Candida boidinii, Hanseulapolymorpha, Torulopsisglabrata), fungi(Trichoderma lignorum, Gliocladiumdeliquescens) utilizes methanol to produce SCP. This SCP contains the essential amino acids lysine, threonine, cysteine, valine, methionine, iso-leusine, leucine and phenylalanine[6]. Industrially produced protein pruteen from methylotrophus at 35-40 degree centrigrade is used as milk substitute in calf feeding.

**ETHANOL :**Ethanol can be obtained from organic substance by alcoholic fermentation or from ethylene br catalytic addition of H2O molecule. As a substrate it is good for human consumption. Bacteria, yeast and mycelium fungi utilize ethanol. This process is not economically effective. However several factor such as local raw materials, innovative fermentation technology, political decisions and foreign trade balance influence SCP production.

**b) FROM WASTES :**There are several materials that no longer has a use and they are collectively known as wastes e.g., molasses, whey, animal manures etc.These waste produces from various industries and other biological processes. Several advantages are present to utilize waste for SCP production. By using waste for SCP production, environmental pollution reduces, low-cost organic waste converts to useful product. Example: Molases is used for alcoholic fermentation from wheat, yeast biomass obtained as byproduct. The baker's yeast(S. cerevisiae) and Torulla yeast (Candidautilis) are produced on this substrate. Whey is a liquid portion of milk remaining after the curd is separated during cheese production. Yeast like Candida krusei in combination with Lactobacillus bulgaricus and Candida intermedica are the most widely used for SCP production from whey.

**c)FROM WOOD:**Waste wood is attractive natural starting materials for SCP production. It contains cellulose, hemicellulose and lignine. The association of cellulose with lignin in wood makes it intractable to microbial degradation. Breakdown of these cellulosic compounds into fermentable sugar is essential. For this purpose, extracellular cellulases can be used. Certain bacteria like Cellulomonassp and fungi such as Trichoderma sp, Penicillium sp. are suitable sources for celluloses. The cellulosic materials can be directly used for biomass production. This resultant SCP is used as animal feed.

**d)FROM CO2 :**Certain algae such as Chlorella sp. Scenedesmussp and Spirulina sp grown in open pond utilize only CO2 as carbon souce. In the presence of sunlight, they can effectively carry out photosynthesis and ultimately produce SCP.Chlorella is used as a protein and vitamin supplement for enriching ice-creams, breads and yogurt in some

countries. These resultant algae biomass can be harvested, dried and converted to the powder form. Algae SCP are very useful as animal supplements. In some parts of Africa and Mexico, people eat Spirulina sptraditionally . SCP of Spirulina sp has high nutritive value ( 65% protein, 20% carbohydrate, 4% fat,3% fiber, 5% chlorophyll and 3% ash). It is a perfect source of protein for human consumption.

**E) FROM SEWAGE :** The sewage obtained from industrial wastes in cellulose processing, starch production and food processing can be utilized for the production of SCP. Domestic sewage is normally used for large scale production of methane which further may be utilized for the SCP production. The organism Candida tropicalis, Paecilomycesvarioti are appointed to use sulfite waste liquor gor SCP production. Dairy waste water is particularly suitable for the SCP production by using lactose utilizing microorganisms.

**B.MICROORGANISMS :** Several microorganisms such as bacteria, yeasts, fungi, algae and actinomycetes utilize a wide range of substrates and they are used for production of SCP. To produce SCP, a suitable microorganism selection is very important step. So the microorganism selection is based on several criteria. It include their nutritive value, production cost, non-pathogenic nature, which raw materials are used and growth pattern. The protein quality completely depends on the microbe that is chosen for SCP production. In this step, care should be taken and have to be observed that the selected strain should no produce any toxic and undesirable effects in the consumer.

Average composition of the main groups of micro-organisms (% dry weight)

TABLE : 1

| | Fungi | Algae | Yeasts | Bacteria |
|---|---|---|---|---|
| Protein | 30-45 | 40-60 | 45-55 | 50-65 |
| Fat | 2-8 | 7-20 | 2-6 | 1.5-3.0 |
| Ash | 9-14 | 8-10 | 5-9.5 | 3-7 |
| Nucleic Acids | 7-10 | 3-8 | 6-12 | 8-12 |

Miller et al., (1976) [7]

**A)FUNGI AND YEASTS :** Filamentous Fungi such as Chaetomiumcellulolitícum, Fusarium graminearum, Paecilomycesvariotii, Aspergillus fumigates, Aspergillus Niger and Rhizopus cyclopean are used for SCP production. They grows on cellulose waste, starch, and sulphite waste liquor and it contain about 50-55% protein. Certain yeasts such as Candida utilis, Candida lipolytica, Saccharomyces cervicae and Candida tropicalis are also used to produce SCP. Strict aseptic conditions have to be maintained when using yeast as a production of yeast. Saccharomyces contains high protein with good balance of amino acids

and rich in –beta complex vitamins. It is more appropriate as poultry feed. Several disadvantages arises when fungi and yeast are used to produce SCP. These are high nucleic acid content, contamination risk is high,and mycotoxins are produced.

**b) BACTERIA :** Large number of bacterial species have been used to produce SCP and wide variety of substrate can also be used e.g. Brevibacterium uses hydrocarbons. Some of these are used for SCP production at commercial scale . They have more than 80% crude protein of good amino acid composition although in some cases a small deficiency of sulphur containing amino acids. Using bacteria , several disadvantages arises. The disadvantages are high nucleic acid content, cell recovering which is a bit problematic, risk of contamination by pathogenic bacteria, careful evaluation for endotoxins production specifically when we are used gram- bacteria.

**c) ALGAE :**Chlorella, Scenedesmusacutus and Spirulina maxima are grown in ponds for SCP and use CO2 and sunlight as substrate. SCP from algae has about 60% protein which is generally good in amino-acid composition but less in sulphur containing amino acids. They are suitable for animal feed on protein rich supplement. It is sold as pills and powders. Chlorella and Spirulina are used for commercial scale production in Taiwan, Japan, Mexico and USA. The disadvantages are rich chlorophyll content which is no suitable for human use, serious risk of contamination during growth, costly recovery method for unicellular algae , and low cell density e.g., 1-2 grams dry weight per liter.

## Microorganisms used for SCP production :

| Microorganism | Substrate(s) |
|---|---|
| Bacteria | |
| Methylophilus metgylotrophus | methane, methanol |
| Methylomonas sp | methanol |
| Pseudomonas sp | alkane |
| Brevibacterium sp | C1-C4 hydrocarbons |
| Yeast | |
| Saccharomyces lipolytica | alkanes |
| Candida utilis | sulfite liquor |
| Kluyveromyces cerevisiae | molasses |
| Lactobacillus bulgaricus | whey |
| Tosulopsis sp | methanol |
| Algae | |
| Spirulina maxima | carbon dioxide |
| Chlorella pyrenoidosa | carbon dioxide |
| Mushrooms ( a type of fungi) | |
| Agaricus biosporus | compost, rice straw |
| Morchella crassipes | whey, sulfite liquor |
| Auricularia sp | saw dust ,rice bran |
| Lentinus esodes | saw dust, rice bran |
| Volvariella volvaceae | cotton , straw |

## IV. **FERMENTATION**

The fermentation process requires a pure culture of selected organism that is in the correct physiological state. It can be carried out in the fermenter which is equipped with aerator, thermostat, pH etc or in the trenches or ponds. Microbes are cultured in fed-batch culture.

## V. HARVESTING OR PRODUCT RECOVERY AND POST HARVESTING TREATMENT

When the microbial colonies are fully developed, then they are harvested. Here bulk of cells are removed from the fermenter. Different methods are used for harvesting . All of methods are depending upon types of microorganisms used for production of SCP. After harvesting, the cells are subjected to a variety of processes. Post harvesting treatments includes steps such as separation, washing, and drying etc. (a) Algal biomass: It is recovered by concentration, de watering and drying. Flocculant such as Aluminiumsulphate and calcium hydroxide can also be used in sometimes. For Spirullina , it can float on surface water. So it can be filtered and suspension is dried with hot water air to get finepowder. (b)Bacterial biomass : Many problems related with the recovery of bacterial cell such as have cell density in the order of 10-20 gm/litre and they are very small. Centrifugation cost is also high. A device has been developed for Methylomonasclara separation from methanol containing

culture medium. This device is basedon coagulation and centrifugation. (c) Yeast biomass: yeast cells are small in size . It can be recovered by decantation, centrifugation, washing and dried treatment method.

## VI. PROCESSING OF SCP

This step is depending on the substrate material and desired food or feed applications. Various processing steps are required to final SCP product formulation . (I)Cell wall degradation in single cell protein products and (II) Nucleic acid removal in single cell protein products.

(I)Cell Wall Degradation in Single Cell Protein Products : Some SCP are used as whole cell preparations , while in the other cell wall may be broken down to make the protein more accessible. Various methods are used to smash the cell wall :

a) MECHANICAL METHODS : this methods includes Crushing, crumbling , grinding, pressure homogenization etc. b) CHEMICAL METHODS : hydrolytic enzymes( exogenous or endogenous), salts(Nail) etc are used. C) PHYSICAL METHODS: Freeze-thaw, osmotic shock, heating and drying are used.

(II) Nucleic Acid Removal in Single Cell Protein Products :Algae have low nucleic acid content but rapidly proliferating bacteria and fungi have high nucleic acid content. When SCP is produced for human consumption, high nucleic acid content is a major problem because ingestion of purine compounds derived from RNA breakdown increases uric acid concentrations in plasma, which can cause gout and kidney stones. Endogenous RNA degrading enzymes such as ribonucleases can be exploited in degradation of RNA. Degraded RNA components diffuse out of the cells, but biomass lose (35-38%) also occurs and treatment at 65 degree centrigrate,pH 7.5-8.5 to activate endogenous ribonuclease. It also reduce the RNA content to <2%, while the protein content remains 50%.

## VII. THE BEL PROCESS

The Bel process was designed by the Bel industries in France. It was developed with the aim of reducing the pollution load of dairy industry waste and simultaneously producing a Paecilomycesvariotii. This continuous process is operated aseptically and produces over 10000 tones of SCP a year. The dried Pekiloprotein contains 50% protein and used in the animal feed preparation.

## VIII. APPLICATIONS OF SCP

It provides instant energy and the best protein supplemented food for undernourished children. It serves as a good source of vitamins, amino acids, minerals, crude fibers, etc. It is extremely good for healthy eyes and skin. SCP is used in animal nutrition as fattening calves,

poultry, pigs and fish breading, in the foodstuffs area as aroma carriers, vitamin carrier, and emulsifying aids and to improve the nutritive value of baked products, in soup, in ready-to-serve meals , in diet recipes . It also used in technical field as paper processing, leather processing and as foam stabilizers and in therapeutic and natural medicines for controlling obesity, lowers blood sugar level in diabetic patients, reducing body weight, cholesterol and stress, and it can prevent accumulation of cholesterol in body.

## IX. **LIMITATIONS OF USING SCP**

a) The nucleic acid content of microbial biomass is very high such as 3-8% in algae, 8-12% in bacteria, and 6-12% in yeast. This is extremely hazardous, since human have a limited capacity to degrade nucleic acids. High nucleic acid produce uric acid cause gout-kidney stone. b) These include the hydrocarbons, heavy metals, mycotoxins and some contaminants. The nature and production of these compounds depends on the raw materials and what type of organism is used. c) There is a possibility of contamination of pathogenic microorganisms in SCP d)the carcinogenic and other toxic substances are often present in association with SCP. e) The digestion rate of microbial cell is slow. This is frequently associated with indigestion and allergic reactions in individuals. f) Food grade production of SCP is more costly and harvesting not easy. In general, SCP for human consumption is 10 times more expensive than SCP for animal feed.

## X. **CONCLUSION**

The development of SCP(single cell protein) is just a beginning in biotechnology. Use of microbes in the production of proteins gives many advantages over the accepted methods. By the improvement in the production of SCPs, We can solve the malnutritive conditions of the progressing countries and can also introduce better quality of food and taste with lower chances of occurrence of side effects . Moreover, genetic modification in microorganisms can lead to a better future of SCPs in biotechnology, chemical and process engineering, food technology, agriculture, animal nutrition, ecology, toxicology, medicine ,veterinary science etc.

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