Microbial Protein: A Valuable Component for Future Food Security

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Microbial Protein: A Valuable Component for Future Food Security

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ABSTRACT

The dried cells of microorganisms (algae, bacteria, actinomycetes and fungi) used as food and feed are collectively known as 'microbial protein'. Since, ancient times a number of microbes have been used as a part of diet all over world. In the last 60s the term 'microbial protein' was substituted with the 'single cell protein' (SCP). In India, little attention was paid on the mass production of SCP, though mushroom cultivation in early 50's. There may not be enough animal or vegetable proteins to fulfil the requirements of population pressure in near future, especially in several developing countries. Therefore, in the light of protein deficiency, microorganisms offer viable alternative of protein supplements. SCP can substitute entirely or moderately the valuable amount of conventional protein feed. For the same, expansion of technologies employing agriculture and food waste products would play a major role for the production of SCP and may also be possible solutions to meet out the requirements of protein. The SCP production is useful to alleviate waste disposal problems showing an eco-friendly and sustainable route including reduction of production cost. The use of microbial protein as food has several advantages over conventional proteins. Microbial proteins are healthy source of vitamins, carotenes and carbohydrates. Additionally, the microbial proteins can be produced under normal settings. Thus, land shortage and environmental calamities (such as drought or flood) cannot be a bottleneck in SCP production. Therefore, looking over the significance of SCP as protein supplements, it is required to develop clean-green technology for its production on large scale to fulfil the future global requirements.

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1. INTRODUCTION

The global population of human beings has been increased up to 250% in last six decades with a boost from 2.6 to 7 billion and it is expected that if the growth will continue with the same rate population may be 9 billion by 2042 according to Census Bureau of United States (Gabriel *et al*., 2014). The rising global population pressure generates challenges to fulfil the requirements of foodstuff. Population cannot entirely dependent over agriculture, animal husbandry or fisheries for food. However, agricultural sector has strengthened in most of the developed countries. However, some of them are still facing problems like hunger, malnutrition, food insecurity and food related diseases (Gabriel *et al.*, 2014). The World Health Organisation (WHO) has estimated that in many developing countries, starvation, malnutrition and related diseases are very common today and up to 12,000,000 individuals lose their life every year (Israelidis, 2008; Gabriel *et al.,* 2014). Therefore, it is required to explore alternative sources of food for sustenance of future indiscriminate increasing population. Microbes are emerging tools for production of quality food resources and single cell protein has been recently recognised as an emerging food alternatives. Among the microbes *Spirullina* (blue green alga) has been identified as rich source of SCP. This micro alga can be cultivated in laboratory as well as outdoor water reservoirs in mass by providing very normal nutrient requirements. This chapter describes about the role of microbes in providing the quality food resources for future generation.

2. SINGLE CELL PROTEIN

Although man has been familiar with micro-organisms as food and feed for centuries, a number of microorganisms have been used as a part of diet since ancient times. Fermented yeast (*Saccharomyces* sp.) was recovered as a leavening agent for bread as early as 2500 B.C. (Frey, 1930). Fermented milk and cheese produced by lactic acid bacteria (*Lactobacillus* and *Streptococcus*) was used by Egyptian and Greeks during 50-100 B.C (Frey, 1930). The protein obtained from microbial source is designated as 'Single Cell Protein' (SCP) (Vincent, 1969; Becker and Venktaraman, 1982) it refers to the "source of mixed protein extracted from pure and mixed culture of bacteria, fungi, algae, and yeast". Single cell protein 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 protein, petro crop and mini food. It was introduced by Prof. Scrimshow of M.I.T. (Massachusets Institute of Technology) to give a better image than microbial or bacterial protein (Adedayo *et al*., 2011). The term single cell protein was coined by Carol L. Wilson in 1966 (Adedayo *et al.*, 2011) and may be more appropriate as most of micro-organism grow as single or filamentous individual. Microorganisms have capability to upgrade low quality organic material to highly nutritive proteineous food, and this has been exploited by industry.

The technology for SCP production for food was developed over the last 100 years while large scale production came into being in the 20th century. The importance of mass production of microorganisms as a direct source of microbial protein was realized around First World War (1914-1915) in Germany and consequently, baker's yeast (*Saccharomyces cerevisiae*) was produced in an aerated molasses medium supplemented with ammonium salts (Litchfield, 1983; Boze *et al.*, 1992; Gabriel *et al.*, 2014). However, aerobic yeasts (*e.g., Candida utilis*) were produced during Second World War (1939–1945) as food and feed supplement in Germany (Gabriel *et al.*, 2014). Moreover, considerable effort has been made to develop technologies for mass cultivation of SCP by formulating different types of growth media and improved culture of micro-organisms in entire Europe since World War II (Gabriel *et al.*, 2014). For example, with the growth of *S. cerevisiae* on a production level Germany had been importing single cell protein to replace up to 60% of the foodstuffs after the war (Litchfield, 1983; Gabriel *et al.*, 2014). In the fifth decade, some oil industries interested for the growth of micro-organisms on alkanes and thereafter, in the 60s, researchers at British Petroleum developed "proteins-from-oil process" technology for producing single cell protein by yeast on waxy n-paraffins, a product produced by oil refineries (Ageitos *et al.*, 2011; Gour *et al.*, 2015). Natural substrates and industrial waste products were initially used for cultivating microorganisms (Grewal *et al.*, 1990; Osho, 1995). In dealing with microbial protein production several natural products were used as a carbon source (Kuzmanova *et al.*, 1989), such as grape juice, cashew apple juice, cellulose and hemicellulose (Osho, 1995; Haider & EL-Hassy, 2000; Azzam, 1992; Pessoa *et al.*, 1997; Bozakuk, 2002; Zubi, 2005). Some micro-organisms were developed into a potential diet for food and feed. Many companies producing single cell protein including BP (UK), Kanegafuichi (Japan), and Liquichimica (Italy) appeared into the scene. Other potential used as substrates for single cell protein include bagasse, citrus wastes, sulphite waste liquor, molasses, animal manure, whey, starch, sewage, etc. (Gour *et al.*, 2015). In India, little attention has been paid on the production of single cell protein though mushroom cultivation started in the early 1950s. However, work on mushroom culture at Solan (Himachal Pradesh,) has brought satisfactory results since 1970. Recently, National Botanical Research Institute (NBRI), Lucknow and Central Food Technologies Research Institute (CFTRI), Mysore, have established centres for mass production of single cell protein from cyanobacteria. At the NBRI single cell prtein is produced on sewage which is further utilized as animal feed (Anonymous, 1980).

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.

3. MICROORGANISMS FOR SINGLE CELL PROTEIN PRODUCTION

Bacteria, yeasts, fungi and algae are used to produce biomass. The choice of microorganism depends on numerous criteria such as, the growth of microorganism 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, amino acid balance), technical (type of culture, type of separation, nutritional requirements). The desired microorganisms should be cultured on the medium under sterile condition. 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.

The bacteria include *Brevibacterium, Methylophilus methylitrophus, Acromobacter delvaevate, A. calcoacenticus, Aeromonas hydrophila, Bacillus megaterium, B. subtilis, Lactobacillus, Cellulomonas, Methylomonas methylotrophus, Pseudomonas fluorescens, Rhodopseudomonas capsulata, Flavobacterium, Thermomonospora fusca* and algae used are *Chlorella pyrenoidosa, Chlorella sorokiniana, Chondrus crispus,, Scenedesmus acutus* and *Sprulina maxima, S. platensis* (Adedayo *et al.,* 2011). The filamentous fungi that have been used include *Chaetomium cellulolиticum*, *Fusarium graminearum*, *Aspergillus fumigatus, A. niger, A. oryzae, Cephalosporium cichhorniae, Penicillium cyclopium, Rhizopus chinensis, Scytalidium acidophlium, Tricoderma viride, T. alba,* etc. (Adedayo *et al.,* 2011). Yeasts such as *Candida utilis, C. lipolytica, C. tropicalis, C. intermedia* and *Saccharomyces cerevisiae* (Adedayo *et al.,* 2011).

The microorganism used for single cell protein production should possess the following characteristics:

- High specific growth rate (m) and biomass yield
- High affinity for the substrate
- Low nutritional requirements, *i.e.* few indispensable growth factors
- Ability to utilize complex substrates
- Ability to develop high cell density
- Stability during multiplication
- Capacity for genetic modification
- Good tolerance to temperature and pH
- Balanced protein and lipid composition.
- Low nucleic acid content, good digestibility and non-toxic.

4. PROTEIN CONTENT IN MICROORGANISMS

Microorganisms have been employed for several years in the production as food especially protein content such as cheese and fermented soybean products. Since a large proportion of cell dry weight is accounted for protein, the nutritional value of a microbial derived food source is determined by the levels of protein present (Patel, 1995). Table 1 and 2 elaborates that amino acid composition in the protein provided by microorganisms is much more suitable for consumption as compared to the vegetables and other products and it is appropriate according to the recommendations of Food and Agriculture organisation (FAO).

Table 1: Daily requirements (g) of essential amino acids for the human adult (Data retrieved from FAO) (http://www.fao.org).

Essential amino acids	*FAO recommendation*	*Minimum*
Phenylalanine	2.2	1.1
Methionine	2.2	1.1
Leucine	2.2	1.1
Valine	1.6	0.8
Lysine	1.6	0.8
Isoleucine	1.4	0.7
Threonine	1.0	0.5
Tryptophan	0.5	0.25
Total	12.7	6.35

4. SUBSTRATES FOR MICROBIAL BIOMASS PRODUCTION

As stated earlier the agricultural doesn't seem to be sufficient to satisfy the food demand for most rapidly growing human population because of different reasons. Microorganisms seem to be important source of food and can be exploited to satisfy the future food demand of indiscriminate increasing human population. Since the microorganisms can grow over huge substrate

Table 2: Essential amio acid content of microorganisms for SCP production (g per 16 g N) (Lichtfield, 1979; *Boze et al.*, 1992; Gabriel *et al.*, 2014)

Protein source	***Cys***	***Ile***	***Leu***	***Lys***	***Met***	***Phe***	***Thr***	***Try***	***Val***
Algae									
Chlorella sorokiniana	3.4	4.0	7.8	1.8	2.7	3.2	1.4	5.1	-
Spirullina maxima	0.4	5.8	7.8	4.8	1.5	4.6	4.6	1.3	6.3
Bacteria and Actinomycetes									
Cellulomonas alcaligenes	5.4	7.4	7.6	2.0	4.7	5.5	7.1	-	-
Methylophilus methylotrophus	0.6	4.3	6.8	5.9	2.4	3.4	4.6	0.9	5.2
Thermomonospora fusca	0.4	3.2	6.1	3.6	2.0	2.6	4.0	13.0	-
Fungi									
Candida lipolytican-	1.1	4.5	7.0	7.0	1.8	4.4	4.9	1.4	5.4
Candida utilis	-	0.4	4.5	7.1	6.6	1.4	4.1	5.5	1.2
Kluyveromyces fragilis	4.0	6.1	6.9	1.9	2.8	5.8	1.4	5.4	-
Saccharomyces cerevisiae	1.6	5.5	7.9	8.2	2.5	4.5	4.8	1.2	5.5
Aspergillus niger	1.1	4.2	5.7	5.9	2.6	3.8	5.0	2.1	5.2
Morchella crassipes	0.4	2.9	5.6	3.5	1.0	1.9	3.0	1.5	3.0
Paecilomyces variotii	1.1	4.3	6.9	6.4	1.5	3.7	4.6	1.2	5.1

range (Table 3) from fruit juices to hydrocarbon as well waste materials (Gabriel *et al.,* 2014) and able to recycle different polluting agents. Therefore, microorganisms may not only be cultivable properly on different cost effective substrates to fulfil the requirements of our daily diet but also be the mediators of environmental renovation.

5. NUTRITIONAL BENEFITS OF SINGLE CELL PROTEINS

Factors like nutrient composition, amino acid profile, vitamin and nucleic acid content as well as palatability, allergies and gastrointestinal impact should bring under consideration for the assessment of the nutritional value of single cell protein (Lichfield, 1968). In addition, long term feeding trials should also be tested for toxicological effects and carcinogenesis (Israelidis, 2008). In fact, nutritive values vary with the microorganisms used for single cell protein production. The way of harvesting, drying and processing also impact on the nutritive value of the product. Single cell protein includes primarily proteins, fats, carbohydrates, ash ingredients, water, and other elements such as phosphorus and potassium. The composition depends upon the microorganism and the substrate on which it grows. Despite, the proteins as nutritional component in a food system they also perform several other functions (Mahajan and Dua, 1995).

Single cell protein are good source high quality protein with low fat content, vitamins predominantly B-complex, superior amino acid composition and furnished with thiamine, riboflavin, glutathione, folic acid and other amino acids but less in sulphur containing amino acids. Fungi and yeast possess up to 50–55% protein and rich in lysine, although deprived

Table 3: Microorganisms used for SCP production according to carbon source (Boze *et al.*, 1992; Gabriel *et al.*, 2014)

Source of carbon	***Microorganisms***
	Algae
CO_2	*Chlorella pyrenoidosa, C. regularis, C. sorokiniana, Oocystis polymorpha, Scenedesmus quadricauda, Spirullina maxima, Spirullina platensis, Dunaliella bardawil*
	Bacteria and Actinomycetes
n-Alkanes	*Acinetobacter cerificans, Achromobacter delvacuate, Mycobacterium phlei.*, *Nocardia* sp., *Pseudomonas* sp.
Methane	*Corynebacterium hydrocarbonoclastus, Nocardia paraffinica, Acinetobacter* sp.,*Flavobacterium* sp., *Hyphomicrobium* sp., *Methylomonas methanica, Methylococcus capsulatus.*
Methanol	*Methylomonas methylovora, M. clara, M. methanolica, Flavobacterium* sp., *Methylophilus methylotrophus, Pseudomonas* sp., *Streptomyces* sp., *Xantomona* sp.
Ethanol	*Acinetobacter calcoaceticus*
Cellulosic wastes	*Thermomonospora fusca*
Sulfite waste liquor	*Pseudomonas denitrificans*
	Yeasts
n-Alkanes, *n*-paraffins	*Candida lipolytica, C. tropicalis, C. guilliermondii, C. maltosa, C. paraffinica, C. oleophila, Yarrowia lipolytica*
Methanol	*Candida utilis, Hanseniaspora* sp., *Pichia pastoris, Hansenula* sp., *Kloeckera* sp.
Ethanol	*Candida enthanothermophilum, C. utilis, C. kruzei*
Whey	*Kluyveromyces fragilis, Candida intermedia*
Cane molasses	*Saccharomyces cerevisiae*
Starch	*Schwanniomyces alluvius, Lipomyces kononenkoe*
Lipids	*Candida rugosa, C. utilis, C. lipolytica, C. blankii, C. curvata, C. deformans, C. parapsilosis*
Cellulose	*Candida utilis*
Sulfite waste liquor	*Candida utilis, C. tropicalis*
	Fungi
Glucose	*Agaricus blazei, A. campestris*
Malt – molasses	*Agaricus campestris*
Starch	*Aspergillus niger, Fusarium graminearum*
Sulfite waste liquors	*Paecilomyces variotii*
Cellulose	*Chaetomium cellulolyticum, Trichoderma viride*
Brewery waste	*Calvatia gigantean*

of cysteine and methionine amino acid content. However, bacteria can produced single cell protein more than 80% protein, albeit it is poor in sulphur containing amino acids with high nucleic acid content. (Kurbanoulu, 2011; Adedayo *et al.,* 2011). Yeast single cell proteins are playing major role in the growth of aquaculture feeds. With admirable nutrient profile and capability to be large scale production economically, single cell proteins have are partially replacing fishmeal as aquaculture feed (Coutteau and Lavens, 1989; Olvera-Novoa *et al.*, 2002; Li and Gatlin, 2003). Some yeast strains such as *Saccharomyces cerevisiae* (Oliva-Teles and Goncalves, 2001) and *Debaryomyces hansenii* (Tofar *et al*., 2002; Adedayo *et al.,* 2011) keeps probiotic properties that can be used to fulfil health requirements replacing chemical drugs. However, several yeast supplements lacking sulphur containing amino acids, especially methionine (Oliva-Teles and Goncalves, 2001; Adedayo *et al.,* 2011), which limits their wide use as the exclusive protein source.

6. CULTIVATION OF SINGLE CELL PROTEIN

Single cell protein can be produced by two types of fermentation processes, namely submerged fermentation and semisolid state fermentation (Fig. 1) (Varavinit *et al*., 1996; Ageitos *et al*., 2011). In the submerged fermentation, the substrate to be fermented is always in a liquid phase with the nutrient required for growth of microorganism. The substrate is held in the fermenter which is operated continually whereas the product biomass is also harvested continuously. The product is filtered or centrifuged and dried for the production of single cell protein. However, in semisolid fermentation process, the preparation of the substrate much elaborated; it is more favourable to a solid substrate, for *e.g*. cassava waste. Submerged culture fermentations are more investment concentrated and with higher operating cost as compared to semisolid fermentations which have a lower protein yield. The microbial cultivation occupies various basic process engineering operations, such as stirring and mixing of a multiphase system (gas-liquid-solid), transport of oxygen from the gas bubbles through the liquid phase to the organisms, and heat transfer from the liquid phase to the environment.

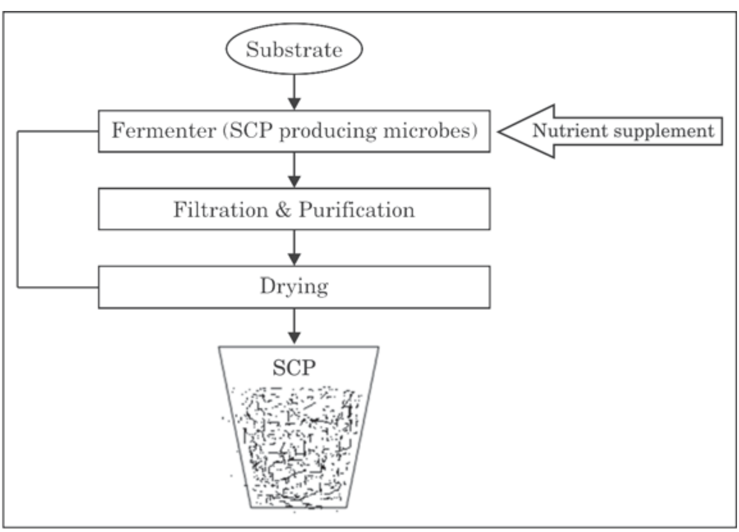

Fig. 1: Flow chart of Single cell protein production (modified from Ageitos *et al*., 2011)

Another U-loop fermenter is a special bioreactor type designed for increasing mass and energy transport phenomena by enhancing the mixing of the multiphase system and favouring heat transfer in single cell protein production (Andersen, 2005) and the production of single cell protein occupy some basic steps such as preparation of suitable growth medium with appropriate carbon source, avoidance of medium contamination and fermentation, for the production of the desired microorganisms and separation of microbial biomass and its processing. The medium should have a carbon source for cultivation of heterotrophic organisms, for *e.g.* fossil carbon sources like n-alkanes, gaseous hydrocarbons, methanol and ethanol, renewable sources like molasses, whey, polysaccharides, effluent of breweries, distilleries, confectioneries and canning industries or other solid substrates such as salts of potassium, manganese, zinc, iron and gaseous ammonia are also added for cultivation of various microorganisms (Nasseri, 2011). Aeration is a vital process in the cultivation and cooling devices are already installed for the removal heat generated during cultivation. Single cell organisms like bacteria and yeast are recovered by centrifugation process while filamentous fungi are recovered by filtration. It is important to recover possible water content prior to completion of final drying under clean and hygienic conditions.

7. SIDE EFFECTS OF SINGLE CELL PROTEINS

Despite very striking features of single cell protein as a nutrient for humans and animals there are various problems associated that deters its adoption globally. Such problems include:

- The concentration of nucleic acid is higher than other conventional protein sources and it is the characteristics of all rapidly growing organisms.
- The problem which occurs with the consumption of high nucleic acid containing protein (18-25 g/100g protein dry weight) is the production of high concentration of uric acid level in the blood causing health disorders such as gout and kidney stone (Nasseri *et al.*, 2011).
- Single cell protein from bacteria has also been found to be associated with these pitfalls which include: high ribonucleic acid content, high risk of contamination during the production process and recovering the cells is a bit problematic
- About 70 to 80% of the total nitrogen is represented by amino acids while the rest occur in nucleic acids.
- It is also point to be noted that the microbial cell wall may be indigestible.
- There may be intolerable colour and flavours (especially in algae and yeast there may also be possible skin reactions from consumption

of foreign protein and gastrointestinal reactions may occur resulting in nausea and vomiting.

- Single cell protein from algae may not be suitable for human consumption because they are rich in chlorophyll (except *Spirullina*)
- It has low density *i.e.* 1-2 gm dry weight/litre of substrate and there is lot of risk of contamination during growth.
- The filamentous fungi show slow growth rate than yeasts and bacteria there is high contamination risk and some strains produce mycotoxins and hence they should be well screened before consumption..

All these detrimental factors affect the acceptability of single cell protein as global food.

8. BACTERIAL BIOMASS

Bacteria are extensively used as a source of single cell protein because of their short life cycle (20-30 minutes) and capability to utilize a wide range of substrates as a source of energy. The specific growth rate and biomass yield of bacteria are greater than those of the other categories of microorganisms. Total protein content may reach up to 80% as compared to other microorganisms used for single cell protein production. Bacterial species can be used in foodstuffs as many are pathogenic, therefore it is required an intensive screening before utilization as food supplement. In addition, separation is difficult because of their small size range.

Rapid growth and high protein content are well known properties of bacteria as single cell protein production (Stringer, 1982; Kuhad *et al.,* 1997; Anupama and Ravindra, 2000). In the 1960s and 1970s, considerable research and industrial development were devoted to production of microbial protein (single cell protein) from hydrocarbon substrates such as methanol and methane, with the aim of supplying protein for human and animal nutrition (Roth, 1980; Stringer, 1982). Imperial Chemical Industries Ltd (ICI) has produced a commercially available product (Pruteen) from methanol (Overland *et al*., 2010), using the bacteria *Methylophilus methylotrophus*. The production of bacterial protein from methanol was a major biotechnological breakthrough, and the results of feed evaluation studies, with a number of species, were generally encouraging (Waldroup and Payne, 1974; D'Mello and Acamovic, 1976; D'Mello *et al*. 1976; Roth and Kirchgessner, 1976; Whittemore *et al*. 1976; Braude and Rhodes, 1977; Braude *et al.,* 1977; Kaushik and Luquet, 1980; Overland *et al*., 2010). However, commercial production was terminated, mainly due to economical considerations related to enhancement of oil prices and the low price of conventional protein sources.

Childress *et al*. (1986) reported that a mussel in the Mexican Gulf obtain required energy and protein through symbiotic relationship with a methane-oxidising bacterium residing in its gill cells. Therefore, the conversion of methane to produce protein-rich biomass by methanotrophic bacteria may be an important process in the natural nutritional chain. Methane contains carbon in a reduced and energy efficient form, and can support a high yield of microbial biomass into the bioconversion of the substrate to quality protein (Hanson and Hanson, 1996). D'Mello (1972) reported that a couple of strains of methanotrophs were found to have a nutritionally favourable profile of essential amino acids. Further studies suggested that methane-consuming bacteria were useful sources of protein for the monogastric animals (D'Mello, 1973). *Methylococcus capsulatus* has shown high efficiency in production of bacterial protein from methane (Bothe *et al.,* 2002). Significant study has been carried out on the bacterial protein produced mainly from methane by natural gas fermentation for a number of animal species, including pigs, chickens, mink, dogs, Atlantic salmon (Salmo salar), rainbow trout (*Oncorhynchus mykiss*), and Atlantic halibut (Skrede *et al*., 1998; 2003; Øverland *et al.,* 2001, 2005, 2006a; Skrede and Ahlstrøm, 2002; Hellwing *et al*., 2005, 2006, 2007a, 2007b; Schøyen *et al*., 2005, 2007a; Aas *et al*., 2006a, 2006b, 2006c). It was suggested from the feeding experiments with target species that bacterial protein derived from natural gas could be utilised as a sustainable future protein source for animal production. The high nucleic acids content of bacteria may make inappropriate for human consumption unless the nucleic acids are partly removed (Kuhad *et al.,* 1997; Anupama and Ravindra, 2000). Although the main focus on nucleic acids has been directed towards the constraints on their use directly as human food, their potential usefulness should be part of the evaluation as animal feed ingredients (Rumsey *et al.,* 1992; Mydland *et al*., 2008).

There is increasing awareness about the microbial protein sources in the utilization of sustainable supply of protein for monogastric animals. Bacterial protein can be grown rapidly and may relieve the pressure on limited and expensive high quality protein sources like fishmeal. Furthermore, production of microbial protein requires a small physical footprints, as well as constraints on the production of plant proteins including limited land area, water and fertiliser supply and sustaining environmental challenges.

9. FACTORS AFFECTING BACTERIAL BIOMASS PRODUCTION

Bacteria growth is affected by several factors; as a result of this the production of biomass on a given substrate significantly may differ. These are:

- Suitable strain of bacterial culture

- Genetic stability of bacterial strain
- Absence of bacteriophages
- Suitable pH (5-7) of growth medium
- Temperature
- Oxygen availability/agitation to create aerobic condition
- Organic substrate and nitrogen concentration
- Maintenance of sterile conditions throughout growth period

10. ALGAL BIOMASS

The protein is an essential component of diet and one of the big problems in present scenario is its global insufficiency. With the current system of production, agriculture cannot be relied upon to feed an ever increasing global population. Hence, there is an urgent need to find additional protein sources.

Spirullina platensis has been used as food for centuries in many spheres by the populations on earth and only revived in recent years (Usharani *et al.,* 2012). It is utilized as a complementary nutritional ingredient for shrimp, fish and poultry and increasingly as a protein and vitamin supplement to aqua feeds. China is using *Spirullina* as a partial substitute of imported forage to promote the growth, immunity and viability of shrimp (Usharani *et al*., 2012). There has also been ample research on the use of *Spirullina* as aquaculture feed supplement in Japan. A group of countries (Burundi, Cameroon, Dominican Republic, Nicaragua and Paraguay) submitted proposal in the United Nations general assembly (sixtieth session, second committee, agenda item 52) for "Use of *Spirullina* to combat hunger and malnutrition and help achieve sustainable development" (Usharani *et al*., 2012). Subsequently, FAO was directed to prepare a draft on *Spirullina*, therefore, there could have been a clear understanding on its use and to convey FAO recommendations on this (Usharani *et al*., 2012).

Spirullina are unicellular and filamentous blue-green algae that has gained significant popularity in the health and food industry and increasingly as a protein and vitamin supplement to aquaculture diets (Usharani *et al*., 2012). It has long been used as a dietary supplement by the people living close to the alkaline lakes where it is found naturally. Among a variety of *Spirullina* species, the blue green alga *Spirullina platensis* has drawn more attention because it shows high nutritional content up to 70% protein content including minerals, vitamins, amino acids, essential fatty acids etc. may be used as protein supplement in diets of undernourished poor children in developing countries (Usharani *et al*., 2012). Wahal *et al*. (1974) showed that water-soluble sugars constitute the major carbohydrates of *Spirullina*.

Spirullina can play an important role not only as human and animal nutrition but also in environmental protection through wastewater recycling and energy conservation. The amino acid composition of *Spirullina* protein held position amongst the excellent proteins of plant, more than that of soya bean. Presence of minerals and vitamins, Gamma-linolenic acid in this alga has been reported to stimulate the hormone prostaglandin synthesis and regulation of blood pressure, cholesterol synthesis, cell proliferation and inflammation (Usharani *et al*., 2012). *Spirullina* provides nearly all necessary nutrients without excess calories and fats. Athletes take *Spirullina* for instant energy requirement as well as *Spirullina* has been recommended to control obesity and premenstrual stress. Moreover, several herbal products like face cream, biolipstics, hair gel have been formulated with phycocyanin pigment of *Spirullina* (Usharani *et al*., 2012). The beta carotene and other carotenoids found in *Spirullina* are useful in control of being cancerous in human beings and increase in pigmentation of eggs, meats and coloration of ornamental fish.

The large cultivation of *Spirullina* may be achieved both in fresh as well as waste water (Usharani *et al*., 2012). *Spirullina* grown under strictly controlled conditions in clean waters could be used to fulfill human nutritious requirements. However, micro alga grown in waste water is used as animal feed and may be a good source of the fine chemicals and fuels. The reclamation waste water system using micro alga is highly applicable in densely populated countries including India where waste materials are generated in high quantities and pose severe environmental problem (Usharani *et al*., 2012). Large scale cultivation of *Spirullina* is feasible in tropical conditions in underdeveloped countries, where costs of labor and land are comparatively cheaper. In spite of micro alga as potential source of food and feed they can be exploited as therapeutic uses. Looking over the rationality of *Spirullina* it is required to study its various features and application in different fields.

10.1. Mass Cultivation of Spirullina

Spirullina is economically significant filamentous blue green algae. The annual production of the cyanobacteria is about 10,000 tons which make this organism the largest micro algal cultivation industry on global level (Usharani *et al*., 2012). Due to nutritious value it has been regarded as a model bio-resource and has drawn increasing attention in recent decades. A special attention has been paid towards *Spirullina platensis* as a potential resource in the field of pharmaceuticals, therapeutics and other high value products such as chlorophyll. The micro alga *Spirullina platensis* have a high tolerance capability to alkaline pH. Stanca and Popovici (1996) reported that the use of urea as a nitrogenous source in *Spirullina platensis* farming leads to amplification in both the biomass and the chlorophyll content (Usharani *et al*., 2012). Urea is easily taken by *Spirullina platensis*, probably due to its spontaneous hydrolysis to ammonia under alkaline cultivation

(Usharani *et al*., 2012). Phang *et al.* (2000) reported that, the waste water is frequently released into the rivers, most of the bigger factories are producing up to 10-22 tons of waste water day by day containing high level of carbon to nitrogen ratio (105:0.12), but it has been made more appropriate for anoxic fermentation in an up-flow packed bed digester. The digested waste water with an average ratio of C: N: P may be 24:0.14:1 support the growth of *Spirullina*.

Algal cultivation on wastewaters aspires at producing algal biomass economically and, at the same time, eliminating organic and inorganic contaminants. The use of microalgae can offer a valuable alternative to the conservative purification treatments and provides many benefits, such as:

- It is not environmentally hazardous, because it is based on the principles of natural ecosystems
- Algal biomass able to recycle decreasing the problem of secondary pollution
- Algal growth on effluent get rid of xenobiotic substances and heavy metals
- Oxygen is released due to photosynthetic reactions, thus increasing the auto- purification potential of water bodies.

10.2. Biochemical Composition of Spirullina

The fundamental biochemical composition of *Spirullina* can be reviewed as follows:

- ***Protein:*** *Spirullina* contains unusually high amounts of protein between 55-70% by dry weight. It is a complete protein, containing all essential amino acids (Usharani *et al*., 2012).
- ***Essential fatty acids:*** *Spirullina has* a high amount of polyunsaturated fatty acids, 1.5-2.0% total lipid found in *Spirullina.* The micro alga is rich in linolenic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid and arachidonic acid (Usharani *et al*., 2012).
- ***Vitamins:*** *Spirullina contains* vitamin B1, B2, B3, B6, B12, vitamin C, vitamin D and vitamin E (Usharani *et al*., 2012).
- ***Minerals:*** *Spirullina is* a significant source of potassium, calcium, chromium, copper, iron, magnesium, manganese, phosphorous, selenium, sodium and zinc (Usharani *et al*., 2012).
- ***Photosynthetic pigments:*** *Spirullina contains* many pigments including chlorophyll-a, xanthophylls, â-carotene, echinenone, myxoxanthophyll, phycocyanin, phycobilins, and allophycocyanin (Usharani *et al*., 2012), etc.

11. YEAST BIOMASS

Yeasts are the first microorganisms to be recognized scientifically (Barnett, 2003), the best studied and generally best accepted by consumers. Yeasts are rarely lethal or pathogenic and can be used in human food supplement. Although the protein content of yeasts hardly exceeds above 60% and the concentration of essential amino acids such as lysine, tryptophan and threonine (Gabriel *et al*., 2014) is satisfactory. In contrast, they contain little amounts of the sulphated amino acids *viz*. cysteine and methionine. They are also rich in vitamins (B group), and nucleic acid content ranges from 4 to 10%. They are even larger and their separation easy as compared to bacteria. They can be used in a raw state. However, their specific growth rate is comparatively slow having generation time 2 to 5 hours. In Germany, during First World War consumption of *S. cerevisae* as food increased rapidly. Since then, its rapid growth took place in biotechnological applications as far as culture development, process optimization and scale up production to substitute the scarcity of conventional plant and animal protein. Global production of yeast biomass ranges up to 0.4 million metric tonnes per annum including 0.2 million tonnes baker's yeast (*S. cerevisae*) alone (Gabriel *et al*., 2014). Yeast produces amino acids from inorganic acids and sulphur supplemented in the salts. They obtain carbon and energy from the organic wastes, *viz*. molasses, starchy materials, milk whey, fruit pulp, wood pulp and sulphite liquor.

11.1. Factors Affecting Yield of Yeast Biomass

Like bacteria, growth and yield of yeasts are also affected by the several factors:

- Organic substrate and nitrogen ratio (optimum C: N ratio favouring high protein component should be between 7:1 & 10:1)
- pH of nutrient medium (in the range of 3.5 to 4.5 to suppress growth of bacterial contamination)
- Temperature (most of the yeast have specific growth rate in the range of 30-34 °C while some strains grow in the range of 40-45 °C
- Oxygen (should be 1g/g dried cells)
- Proper maintenance of sterile form throughout the process
- Suitable strain of yeast for cultivation.

12. FUNGAL BIOMASS

In Europe, people made effort to produce *Fusarium* and *Rhizopus* culture for protein supplement (Gour *et al*., 2015) around the Second World War. Moreover, inoculum of *Aspergillus oryzae* or *Rhizopus arrhizus* was chosen because of their harmless nature (Riviere, 1977). 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 upon type of fungi and substrates on which it is grown. Chahal (1982) 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 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 filamentous fungi possess a faint mushroom like flavour 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 component of fungal protein are lower in comparison with yeast and bacteria.

12.1. Growth Conditions for Fungi

There are some factors which (similar to bacteria) affect the growth of moulds:

- Carbon, nitrogen (C: N) ratio is required to be in the range of 5:1 to 15:1
- Ammonium salts are used as a nitrogenous source and phosphoric acid for phosphorus in continuous culture. In addition, most of fungi use minerals, such as potassium, sulphur, magnesium, calcium, iron, manganese, zinc, copper and cobalt for their growth. Their concentration may differ with respect to fungal species
- pH required for growth medium ranges between 3.0 to 7.0 but the pH 5-6 or below is more desirable to most of the fungi due to bacterial contaminants
- Generally the temperature ranges between 25-30 °C
- Good quantity of oxygen is also required for the normal growth of fungi

13. CONCLUSIONS

The rapidly growing world population generates challenges for providing necessary foodstuff. One possible solution to this problem may be microbial mediated generation of single cell protein. Bacteria, algae, yeast and molds are candidates for the synthesis of single cell protein. They are rich in proteins, minerals, vitamins and essential fatty acids. The digestibility of these proteins as well as most of the other constituents ranges from 65 to 95%. Therefore, in the light of protein shortage, micro-organisms may offer many possibilities for protein production. They can be used to replace totally or partially the valuable amount of conventional vegetable and animal protein feed. Microbes are able to grow on waste materials and used them as substrate for the production of proteins; therefore it reduces the environmental pollution and helps in recycling of materials. Single cell producing organisms grow faster and produce large quantities of protein from relatively small area of land and time. Thus, land shortage and environmental calamities (such as drought or flood) cannot be a bottleneck in SCP production. In addition to nutritional values of single cell protein, it can be produced throughout the year since it is independent of seasonal as well as climatic conditions. Although some organisms that produce single cell protein are multicellular, they are useful in designating a potential source of protein and may become awesomely so important in years to come.

Microbial protein may be sustainable future protein source to fulfil the requirements of human and animal food supplement. There are some drawbacks such as presence of high quantity nucleic acids in some organisms which makes unsuitable for human consumption unless the nucleic acids are removed. Moreover, processing of single cell protein is also important because of susceptibility to contamination. Looking over the importance of microorganisms as an important source of nutrition in the coming years and their capability to grow on waste materials successfully and producing high amount of quality protein. It is required to develop green-clean technology for the large scale production to fulfil the needs of future and sustainable environment.

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