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Value-added food: Single cell protein

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Abstract

The alarming rate of population growth has increased the demand for food production in third-world countries leading to a yawning gap in demand and supply. This has led to an increase in the number of hungry and chronically malnourished people. This situation has created a demand for the formulation of innovative and alternative proteinaceous food sources. Single cell protein (SCP) production is a major step in this direction. SCP is the protein extracted from cultivated microbial biomass. It can be used for protein supplementation of a staple diet by replacing costly conventional sources like soymeal and fishmeal to alleviate the problem of protein scarcity. Moreover, bioconversion of agricultural and industrial wastes to protein-rich food and fodder stocks has an additional benefit of making the final product cheaper. This would also offset the negative cost value of wastes used as substrate to yield SCP. Further, it would make food production less dependent upon land and relieve the pressure on agriculture. This article reviews diversified aspects of SCP as an alternative protein-supplementing source. Various potential strains and substrates that could be utilized for SCP production are described. Nutritive value and removal of nucleic acids and toxins from SCP as a protein-supplementing source are discussed. New processes need to be exploited to improve yield. In that direction the solid state fermentation (SSF) method and its advantages for SCP production are highlighted.

Keywords: Single cell protein (SCP); Staple food; Nutritive value; Nucleic acids; Toxins; Solid state fermentation (SSF)

1. 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 Pop-*

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ulation and extensively exemplified by Charles Darwin in his theory of natural selection. A similar trend is observed in the recent statistical analysis carried out by the Food and Agricultural Organization (FAO) with respect to the human population and availability of affordable proteinaceous food sources. The scarcity of protein-rich food and global existence of impoverished millions of people have forced mankind to search for alternative protein sources that can replace conventional, but expensive, soymeal or fishmeal. Hence, the focus has shifted in recent times to exploit microbes as food sources for fortification of the food supply or for consumption as single cell protein (SCP). The term 'single cell protein' was coined in 1968 at a meeting held at the Massachusetts Institute of Technology (MIT) to replace the less aesthetic 'microbial protein' and 'petroprotein' which were the terms originally used (Mateles and Tannenbaum, 1968; Tannenbaum and Wang, 1975).

Use of microbes as a food source may appear to be unacceptable to some people but the idea of consumption of microbes as food for man and animals is certainly innovative to solve

Table 1
Algae as food in different regions of the world

Algae	Region	Use
Alaria	Japan	Stipes called 'Saumen' are dried, salted and sold
Ascophylluem, Fucus, Laminaria	Japan, USA, New Zealand	Feed for cattle, poultry and pigs
Caulerpa rosemosa	Philippines	Food
Durvillea antartica, Ulva	Chile	Food
Laminaria	Japan	Stipes called 'Kombu' are dried, salted and sold
Laminaria, Ecklonia, Eisenia	Great Britain, France, Scandinavia, Pacific coasts of USA	Chopped and used for chickens and sheep
Nostoc	Brazil	Colonies are boiled and consumed
Pelvwtia	Norway, France, USA, Denmark, New Zealand	Fodder for cattle
Porphyra tenera (Amanori), *Laminaria, Monostroma, Undaria, Sargassum*	England, Korea, China, Japan	Regular diet, Food
Rhodomenia palmata	Not available	Food and salty confection named 'dulse'
Rhodymenia	France	Cattle feed
Rhodymenia palmata, Gelidium, Grateloupia, Fucus	Pacific islands	Chopped and added to other dishes
Rhodymenia, Chlorella pyrenoidosa, Spirulina, Synechococcus	Pacific islands, Oriental region	Regular portion of diet
Sargassum	China	Fodder
Spirogyra, Oedogonium	India	Dried and used to make soup
Ulva	Europe	As regular diet
Ulva lactuca	Scotland	Used in salads and soups

the global food problem. For thousands of years man has been consuming, either intentionally or unintentionally, products such as alcoholic beverages, cheese, yogurt, soya sauce, and along with these products, the biomass responsible for their production (Tuse, 1984). The first written record of the utilization of microbes dates back to 2600 BC in Babylonia, as traces of bread were found there. By the time of Hammurabi, who ruled during the 12th century BC, baking had developed into a special craft. The discovery of leavened bread is generally attributed to the Egyptians (Jacob, 1944). Even today there are reports regarding the use of microbes in food and feed from many parts of the world (Singh, 1998).

Currently SCP is produced from many species of microorganisms. These include algae, fungi and bacteria. It is convenient to use fungi and bacteria for production of SCP when grown on inexpensive waste material. Their rapid growth and high protein content have made them the prime candidates for use as sources of SCP. Several species of algae that are currently being used are cultivated on aquatic media (Tuse, 1984).

2. Sources

Algae, fungi and bacteria are the chief sources of microbial protein that can be utilized as SCP. Since ancient times, people near Lake Chad in Africa and the Aztecs near Lake Texcoco in Mexico have been harvesting *Spirulina* from the waters and using it as food after drying (Singh, 1998). Among the algae, *Spirulina* is most extensively used and is even carried by astronauts during space travel. Similarly, biomass from *Chlorella* and *Senedesmus* is har-

Table 2
Single cell protein production from fungi

Organism used	Substrate	References
Aspergillus niger AS 101	Corn cobs	Singh et al., 1991
Aspergillus niger, Sporotrichum pulverulentum	Maize and Cotton stalk	El-Saadany et al., 1988
Candida krusei SO1& *Saccharomyces* spp. LK3G	Sorghum hydrolysate	Kolani et al., 1996
Candida tropicalis ceppo 571	Sulfite waste liquor	Guven and Cansunar, 1989
Chaetomium cellulolyticum	Cellulosic wastes	Singh, 1998
Chrysonilia sitophilia	Lignin	Rodriguez et al., 1997
Fusarium graminearum	Starch hydrolysates	Singh, 1998
Marine yeast	Pawn shell wastes	Rhishipal and Philip, 1998
Mixed cultures of yeasts	Dairy wastes	Saliceti-Piazza et al., 1992
Paecilomyces variolii	Sulfite liquor	Singh, 1998
Penicillium cyclopium	Whey	Kim and Lebeault, 1981
Penicillium roqueforti, Penicillium camemberti	Citus fruit peel	Scerra et al., 1999
Pichia pastoris	Methanol	Wagner, 1990
Saccharomyces cereviceae	Molasses, Stillage	Singh, 1998
Schwanniomyces occidentalis	Starch	Deibel et al., 1988
Scytalidium acidophilum	Waste paper	Invarson and Morita, 1982
Trichoderma album	Not disclosed	Staron, 1981
Trichoderma reesei & *Kluyveromyces marxianus*	Beet-pulp	Ghanem, 1992
White rot fungi	Sugarcane bagasse	Zadrazil and Puniya, 1995
Yeast	Plant origin liquid waste	Chanda and Chakrabarti, 1996

vested and utilized as food and feed by tribal communities in certain parts of the world. A variety of algae form an important food source for fish, aquatic amphibians, mammals and other animals. Algae can be used as a food source in many ways as listed in Table 1 (Vashista, 1989).

Many fungal species are used as protein-rich food. Most popular among them are the yeast species *Candida, Hansenula, Pitchia, Torulopsis* and *Saccharomyces*. Many other filamentous fungi also serve as sources of SCP (Table 2). A recent trend in SCP production is the exploitation of fungal species for bioconversion of lignocellulosic wastes.

Cellulomonas and *Alcaligenes* are the most frequently used bacterial species as an SCP source (Trehan, 1993). Sasikala and Ramana (1995) reviewed the potential phototrophic bacterial strains for SCP production. There are also reports regarding the use of methanotrophic and other bacteria for SCP production (Murrell, 1992; Table 3).

3. Production process

Technically, SCP is the manufacture of cell mass using microorganisms by culturing on abundantly available agricultural and industrial wastes. The production of microbial biomass is done either by a submerged or solid state fermentation process. After fermentation, biomass is harvested and may be subjected to downstream processing steps like washing, cell disruption, protein extraction and purification (Faust, 1987).

4. Key substrates

A variety of substrates have been utilized to cultivate bacteria, fungi and algae. The main substrates required for growth of algae are carbon dioxide and sunlight. Principal algal species for SCP production are reported in Table 4. Fungal species are cultured on different substrates, mostly cheap wastes, which supply the carbon and nitrogen for growth (Table 2). Lignocellulosic wastes from different sources have varying composition of hemicellulose, cellulose and lignin. Some sources of lignocellulosic material are wood from angiosperms and gymnosperms, grasses, leaves, wastes from paper manufacture, sugarcane bagasse,

Table 3
Single cell protein production from bacteria

Organism used	Substrate	References
Bacterial species	Variety of substrates	Tannenbaum and Wang, 1975
Bacteria of *Methylococcaceae*	C_1 compounds	Ekerott and Villadseer, 1991
Brevibacterium spp.	C_1–C_4 compounds	Singh, 1998
Cellulomonas spp.	Agricultural wastes	Callihan and Clemmer, 1979
Different species of bacteria	Fruit processing wastes	Litchfield, 1979
Methanomonas methanica	Methane	Singh, 1998
Methylophilus methanotrophus	Methanol	Singh, 1998
Pseudomonas fluorescens	Manure, Animal wastes	Shuler et al., 1979
Rhodopseudomonas gelatinosus	Wheat bran	Shipman et al., 1975
Streptomyces spp.	Methanol	Singh, 1998

wheat straw, wheat bran, rice bran, groundnut shell and other agricultural wastes (Tanaka and Matsuno, 1985; Gupte and Madamwar, 1997). Based on the dominant component in the waste used, specific fungi can be utilized for biomass production. Bacteria can also be cultivated on wastes or byproducts obtained from industrial processes (Table 3). The biomass thus produced can be harvested and used as SCP.

5. Nutritive value

The food value and usefulness of SCP from any source is based on its composition. The nutrients, vitamins, nitrogen, carbohydrates, fats, cell wall components, nucleic acids, protein concentration and amino acid profile, should be analyzed before the product is used for food or as feed supplementation.

Algae are rich in protein, fats and vitamins A, B, C, D and E. Planktonic algae are the original source of vitamins A and D. *Macrocystis* is rich in vitamins A and E, while the diatom *Nitzschia* is rich in vitamin A. Vitamin B is found in *Ulva, Enteromorpho, Laminaria, Alaria valida* and *Porphyra*. Vitamin C is present in *Ulva, Enteromorpho, Alaria valida,* among others. *Dulse* contains half as much vitamin C as oranges, and *Fucoids* and *Porphyra* are even richer. The milk-yielding capacity of cattle is reportedly enhanced when *Pelvetia* forms an ingredient in cow feed (Vashista, 1989). Apart from vitamins, algae also contain 40–60% protein, 7% mineral salts, chlorophyll, bile pigments, fiber, and have a very low nucleic acid content (4–6%) (Brock, 1989).

While algae are rich in all vitamins, fungi provide, to some extent, the B-complex group of vitamins. Fungi also show a low level of nucleic acid content (9.7%) (Frazier and Westhoff, 1990). The amino acid composition of *Aspergillus niger* according to FAO standards is well balanced (Kuzmanova et al., 1989; Table 5). Yeast contains thiamine, riboflavin, biotin, niacin, pantothenic acid, pyridoxine, choline, streptogenin, glutathione, folic acid and p-amino benzoic acid (Frazier and Westhoff, 1990). SCP from mixed cultures of *Trichoderma reesei* and *Kluyveromyces marxianus,* when grown on beet pulp, are reported to con-

Table 4
Single cell protein production from algae

Organism used	Substrate	References
Caulerpa rocemosa	Carbon dioxide + sunlight	Vashista, 1989
Chlorella salina CU-1(28)	Saline sewage effluent	Wong and Chan, 1980
Chlorella spp.	Carbon dioxide	Singh, 1998
Chlorella spp. (M109, M121, M122, M138, M150)	Carbonate and seven other compounds	Mahasneh, 1997
Dunaliella	Carbon dioxide + sunlight	Trehan, 1993
Chlorella & Diatoms	Carbon dioxide + sunlight	Trehan, 1993
Laminaria	Carbon dioxide + sunlight	Vashista, 1989
Porphyra	Carbon dioxide + sunlight	Vashista, 1989
Sargassum	Carbon dioxide + sunlight	Vashista, 1989
Spirulina maxima	Carbon dioxide + sunlight	Singh, 1998
Spirulina spp.	Carbon dioxide	Singh, 1998

tain essential amino acids which compares favorably with FAO guidelines and soybean oil meal (Ghanem, 1992). The amino acid composition of *Chaetomium cellulolyticum* was found to be better than *Trichoderma viride* on comparison with the FAO reference protein, alfalfa and soymeal (Moo-Young, 1977, Moo-Young et al., 1977). Proteins of *K. fragilis* obtained by culturing on whole whey along with the unconsumed whey proteins yielded a SCP with high crude protein and low ash content. The product was also rich in sulfur-containing amino acids (Galvez et al., 1990; Singh et al., 1991).

Bacterial SCP is high in protein and certain essential amino acids. The crude protein content is around 80% of the total dry weight. The nucleic acid content, especially RNA, is very high on a dry weight basis 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; Frazier and Westhoff, 1990; Singh, 1998). The essential amino acid composition of different *Lactobacilli* appears comparable to the FAO reference protein and SCP from other sources (Erdman et al., 1977).

6. Limitations for use of SCP from various sources

Although algae are very good nutrition sources, there are some limitations for human consumption. The most important one 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. The cellulose digestion step is not required if the SCP is used as feed for cattle as they have cellulose-degrading symbiotic bacteria and protozoa in their rumen. Algal production is generally done outdoors and is dependent on the climatic conditions. Hence, productive algal species and favorable conditions are important. Elaborate methods and preparations are required to eliminate contamination (Trehan, 1993).

The presence of mycotoxins in certain fungal species especially *Aspergillus parasiticus* and *A. flavus* is a major hindrance in their use. These toxins are known to produce many allergic reactions, diseases and liver cancer in humans as well as animals. Hence, it is a prerequisite that mycotoxins are eliminated before fungal SCP is consumed.

Use of bacterial SCP is limited due to its high cost. Harvesting protein from bacteria is costly due to the smaller cell size and hence the cells must be flocculated to give a higher solids slurry prior to centrifugation (Trehan, 1993). Bacterial cells also have a very high content of nucleic acids. Moreover, there is a psychological barrier to the use of bacteria as major food source.

Table 5
Composition of SCP from *Aspergillus niger* as compared to the FAO standard

	Amino acid (%)							
	Valine	Leucine	Isoleucine	Lysine	Methionine	Phenylalanine	Cystine	Tyrosine
FAO Standard	4.20	4.80	4.20	4.20	2.20	2.80	2.80	2.80
Aspergillus niger	4.36	6.80	3.75	4.50	0.35	5.70	Trace	3.00

Algal species are extensively used for commercial purposes owing to their very low nucleic acid content. The removal of mycotoxins and reduction in nucleic acid content, make it possible for some fungal species to be used as SCP sources on commercial basis, but SCP from bacteria has yet to carve out its niche in the global SCP market. Extensive use of SCP as food is possible if it is tasty, appealing to the eye and possesses a higher nutritive value than conventional protein sources. It should be economically viable with a high yield coefficient (Ys) to reach the poorest of the poor (Frazier and Westhoff, 1990).

7. Ranking of algae, fungi and bacteria derived SCP products

A comparison between algae, fungi and bacteria highlighting the nutritive value of the final product is cited in Table 6. The acceptability of a particular species as food or feed depends on the growth rate, substrate used, contamination and associated toxins. These are briefly presented and compared in Table 7. The parameters indicate that nucleic acid safety in algae is better than fungi and bacteria. Further, due to their low nucleic acid content, fungi are better than bacteria. Moreover, fungal SCP is rich in methionine and lysine. Fungal lysine content is higher than bacteria or algae. Hence, the order of preference can be given as algae $>$ fungi $>$ bacteria. Fungal sources can be exploited as nutritive SCP if the nucleic acid content is considerably reduced to levels comparable to that of algae and also if the mycotoxins are removed.

However, among all SCP sources, the species, which find global acceptability presently, is the yeast in breweries and bakeries. Further, *Spirulina* and *Chorella* are most popular among the algae. These are used as food and food supplements, in many parts of the world.

Table 6
Composition of SCP from the representative types

Component	Percentage composition of weight		
	Algae	Fungi	Bacteria
True proteins	40–60[a]	30–70[a]	50–83[a]
Total nitrogen (Protein + nucleic acids)	45–65[a]	35–50[a]	60–80[a]
Lysine	4.6–7.0[a]	6.5–7.8[a]	4.3–5.8[a]
Methionine	1.4–2.6[a]	1.5–1.8[a]	2.2–3.0[a]
Fats/Lipids	5–10[a]	5–13[a]	8–10[a]
Carbohydrate	9	NA	NA
Bile pigment and Chlorophyll	6	NA	NA
Nucleic acids	4–6[a]	9.70	15–16[a]
Mineral salts	7	6.6	8.6
Amino acids	NA	54	65
Ash	3	NA	NA
Moisture	6.0	4.5–6.0[a]	2.8
Fiber	3	NA	NA

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

NA- Not available.

Sources: Brock, 1989; Frazier and Westhoff, 1990; Powar and Daginawala, 1995; Ziino et al., 1999.

Table 7
Comparison of various parameters for SCP production from algae, fungi and bacteria

Parameter	Algae	Bacteria	Fungi (Yeast)	Fungi (Filamentous)
Growth rate	Low	Highest	Quite high	Lower than bacteria and yeast
Substrate	Light, carbon dioxide or inorganic samples	Wide range (Refer Table 3)	Wide range except carbon dioxide	Mostly lignocellulosics
pH range	Upto 11	5–7	5–7	3–8
Cultivation	Ponds, Bioreactors	Bioreactors	Bioreactors	Bioreactors
Contamination risks	High and serious	Precautions needed	Low	Least if pH is less than 5
S-containing amino acids	Low	Deficient	Deficient	Low
Nucleic acid removal	–	Required	Required	Required
Toxin	–	Endotoxins from gram-negative bacteria	–	Mycotoxins in many species

Source: Singh, 1998.

8. Toxic compounds in SCP and their effects

The extent to which SCP is extracted and purified when used as food should be comparable to global standards. The final product should not only be nutritious, but should also pass all toxicity tests to be commercialized as a food product. Apart from the nucleic acid content, several toxins and unwanted compounds accumulated during the course of growth on substrates like hydrocarbons and petroleum contaminated with heavy metals should be removed.

8.1. Nucleic acids

Intake of a diet high in nucleic acid content 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).

In rapidly proliferating microbial cells RNA forms the bulk of the nucleic acids (Singh, 1998). The RNA content of yeast cells is known to be dependent on the culture conditions and C/N ratios. The nucleic acid level can be reduced by several means. These include activation of endogenous RNAase by brief heat treatment up to 60–70°C for 20 min, alkaline hydrolysis of nucleic acids, modifications of cultural conditions with respect to nitrogen, carbon, phosphorous and zinc content or chemical extraction and removal of nucleic acids.

Some researchers have extensively discussed safety evaluation of SCP products for human food and animal feed, and specific methods to reduce the nucleic acids are also reported (Taylor et al., 1974). Purine content in baker's yeast can be reduced by chemical treatment and autolytic methods (Trevelyan, 1976a,b). Reduction of nucleic acids can be achieved by

the endogenous polynucleotide phosphorylase and RNase in *Brevibacterium* (Yang et al., 1979). Two derivatives of pancreatic RNase and an endonuclease of *Staphylococcus aureus*, immobilized on corncobs, have been used to reduce the percentage of nucleic acids in SCP concentrates of yeasts, from 5–15% to 0.5% with a protein loss of only 6% after treatment (Martinez et al., 1990). Immobilized nucleases like benzonases on corncobs were also used to reduce the nucleic acid content in protein concentrates. The percentage of DNA was reported to be reduced to 3–6% and RNA to 50% with loss of protein in the process being only 1% (Moreno et al., 1991). An immobilized pancreatic RNase was also investigated for the degradation of yeast ribonucleic acid. The rapid reaction rates obtainable at relatively low temperatures offer a potential alternative method of purifying yeast SCP with minimal loss of derived protein (Dale and White, 1979). Methods for reduction of nucleic acid content in SCP obtained from gas oil are also reported (Abou-Zeid et al., 1995).

8.2. Toxins

Toxins, if present in SCP, also act as contaminants. Toxins are actually secondary metabolites produced by certain fungi (Bennett and Keller, 1997) and bacteria (Blancou et al., 1978) during growth. Algae generally do not produce harmful toxins. The toxicity of the SCP product must be assessed before marketing it. When used as animal feed, toxicity levels are generally higher than when consumed by humans. Some toxicity tests can only be done on live model animals. Hence, all these tests and analyses are primarily made to assess the suitability of the final product for food, feed or supplementation purposes. For example, Gupta and Sharma (1984) reported the effect of aflatoxins on various animal models. Based on the age, body weight and sex of the animals the toxicity effect was different even when equal amounts of aflatoxin was administered to animals. Hence, the limit of unavoidable consumption varies based on toxicity levels and decontamination.

8.2.1. Mycotoxins

Human mycotoxicoses dates back to 1100 AD (Gupta and Sharma, 1984). There are many reports on mycotoxins but aflatoxins are the most important and best understood (Eaton and Groopman, 1994). Mycotoxins generally display great chemical heterogeneity and are traditionally analyzed by thin layer chromatography or high performance liquid chromatography techniques. The best single source for mycotoxin methodology, including structural elucidation, is given by Cole (1986).

Prominent fungal toxins include aflatoxins of type B_1, B_2, G_1 and G_2 from *Aspergillus flavus*, citrinin from *Penicillium citrinum*, trichothecenes and zearalanone from *Fusarium* species and ergotamine from *Claviceps* species (Bennett and Keller, 1997). There is strong epidemiological evidence linking aflatoxins to human liver cancer (Eaton and Groopman, 1994). Aflatoxins are produced by *A. flavus*, *A. parasiticus* and *A. oryzae* (Schlegel, 1996). Apart from aflatoxins, ochratoxins are important mycotoxins. These are structurally related groups of pentaketides. Ochratoxin A is the most abundant and toxic of the five metabolites in the Ochratoxin group. These metabolites have been found to occur in the *Aspergillus* and *Penicillium* species and cause damage to the liver as well as kidneys (Kerkadi et al., 1998; Varga et al., 1996).

Trichothecenes are considered the next most important group among mycotoxins. They have a 12, 13 epoxytrichothec-9-ene nucleus. There are 80 types. These cause dermal toxicity and several hematopoietic effects (Cole and Cox, 1981; Marasas et al., 1984; Joffe, 1986). Mycotoxins recently became the objects of general public interest when thousands of young turkeys died due to aflatoxin contamination in the feed by a mycotoxin-producing fungal species (Schlegel, 1996). Even though non-toxin-producing strains are used for SCP production, contaminating fungal strains sometimes produce toxins in the medium. Minute quantities of mycotoxins are capable of producing allergies, diseases, rashes on skin, neurotoxicity and other disorders. Hence, contaminating strains should be checked for toxin production and eliminated before and during the SCP production process. Eliminating or minimizing mycotoxin contamination is a continuing biotechnological challenge (Lee et al., 1992).

Research in the removal of mycotoxins from SCP has been focused chiefly on aflatoxins. Among the many methods tested, ammoniation is the most successful. It can reduce aflatoxin levels by 99% (Park and Liang, 1993; Lee et al., 1992). Further, when *A. flavus* is co-cultured with other microbes, its toxin-producing capacity is found to be reduced (Widstrom, 1992; Cotty et al., 1994).

Most recently, molecular biology techniques have been exploited for detoxification. Molecular dissection and elimination of genes responsible for mycotoxin synthesis is developing into an exciting area of contemporary mycotoxin research (Keller et al., 1992). The biosynthetic pathways for both aflatoxins (Dutton, 1988) and trichothecenes (Desjardins et al., 1993) have been studied. The techniques of cloning, probing, sequencing, expression libraries, transcript mapping, gene disruption, and chromosome walking have been employed to isolate the aflatoxin pathway clusters from *A. flavus* and *A. parasiticus* (Bennett et al., 1994; Trail et al., 1995). Studies that identify the molecular determinants regulating mycotoxin production are promising rational control strategies. For example, aflR is the regulatory gene controlling the production of both aflatoxins and sterigmatocystin in *Aspergillus.* This forms the target gene to be inhibited for the future control of mycotoxin production in *Aspergillus* (Chang et al., 1993; Woloshuk et al., 1994; Yu et al., 1996). When *Aspergillus* is impaired in asexual reproduction the production of aflatoxins is inhibited (Kale et al., 1994). A recent report describes how a plasmid vector (pDEL2) was engineered to introduce a deletion within the aflatoxin biosynthesis gene cluster of *A. parasiticus*. Subsequent aflatoxin precursor feeding studies confirmed that the enzyme activities associated with the deleted genes were absent (Cary et al., 1999). There are also reports that products of plant defense pathways (i.e. lipoxygenase or the jasmonate pathway) can inhibit aflatoxin production (Goodrich-Tanrikulu et al., 1995; Zeringue, 1996).

Hence, it can be seen that detoxification of SCP from fungal sources is possible by gaining an understanding of toxin gene regulation. Though the research in this field is in the initial stages, reliable and easily applicable techniques can be expected in the near future.

8.2.2. Bacterial toxins

Bacteria produce either endo or exotoxins. Exotoxins are secreted by gram-positive bacteria into the surrounding medium. They are proteins of molecular weight 10 000–900 000 Dalton. They do not produce fever in the host but cause generalized symptoms and lesions of various kinds. Administration of filtrates, lacking bacterial cells, to experimental animals

also results in these symptoms. The toxins are fatal for laboratory animals at nanogram levels. Some exotoxins are: enterotoxin, erythrogenic toxin, alpha-toxin, and neurotoxin.

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. They often produce fever in the host and are fatal for laboratory animals at slightly higher doses than exotoxins (Powar and Daginawala, 1995). SCP from *Pseudomonas* species and *Methylomonas methanica*, when used for animal feed purposes, caused febrile reaction and high titres of IgG and IgM due to endotoxins (Ekenvall et al., 1983).

Pathogenic bacteria can be avoided for use as SCP. Some non-pathogenic species are highly nutritive due to their high protein content. But if these species produce toxins their use as SCP sources is not possible. Hence, removal of toxin can be done for SCP derived from non-pathogenic species prior to their use as SCP source. Exotoxins can be easily removed, as these are present in soluble form in the medium. They are sensitive to temperatures above 60°C. Moreover, 50% alcohol, formaldehyde and dilute acids can denature exotoxins or convert them into non-toxic toxoids. Toxoids are beneficial for artificial immunization (Powar and Daginawala, 1995).

Because endotoxins are the part of cellular components of some of the gram-negative bacteria and are not released into the medium by the living bacterial cell, their removal is somewhat difficult. Their formation can only be prevented by genetic engineering, where the activity of genes controlling the formation of the unwanted toxins can be modified or suppressed. This may be a difficult task to achieve, as they are integral structural components of the bacterial cell wall.

9. SCP safety

The foreign protein in SCP can be unsuitable for humans and lead to skin reactions, allergies or gastrointestinal reactions resulting in nausea and vomiting. The SCP may even carry carcinogenic factors as contaminants derived from the substrates used. Hence, prior decontamination and purification of the final product is required before it is used as a food source. Bacterial SCP appears to be safe, as it has no effects on the rat immune system when tested both in vitro and in vivo. Immunogenicity of the product was checked by lymphocyte transformation tests for systemic response and foot-pad swelling assay to determine immunological risk of food stuffs to man. There was no apparent systemic response when lymphocyte transformation tests were carried out. The foot-pad-swelling assay also did not show any effects (Steinmann et al., 1990).

SCP may also contain contaminating heavy metals or other metallic compounds, which can cause mutations, even in minute quantities. Further, foreign protein consumed in the form of SCP should not be mutagenic. SCP from bacteria, grown on methanol as a substrate was tested for mutagenicity in five in vivo tests in different mammalian test systems. Statistical evaluation of all test results revealed no evidence of mutagenic activity (Renner and Munzner, 1978).

High metabolic efficiency of SCP from any source is essential in order to extract the maximum possible benefits. SCP from bacteria grown on methanol was evaluated in dogs (Dal-

matian variety). There were no effects on metabolism or any other harmful influence (Giesecke et al., 1982). Separate 90-day trials were done on egg-laying hens at peak production to evaluate two SCP sources, namely Pruteen, produced from methanol utilizing bacteria and Lavera-type yeast grown on n-paraffins of heavy oil. At the same concentrations (50 g/Kg of diet) yeast SCP had little effect on performance, but Pruteen decreased the production rate and egg size due to a significant reduction (5%) in food consumption (Bornstein et al., 1982).

A major limiting factor in the use of SCP as food is its nucleic acid content. The relationship between SCP nucleic acid and human feeding was reviewed (Araujo-Neto and Ferreira-Pinto, 1975) and analysis of nitrogen, cell wall, protein and RNA content was carried out by Kellems et al. (1981). It was concluded that SCP was higher in methionine and lysine than cottonseed meal. True proteins based upon amino acids recovered in SCP samples ranged from 51.6 to 65.9% of crude protein. In digestion trials, sheep consumed the SCP diets readily and without any digestive disturbances. Based on in vitro and laboratory results the SCP from secondary clarifiers of pulp mill had the potential to be a viable protein supplement for live stock. To prove that nucleic acid consumption increased levels of uric acid in the body, rats were fed *Fusarium* derived SCP. Plasma and kidney uric acid concentrations showed an increase after 21-day trials in the absence of uricase. During the trials, the uricase activity was inhibited by oxonate, a uricase inhibitor in the diet (Winocour et al., 1978). Hence, SCP for human consumption should be free from nucleic acid as humans lack uricase in their system. Digestibility also plays an important role in the efficient utilization of SCP in a diet supplemented by SCP. Protein digestibility values expressed as a percentage, range from 65 to 96% for the various cultures tested. Protein efficiency ratio (PER) values range from 0.6 to 2.6 (Frazier and Westhoff, 1990). An analysis was done for the apparent digestibility of diets containing fishmeal, soybean meal and bacterial meal when fed to *Salmo salar*. The digestibility of the diet with bacterial meal was comparable with that of the other supplements (Storebakken et al., 1998).

SCP, being a novel product, demands extensive sanitation and purification processes before the final product is cleared for consumption as per quality control standards. The US Food and Drug Administration and the Protein Evaluation Group of the United Nations have developed guidelines for the safety evaluation of SCP products in humans and domestic livestock (Litchfield, 1985). Rigorous sanitation and quality control procedures must be maintained throughout the process to avoid spoilage and contamination by pathogenic and toxigenic microorganisms when biomass for SCP is being cultivated.

10. Optimum SCP production parameters

Substrate, yield, and nutritive value in terms of proteins and vitamins dictate the cost of the final product. The yield is controlled by the organism and the substrate used for fermentation. Mixed cultures of *Trichoderma reesei* and *Kluyveromyces marxianus* gave a SCP yield of 51% and the efficiency of conversion of beet-pulp into protein was reported to be 39.4%. The yield was 54% when yeast extract was substituted in the basal medium and beet-pulp level was increased from 2 to 4%. The conversion efficiency in the latter case was increased

to 41.8% (Ghanem, 1992). Among 67 potential yeasts analyzed, *Candida tropicalis* and *Yarrowia lipolytica* were found to be the most efficient for SCP production on diesel oil as the sole carbon source. Maximum yield was reported at 168 h for diesel oil concentrations in the range of 40–60 mL/L (Ashy and Abou-Aeid, 1982).

Culture conditions, pretreatment of substrates, nutrient supplementation, types of fermentation processes, and strain improvement can all alter the final SCP composition. Several fungal cultures produced double the amount of crude protein on alkali-treated rice straw when compared to that of untreated straw (Darmwal and Gaur, 1991). When *Kluyveromyces fragilis* was grown on whole whey as the substrate, the chemical score of the protein produced was 91%. Whey protein product, a protein isolate could be recovered with a yield of 80%. The protein content of the isolate was reported to be 75% and the nucleic acids were reduced by 90.8%. Cell wall debris was also considerably reduced in the final product (Galvez et al., 1990). Addition of yeast extract and manganese chloride resulted in the highest yield of SCP from *Lactobacillus delbrueki* subsps. *bulgricus* 369 when cultured on dried whey. The yield was reported to be 32.8% (el-Sabaeny, 1996). Conversion of substrate into final product in terms of protein content is shown in Table 8. Here, mycoprotein (*Fusarium graminearum*) production shows greater efficiency in conversion of substrate to protein when compared to the conversion of feed by farm animals.

Cloning techniques are also used for improvement of SCP production. *Pseudomonas methylotrophus* was isolated by Imperial Chemical Industries (ICI) and then significantly improved through genetic engineering and physiological development. The glutamine-keto-acid-transaminase (GOGAT) enzyme of *P. methylotrophus* forms glutamate and NAD(P) from alpha-ketoglutarate, NADP(H) and glutamine. When the glutamate dehydrogenase system isolated from *Escherichia coli* was transferred to a GOGAT mutant of *P. methylotrophus* by genetic engineering, improved nitrogen assimilation was observed. It was used commercially and yield improvements in terms of raw protein, pure protein, cell dry weight and maximum growth rate were significant (Gow et al., 1975). Yields of the engineered organisms were about 5% greater than the parent organism (Balasubramanian et al., 1998). The use of genetic engineering is not restricted to the design and production of microbes with high protein yield alone, but also finds application in the improvement of downstream processing in the post-fermentation stage. It is used in the production of ‘designer’ or ‘recombinant’ proteins of high nutritive value and their subsequent purification. Novel techniques like specifically designed tags or modification of sequences with target-gene product are used for effi-

Table 8
Conversion rates in protein formulation in mycoprotein and animals

	Starting material (1 Kg)	Product	
		Protein (g)	Total (g)
Cow	Feed	14	68 Beef
Pig	Feed	41	200 Pork
Chicken	Feed	49	240 Meat
Fusarium graminearum	Carbohydrate + inorganic nitrogen	136	1080 Wet cell mass

Source: Beech et al., 1984.

cient recovery of native and modified proteins (Nygren et al., 1994; Murby et al., 1996). Improvement in protein recovery can also be achieved by suppression or elimination of protease activity in the microbial cells. Mutants with a modified protease gene sequence can be isolated and used for this purpose. For example, the LDHP1 strain of *S. cerevisiae*, a protease-deficient mutant produced by osmotic shock, showed an improved beta-galactosidase yield (Becerra et al., 1997).

Recombinant DNA technology can be used to isolate mutant genes that can produce high amounts of specific amino acids like glutamate, tryptophan, and phenylalanine along with high protein yields. Such genes were combined by recombination techniques and incorporated into an organism with a wide substrate range. A mutant variety of *S. cerevisiae* produced a high concentration of intracellular glutamate (Hill, 1994). Mutants of *S. cerevisiae*, which were resistant to toxic ethionine, showed accumulations of methionine as high as 163 times that of parental strain. These high levels were achieved due to enhanced specific activity and reduced feedback inhibition by threonine of the enzymes aspartokinase and homoserine dehydrogenase (Martinez-Force and Benitez, 1993). About 180 000 tons of lysine is needed annually to improve the nutritive value of food and SCP. An alanine auxotroph of *Corynebacterium glutamicum*, sensitive to beta-fluoropyruvate and resistant to alpha-chlorocaprolactum, could produce 70 g/L lysine. Upon strain improvement by recombinant DNA techniques, the yield was enhanced to 98 g/L of lysine (Sahm, 1995). The yield of expensive amino acids like threonine can also be improved by the use of recombinant *Escherichia coli* (Tsukada and Sugimori, 1971), and that of phenylalanine by using a genetically manipulated strain of *Rhodotorula rubra* (De Boer and Dijkhuizen, 1990).

High cost restricts the use of tryptophan by humans. Using genetic recombination to combine four mutations into one strain of *S. cerevisiae* helped to produce 12 g/L tryptophan (Moller, 1994). Strain improvement is done by complementing the auxotrophic mutants with wild-type genes (Jungehulsing et al., 1994; Niederberger, 1989). The substrate range of such strains can be extensively widened when compared to the auxotrophic variety. This would allow the utilization of a variety of unused substrates for the growth of microbes for SCP production.

Unstructured logistic models were also applied to stimulate the kinetic processes of SCP batch cultures with two species of yeast, in which glucose, xylose or a mixture of the two were used as a carbon source. The aim was to increase the productivity of the final product i.e. SCP (Liu et al., 1995; Ahmad and Holland, 1995).

Solid state fermentation (SSF) involves the growth of microbes on a chiefly insoluble substrate where there is no free liquid. Research in industrial fermentation technology has largely concentrated on submerged fermentation and until recently SSF did not generate much interest. A renewal of interest in SSF over the past 10 years stems from the need to reduce manufacturing costs from wastes by taking advantage of engineering principles and techniques (Pandey and Soccol, 1998). These include simplicity in operating the Koji fermenter, simultaneous saccharification, fermentation of lignocellulosic substrates, and release of concentrated hydrolytic enzymes in the medium during growth. Generally, under conditions of low water activity and an intractable solid substrate, fungi show luxuriant growth when compared to bacteria and algae. Mycelial tips of fungi have immense turgor pressure, which assists in their penetration of hard substrates. Hence better growth of fungi in SSF

gives much higher biomass when compared to submerged fermentation. Further, the SSF process is simple and has many advantages over the submerged fermentation (Table 9). However, substrate particle size, moisture level and C/N ratio are critical factors for SSF (Pandey and Soccol, 1998; Tengerdy, 1985; Rodriguez-Vazquez et al., 1992; Zadrazil and Puniya, 1995; Nigam and Singh, 1994).

A study regarding the cost analysis of SCP reported that the cost of SCP produced by submerged fermentation was 2–5-fold the cost of fishmeal (Perera et al., 1995). Perhaps SSF processes should be used to reduce the cost of SCP production. Currently SSF is being used for the production of protein-enriched feed (Zadrazil and Puniya, 1995; Rodriguez-Vazguez et al., 1992; Chaudhari et al., 1994). Industrial scale application of SSF for production of SCP would help increase yields and improve conversion efficiency, which would reduce the overall cost of the final product.

11. Impact of SCP in solving protein malnutrition

Aside from protein, microbial cells are also rich in carbohydrates, fats, vitamins and minerals. Today SCP is considered as a potential protein source for humans and as feed for animals (Trehan, 1993). According to chemical, microbiological and toxicological analysis, SCP from *Aspergillus niger* proved to be a potentially good animal feed. When cultivated as monoculture it nearly doubled the protein content of the starting material (Kuzmanova et al., 1989). Testing of SCP products on pigs and chickens (Giec and Skupin, 1988) suggested that 10–20% of the protein in foodstuffs could be replaced by SCP. Prospects for SCP as a human food along with development of three generations of SCP products, each having a higher degree of purity and suitability, is discussed by Mauron (1976). For human use, the microbes used are primarily *Saccharomyces cerevisiae*, *Candida utiliz*, *Spirulina* and *Chlorella* species. These are fully accepted for human consumption by public health authorities. *Spirulina maxima* is grown on a commercial basis in Lake Texcoco. Several plants are currently being operated for *Chlorella* and *Spirulina* production in Japan, Taiwan, Israel, Thailand and the United States. The overall production of algal health food is about 2000 metric tonnes/year on dry weight basis. These algae are grown in ponds, fermenters and then spray dried and sold as powder or pills (Trehan, 1993).

Table 9
Comparison of solid state and submerged fermentation processes

Parameter	Submerged fermentation	Solid state fermentation
Substrate condition	Requires continuous agitation and soluble substrate	No agitation required and insoluble polymers as substrate
Moisture	Required in large quantity	Absence of free water
Aerobic conditions maintenance	By agitation	By diffusion
Post fermentation waste	Large quantity, hence effluents polluting the environment	Very little, hence non-polluting
Space	Large	Small
Capital investment	Very high	Low
Aseptic conditions	Highly essential	Not required

Though some species of algae, fungi and bacteria are commercialized, their use and acceptability is poor. Due to the high cost of production, ignorance among masses and poor marketing strategies, these products are found on a limited scale and have yet to form part of a staple diet. Some algae find acceptance in the form of regional recipes, as in some tribal communities in Africa and South America or places where normal food is scarce. SCP is also used as a prescribed health food on a limited scale. Use of SCP as a binder and filler would mean utilization in even greater quantities as well as varieties. To explore the potential of SCP to its maximum extent, it should be produced with direct human consumption in mind. This potential is also limited by available technology. Hence, there should be cooperation among academia, government and industry in the fundamental study for the utilization of SCP for human food.

12. Conclusions

Sustainable food security implies physical and economic access to food for all. This requires greater efforts directed at food production and distribution as well as improving living standards of the people. The recent Human Development Index (HDI) of the United Nations Organization ranks countries on their ability to provide food resources, education and a healthy life to their citizens. Moreover, there is also a growing awareness among people regarding nutritional deficiencies and protein deficiency in particular. In recent times people have begun to accept alternative and innovative protein-rich foods. However, they still hesitate to accept SCP as a food to any great extent.

Recently, there were newspaper reports regarding destruction of genetically modified cloned crops in Europe. People fear that microbial genes cloned into crops would enter the human system and lead to diseases, allergies or even cancer. Precautions and control measures must be taken to alleviate these fears. SCP products should be evaluated extensively for safety purposes, to gain popularity among masses.

The chemical composition of any SCP product must be characterized clearly in terms of percentage protein, type of amino acids, nucleic acid, lipids, fats, toxins and vitamins. Properties like density, particle size, texture, color and storage must be clearly indicated on the package for marketing. A microbiological description indicating species, strains and percentage of contaminants, if any, should be indicated. Final products for human consumption must be made to undergo rigorous testing during the pre-marketing stage. Possible toxic or carcinogenic compounds, heavy metals and polycyclic hydrocarbons must be assayed for and removed. It is of primary importance that SCP products are safe to eat and also inexpensive in order to be popular among masses. Further, genetically improved, high-yielding and non-toxic microbes can be grown for SCP production. We should therefore look forward to extensive use of value-added SCP products in the new millennium and the eradication of chronic malnutrition globally by bridging the gap between demand and supply with SCP.

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References

Abou-Zeid AA, Khan JA, Abulnaja KO. On methods for reduction of nucleic acid content in single cell protein from gas oil. Biores Technol 1995;52:21–4.

Ahmad MN, Holland CR. Growth kinetics of single cell protein in batch fermenter. J Food Eng 1995;26:443–52.

Araujo-Neto JS, Ferreira-Pinto G. Nucleic acid and single cell protein utilization in human feeding: a review. Arch-Latinoam-Nutr 1975;25(2):105–18.

Ashy MA, Abou-Zeid A. Potentialities of yeasts in production of single cell proteins (SCP). Zentralbl Mikrobiol 1982;137(5):387–94.

Balasubramanian D, Bryce CFA, Dharmalingam K, Green J, Kunthala J. Concepts in biotechnology. Universities Press (India) Limited, COSTED-IBN, 1998. pp. 135–74.

Becerra M, Cerdan E, Gonzalez-Siso MI. Heterologous *Kluyveromyces lacti* beta-galactosidase production and release by *Saccharomyces cerevisiae* osmotic remedial thermosensitive autolytic mutants. Biochim Biophys Acta 1997;61335(3):235–41.

Beech GA, Melvin MA, Taggart J. Food and drink biotechnology. In: Higgins IJ, Best DJ, Jones J, editors. Biotechnology: Principles and applications. Oxford: Blackwell, 1984. pp. 73–110.

Bennett JW, Keller NP. Mycotoxins and their prevention. In: Anke T, editor. Fungal biotechnology. Weinheim: International Thompson Publishing Company, 1997. pp. 265–73.

Bennett JW, Bhatnagar D, Chang PK. The molecular genetics of aflatoxin biosynthesis. In: Powell KA, Renwick A, Peberdy JF, editors. The genus *Aspergillus*. New York: Plenum Press, 1994. pp. 51–8.

Blancou J, Calvet H, Riviere R. Single cell protein production from peanut shell. Rev-Elev-Med-Vet-Pays-Trop 1978;31(3):363–8.

Bornstein S, Plavnik I, Lipstein B. Evaluation of methanol grown bacteria and hydrocarbon grown yeast as sources of protein for poultry: trials with egg laying birds. Br Poult Sci 1982;23(6):487–99.

Brock TD. A textbook of industrial microbiology. Sunderland, MA: Sinauer Associates Inc., 1989. pp. 306–16.

Callihan CD, Clemmer JE. Biomass from cellulosic materials. In: Rose A, editor. Microbial biomass-economic microbiology, vol. 4. New York: Academic Press, 1979. pp. 271–88.

Calloway DH. The place of single cell protein in man's diet. In: Davis P, editor. Single cell protein. New York: Academic Press, 1974. pp. 129–46.

Cary JW, Barnaby N, Ehrlic KC, Bhatnagar D. Isolation and characterization of experimentally induced aflatoxin biosynthetic pathway deletion mutants of *Aspergillus parasiticus.* Appl Microbiol Biotechnol 1999;51(6): 587–602.

Chanda S, Chakrabarti S. Plant origin liquid waste, a source for single cell protein production by yeast. Biores Technol 1996;57:51–4.

Chang PK, Cary JW, Bhatnagar D. Cloning of *Aspergillus parasiticus* apa-2 gene associated with regulation of aflatoxin biosynthesis. Appl Environ Microbiol 1993;59:3273–9.

Chaudhari LC, Singh R, Kamra DN. Biodelignification of sugar cane bagasse by *Pleurotus* and *Pleurotus cornucopiae*. Ind J Microbiol 1994;34:55–7.

Cole RJ. Modern methods in analysis and structural elucidation of mycotoxins. San Deigo, CA: Academic Press, 1986.

Cole RJ, Cox RH. Handbook of toxic fungal metabolites. New York: Academic Press, 1981.

Cotty PJ, Bayman P, Egel DS. Agricultural, aflatoxins and *Aspergillus*. The genus *Aspergillus*. New York: Plenum Press, 1994. pp. 1–27.

Dale BE, White DH. Degradation of ribonucleic acids by immobolized ribonuclease. Biotechnol Bioeng 1979;21(9):1639–48.

Darmwal NS, Gaur AC. Isolation of cellulolytic fungi for cellulase and protein production from rice straw. Zbl Mikrobiol 1991;146(7–8):467–70.

De Boer L, Dijkhuizen L. Microbial and enzymatic process for L-phenylalanine production. Adv Biochem Engineer Biotechnol 1990;41:1–27.

Deibel MR, Hiebsch RR, Klein RD. Secreted amylolytic enzymes from *Schwanniomyces occidentalis*: purification by fast protein liquid chromatography (FPLC) and preliminary characterization. Prep Biochem 1988;18(1):77–120.

Desjardins AE, Hohn TM, McCormick SP. Trichothecene biosynthesis in *Fusarium* species: chemical genetics and significance. Microbial Rev 1993;57:595–604.

Dutton MF. Enzymes and aflatoxin biosynthesis. Microbial Rev 1988;52:274–95.

Eaton DL, Groopman JD. The toxicity of aflatoxins. Human health, veterinary and agricultural significance. San Diego, CA: Academic Press, 1994.

Ekenvall L, Dolling B, Gothe CJ, Ebbinghaus L, von Stedingk LV, Wasserman J. Single cell protein as an occupational hazard. Br J Ind Med 1983;40(2):212–5.

Ekerott L, Villadseer J. Biotechnology 1991;18:205–31.

el-Saadany R, Khalaf H, el-Manawaty H, Salom F. The production of single cell protein from agricultural wastes by fungi. Acta-Aliment. Acad Sci Hung 1988;17(4):376–7.

el-Sabaeny AH. Influence of medium composition on lactic acid production from dried whey by *Lactobacillus delbrueckii*. Microbiologia 1996;12(3):411–6.

Erdman MD, Bergen WG, Reddy CA. Amino acid profiles and presumptive nutritional assessment of single cell protein from certain lactobacilli. Appl Environ Microbiol 1977;33(4):901–5.

Faust U. Production of microbial biomass. In: Prave P, Faust U, Sittig W, Sukatsch DA, editors. Fundamentals of biotechnology. Weinheim: VCH Publishers, 1987. pp. 601–22.

Frazier WC, Westhoff DC. Food microbiology. New Delhi: Tata McGraw Hill Publishing Company Limited, 1990. pp. 398–415.

Galvez A, Ramirez MJ, Garcia-Garibay M. Chemical composition of a mixture of single cell protein obtained from *Kluyveromyces fragilis* and whey proteins. Arch-Latinoam-Nutr 1990;40(2):252–62.

Ghanem KM. Single cell protein production from beet-pulp by mixed culture. Microbiologia 1992;8(1):39–43.

Giec A, Skupin J. Single cell protein as food and feed. Nahrung 1988;32(3):219–29.

Giesecke D, Gaebler S, Tiemeyer W. Purine availability and metabolism in dogs fed single cell protein or RNA. J Nutr 1982;112(10):1822–6.

Goodrich-Tanrikulu M, Mahoney N, Rodriguez SB. The plant growth regulator methyl jasmonate inhibits aflatoxin production by *Aspergillus flavus*. Microbiol 1995;141:2831–7.

Gow JS, Littlehailes JD, Smith SRL, Walter RB. SCP production from methanol: bacteria. In: Tannenbaum SR, Wang DIC, editors. Single cell protein, vol. II. Cambridge, MA: MIT Press, 1975. pp. 370–84.

Gupta PK, Sharma YP. Mycotoxins. In: Gupta PK, Salunke DK, editors. Modern toxicology, vol. 2. The adverse effects of xenobiotics. New Delhi: Metropolitan Book Co. Pvt. Ltd., 1984. pp. 317–40.

Gupte A, Madamwar D. Solid state fermentation of lignocellulosic waste for cellulase and β-glucosidase production by cocultivation of *Aspergillus ellipticus* and *Aspergillus fumigatus*. Biotechnol Prog 1997;13:166–9.

Guven M, Cansunar E. Single cell protein production and reduction of pollutants in waste sulphite liquor. Mikrobiyol Bul 1989;23(4):329–35.

Hill F. Yeast with high natural glutamic acid content. Patent EP592785, 1994.

Invarson KC, Morita H. Single cell protein production by the acid tolerant fungus, *Scytalidium acidophilum* from acid hydrolysates of waste paper. Appl Environ Microbiol 1982;43:643–7.

Jacob HI. Six thousand years of bread. Garden City, NY: Doubleday, Doran & Co., 1944.

Joffe AZ. *Fusarium* species: their biology and toxicology. New York: John Wiley and Sons, 1986.

Jungehulsing U, Arntz C, Smit R, Tudzynski P. The *Claviceps purpurea* glyceraldehyde-3-phosphate dehydrogenase gene cloning, characterization and use for improvement of a dominant selection system. Curr Genet 1994;25:101–6.

Kale SP, Bhatnagar D, Bennett JW. Isolation and characterization of morphological variants of *Aspergillus parasiticus* deficient in secondary metabolite production. Mycol Res 1994;98:645–52.

Kellems RO, Aseltine MS, Church DC. Evaluation of single cell protein from pulp mills: laboratory analysis on *in vivo* digestibility. J Anim Sci 1981;53(6):1601–8.

Keller NP, Cleveland TE, Bhatnagar D. A molecular approach towards understanding aflatoxin production. In: Bhatnagar D, Lillehoj EB, Arora DK, editors. Handbook of applied mycology, vol. 5. Mycotoxins in ecological systems. New York: Marcel Dekker, 1992. pp. 287–310.

Kerkadi A, Barriault C, Tuchweber B, Frohlich AA, Marquardt RR, Bouchard G, Yousef IM. Dietary cholestyramine reduces ochratoxin A-nephrotoxicity in the rat by decreasing plasma levels and enhancing fecal excretion of the toxin. J Toxicol Environ Health 1998;53(3):231–50.

Kim JH, Lebeault JM. Protein production from whey using *Penicillium cyclopum*: growth parameters and cellular composition. Eur J Appl Microbiol Biotechnol 1981;13:151–4.

Kolani S, Delgenes JP, Moletta R, Traore A, Doh A. Optimization of cell yield of *Candida krusei* SO1 and *Saccharomyces* sp. LK3G cultured in sorghum hydrolysate. Biores Technol 1996;57:275–81.

Kuzmanova S, Vandeaka E, Dimitrovski A, Doneva D. Microbial procedure for utilization of food industry wastes. Dechema Biotechnology Conferences 3-VCH Vertagsgesellschaff, 1989. pp. 985–8.

Lee LS, Bayman P, Bennett JW. Mycotoxins. In: Finkelstein B, Ball C, editors. Biotechnology of filamentous fungi: technology and products. Boston, MA: Butterworth & Heinemann, 1992. pp. 463–502.

Litchfield JH. Production of single cell protein for use in food and feed. In: Peppler HJ, Perlman D, editors. Microbial technology, vol. I. 2nd ed. New York: Academic Press, 1979. pp. 93–155.

Litchfield JH. Bacterial biomass. In: Moo-Young M, Bull AT, Dalton H, editors. Comprehensive biotechnology, vol. III. New York: Pergamon Press, 1985. pp. 463–81.

Liu G, Zheng Z, Song D, Cen P. Kinetic models of batch and fed-batch culture of single cell protein with double carbon sources. Chin J Biotechnol 1995;11(3):163–70.

Mahasneh IA. Production of single cell protein from five strains of *Chlorella* spp. (Chlorophyta). Cytobios 1997;90:153–61.

Marasas WFO, Nelson P, Toussoun TA. Toxigenic *Fusarium* species: identity and mycotoxicology. Pennsylvania, USA: University Press, 1984.

Martinez MC, Sanchez-Montero JM, Sinisterra JV, Ballesteros A. New insolubilized derivatives of ribonuclease and endonuclease for elimination of nucleic acids in single cell protein concentrates. Biotechnol Appl Biochem 1990;12(6):643–52.

Martinez-Force E, Benitez T. Regulation of aspartate derived amino acid biosynthesis in yeast *Saccharomyces cerevisiae*. Curr Microbiol 1993;26:313–22.

Mateles RJ, Tannenbaum S. Single cell protein. Cambridge, MA: MIT Press, 1968.

Mauron J. New protein sources. Bull Schweiz Akad Med Wiss 1976;31(4–6):335–51.

Moller A. L-Tryptophan production from anthranilic acid by amino acid auxotrophic mutants of *Candida utilis*. Process Biochem 1994;29:521–7.

Moo-Young M. Economics of SCP production. Process Biochem 1977;12(4):6–10.

Moo-Young M, Chahal DS, Swan JE, Robinson CW. Single cell protein production by *Chaetomium cellulolyticum*, a new thermotolerant cellulolytic fungi. Biotechnol Bioeng 1977;19(4):527–38.

Moreno JM, Sanchez-Montero JM, Ballesteros A, Sinesterra JV. Hydrolysis of nucleic acids in single cell protein concentrates using immobilized benzonases. Biotechnol Appl Biochem 1991;31(1):43–51.

Murby M, Uhlen M, Stahl S. Upstream strategies to minimize proteolytic degradation upon recombinant production in *Escherichia coli*. Protein Expr Purif 1996;7(2):129–36.

Murrell JC. Genetic and molecular biology of methanotrophs. FEMS Microbiol Rev 1992;8(3–4):233–48.

Niederberger P. Amino acid production in microbial eukaryotes and prokaryotes other than coryneforms. In: Hunter I, Rhodes M, editors. Society for General Microbiology Symposium, 44. Cambridge, UK: University Press, 1989. pp. 2–24.

Nigam P, Singh D. Solid state (substrate) fermentation systems and their applications in biotechnology. J Basic Microbiol 1994;34:405–14.

Nygren PA, Stahl S, Uhlen M. Engineering proteins to facilitate bioprocessing. Trends Biotechnol 1994;12(5): 184–8.

Pandey A, Soccol CR. Bioconversion of biomass: a case study of lignocellulosic bioconversion in solid state fermentation. Brazilian Arch Biol Technol 1998;41:379–90.

Park DL, Liang B. Perspective on aflatoxin control for human and animal feed. Trends in Food Sci Technol 1993;4:334–41.

Perera WM, Carter CG, Houlihan DF. Feed consumption, growth and growth efficiency of rainbow trout (*Onchorhyncus mykiss* (Walbaum)) fed on diet containing bacterial single cell protein. Br J Nutr 1995;73(4):591–603.

Powar CB, Daginawala HF. General microbiology, vol. 2. Bombay: Himalaya Publishing House, 1995. pp. 88–131.

Renner HW, Munzner R. Mutagenic evaluation of single cell protein with various mammalian test systems. Toxicology 1978;10(2):141–50.

Rhishipal R, Philip R. Selection of marine yeast for generation of single cell protein from prawn shell wastes. Biores Technol 1998;65:255–6.

Rodriguez J, Ferraz A, Nogueira RF, Ferrer I, Esposito E, Duran N. Lignin biodegradation by the ascomycete *Chrysonilia sitophila*. Appl Biochem Biotechnol 1997;62(2–3):233–42.

Rodriguez-Vazquez R, Villanuevaventure G, Riosleal E. Sugar cane bagasse pith dry pretreatment for single cell protein production. Biores Tech 1992;39:17–22.

Sahm H. Metabolic design in the amino acid producing bacterium *Corynebacterium glutamicum*. Folia Microbiol 1995;40:23–30.

Saliceti-Piazza L, Dale MC, Moelhman M, Ooks MR, Wankat PC. Free and immobilized yeasts for BOD reduction in dairy wastes: growth on low levels of lactose, lactic acid and glycerol. Miami, FL: AlChE, Annual Meeting, Paper No. 165k, 1992.

Sasikala C, Ramana CV. Biotechnological potentials of anoxygenic phototrophic bacteria. I. Production of single cell protein, vitamins, ubiquinones, hormones and enzymes and use in waste treatment. Adv Appl Microbiol 1995;41:173–226.

Scerra V, Caridi A, Foti F, Sinatra MC. Influence of dairy *Penicillium* spp. on nutrient content of citrus fruit peel. Animal Feed Science and Technology 1999;78:169–76.

Schlegel HG. General microbiology. Cambridge, UK: University Press, 1996. pp. 357–84.

Shipman RH, Kao IC, Fan LT. Single cell protein production by photosynthetic bacteria cultivation in agricultural byproducts. Biotechnol Bioeng 1975;17:1561–70.

Shuler ML, Roberts ED, Mitchell DW, Kargi F, Austic RE, Henry A, Vashon R, Seeley HR. Process for aerobic conversion of poultry manure into high protein feed stuff. Biotechnol Bioeng 1979;21:19–38.

Singh A, Abidi AB, Agarwal AK, Dharmwal NS. Single cell protein production from *Aspergillus niger* and its evaluation. Zentralbl-Mikrobiol 1991;146(3):181–4.

Singh BD. Biotechnology. New Delhi: Kalyani Publishers, 1998. pp. 498–510

Staron TJ. Production of proteins by mycological process. Br. Pat. 1604781, 1981.

Steinmann J, Wottge HU, Muller-Ruchholtz W. Immunogenicity testing of food proteins: *in vitro* and *in vivo* trials in rats. Int Arch Allergy Appl Immunol 1990;91(1):62–5.

Storebakken T, Kvien IS, Shearer DD, Grisdale-Helland B, Helland SJ, Berge GM. The apparent digestibility of diets containing fish meal, soybean meal or bacterial meal fed to the Atlantic salmon (*Salmon salar*): evaluation of different fecal collection methods. Aquaculture 1998;169:195–210.

Tanaka M, Matsuno R. Conversion of lignocellulosic materials to single cell protein, recent developments and problems. Enzyme Microb Technol 1985;7:197–206.

Tannenbaum SR, Wang DIC. Single cell protein, vol. II. Cambridge, MA: MIT Press, 1975.

Taylor JC, Lucas EW, Gable DA, Graber G. Evaluation of single cell protein for non-ruminants. In: Davis P, editor. Single cell protein. New York: Academic Press, 1974. pp. 179–86.

Tengerdy RP. Solid state fermentation. Trends Biotechnol 1985;3(4):96–9.

Trail F, Manhanti N, Linz J. Molecular biology of aflatoxin biosynthesis. Microbiology 1995;141:755–65.

Trehan K. Biotechnology. New Delhi: Wiley Eastern Limited, 1993. pp. 79–88.

Trevelyan WE. Chemical methods for the reduction of the purine content of the baker's yeast, a form of single cell protein. J Sci Food Agric 1976a;27(3):225–30.

Trevelyan WE. Autolytic methods for the reduction of the purine content of baker's yeast, a form of single cell protein. J Sci Food Agric 1976b;27(8):753–62.

Tsukada Y, Sugimori T. Induction of auxotrophic mutants from *Candida* species and their application to L-threonine fermentation. Agr Biol Chem 1971;35:1–7.

Tuse D. Single cell protein: current status and future prospects. Crit Rev Food Sci 1984;19(4):273–325.

Varga J, Kevei E, Rinyu E, Teren J, Kozakiewicz Z. Ochratoxin production by *Aspergillus* species. Appl Environ Microbiol 1996;62(12):4461–4.

Vashista BR. Botany for degree students-Algae. New Delhi: S. Chand and Co. Ltd., 1989. pp. 503–14.

Wagner GH. Emerging applications of methylotrophic yeasts. FEMS Microbiol Rev 1990;7(3–4):279–83.

Widstrom NW. Aflatoxin in the developing maize: interactions among involved biota and pertinent econiche factors. In: Bhatnagar D, Lillehoj EB, Arora DK, editors. Handbook of applied mycology, vol. 5. Mycotoxins in Ecological Systems. New York, USA: Marcel Dekker, 1992.

Winocour PD, Turner MR, Taylor TG, Munday KA. Platelet aggregation in rats in relation to hyperuricaemia induced by dietary single cell protein and to protein deficiency. Thromb Haemost 1978;39(2):346–59.

Woloshuk CP, Fount KR, Brewer JF. Molecular characterization of aflR, a regulatory locus for aflatoxin biosynthesis. Appl Environ Microbiol 1994;60:2408–14.

Wong PK, Chan K. Algal single cell protein production from sewage effluents with high salinity. Experientia 1980;36(9):1065–6.

Yang HH, Thayer DW, Yang SP. Reduction of endogenous nucleic acid in single cell protein. Appl Environ Microbiol 1979;38(1):143–7.

Yu J, Butchko RAE, Fernandes M. Conservation of structure and function of aflatoxin regulatory gene aflR from *Aspergillus nidulans* and *Aspergillus flavus*. Curr Gene 1996;29:549–55.

Zadrazil F, Puniya AK. Studies on effect of particle size on solid state fermentation of sugarcane bagasse into animal feed using white rot fungi. Biores Technol 1995;54:85–7.

Zeringue HJ. Possible involvement of lipoxygenase in a defense response in aflatoxigenic *Aspergillus*-cotton plant interactions. Can J Bot 1996;74:98–102.

Ziino M, Curto RBL, Salvo F, Signorino D, Chifalo B, Giuffrida D. Lipid composition of *Geotrichum candidum* single cell protein grown in continuous submerged culture. Biores Technol 1999;67:7–11.