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# Microorganisms as an Alternative Source of Protein

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*Demand for human food and animal feed proteins from nonconventional sources has increased, particularly in developing countries. Microbial protein is one such source. It is desirable because it is amenable to controlled intensive cultivation and is less dependent on variations in climate, weather, and soil. Microbial proteins must be evaluated for nutritive value, safety, and economic considerations before mass production is undertaken.*

## Background

The increasing world demand for food and feed proteins has spurred the search for nonconventional protein sources that meet protein requirements. Microbial protein in various forms has attracted particular attention because it is amenable to controlled intensive cultivation and is less dependent on variations in climate, weather, and soil. The term "single-cell protein," coined at the Massachusetts Institute of Technology, refers to the dried cells of microorganisms such as yeasts, bacteria, microalgae, and fungi grown in large-scale culture systems for use as protein sources in human foods or animal feeds.[1] Other terms have also been suggested, including "microbial biomass" and "microbial biomass protein," for proteins or protein concentrates obtained from these cells. The protein can be consumed directly as part of the cell itself, particularly in animal feed formulations, or it can be extracted and processed into fibers or meatlike products for nutritious human food.

Human beings have always utilized certain microorganisms as part of their diet. Since ancient times—as early as 2500 BC—the most widely used fermenting agent, the yeast *Saccharomyces cerevisiae*, was used in the production of bread. Fermented cheese and milk products produced by lactic acid bacteria were invented and enjoyed by the early Egyptians and Greeks. During the Roman era (100–50 BC), the use of such microorganisms was at its peak. Wild mushrooms were considered a delicacy by the Egyptian pharaohs, and there are records of their use as food in China between 26 BC and AD 220.[2] Tribes living near Lake Chad have eaten *Spirulina* algae for many generations, and even Aztec Indians in Mexico were dependent on the *Spirulina* sp. as one of their major sources of protein at the time of the arrival of the Spanish explorers in the 16th century.

The production of microorganisms for direct use in human food or animal feed is a fairly recent development. The first attempt to cultivate microorganisms for food on a large scale was made in Germany during World War I, when *Torula* yeast was produced. The production of microbial food was then continued during the period between the two world wars.[3] *Candida utilis*, an aerobic yeast, was produced during World War II because of its potential as food and feed. Moreover, biomass from a filamentous fungus grown on milk whey was used in Germany during World War II to supplement human food.[4]

Since World War II, several efforts have been made to develop processes for mass cultivation of microbial protein. Because the world's population is now growing rapidly, investigating possibilities to increase food production is important. The present food supply in terms of calories and protein per capita per day (Table 1) indicates that the world's food supply is deficient not only in protein but also in other energy-rich food.[5] Only developed countries have a sufficient protein supply of animal and veg-

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**Table 1.** World Food Supply in Terms of Calories and Protein Per Capita[5]

| Country | Calories Available Per Capita/ Per Day | Protein (g) Available Per Capita/ Per Day |
|---|---|---|
| Bangladesh | 1945 | 42.4 |
| Brazil | 2522 | 61.2 |
| Canada | 3346 | 101.3 |
| Cuba | 2636 | 68.8 |
| India | 1949 | 48.4 |
| Indonesia | 2115 | 43.7 |
| Mexico | 2668 | 66.1 |
| Pakistan | 2255 | 62.0 |
| USA | 3537 | 106.2 |

etable origin, whereas in most developing countries many people suffer from protein-calorie malnutrition.[6,7]

Developing countries like India, Pakistan, Bangladesh, Indonesia, and Brazil are, however, rich in agricultural wastes. If, on average, 70% of the carbohydrates (cellulose and hemicelluloses) available from various crops and forest residues are considered, from 5 to 10 tonnes/year per hectare of carbohydrates from these residues would be available for bioconversion to protein-rich microbial biomass.[5] Thus, an additional 1.25–2.5 tonnes of food/feed per hectare in the form of microbial protein (about a 25% yield from the total carbohydrate portion of crop residues) would be available. This addition would be important to alleviate food and feed shortages in developing countries.

This article reviews information on microorganisms as a protein source, including algal and bacterial protein produced with carbon dioxide and hydrogen as substrates. The nutrition, safety, and economic considerations of microbial protein production are also discussed.

## Microorganisms as a Source of Protein

The potential of microbial protein production must be discussed and evaluated in the context of the present world situation. The 1970s were a time of near crisis in the world protein market. An acute shortage of two important raw materials in the animal feed industry—soybean and fish meal—led to steep price rises. This caused competition between man and animal for cereals, a staple food in many developing countries. Owing to simultaneously growing demands for meat, some meat-exporting countries started to rely more heavily on internally produced grain staples as a component of their animal feed. The recovery of fish meal supplies remained slow in 1974, but returned to normal in 1975 and early 1976. However, prices remained high and supplies uncertain for many years. In addition, long-term high demand and crop failures maintained high prices and shortages of soybeans. Presently, the United States produces 75% of the world's soybean supply, and one in seven acres of U.S. cropland is used for soybean production. Because of increasing demand for soybeans by the manufacturers of textured vegetable products, a shift toward soybean as a meat substitute for human consumption is occurring. In 1980, 20% of the American meat market was satisfied by meat analogues.

The compound feed industry is growing at a rate of more than 10% per year. Owing to uncertainties in crop turnover and an ever increasing world demand for meat products, the compound feed industry is attracted to a product with consistent composition and good nutrition value. Because of advances in research and development in recent years and because of the enormous production potential for microbial protein, it is likely that the industry can be supplied with good-quality proteins that will not only fill the deficit created by expansion but also reduce the flow of soybeans and cereals into animal feed. Increasing supplies of microbial protein for animal feed would improve human nutrition relatively quickly by taking vegetable sources of proteins out of the human-animal competition and making them available for human consumption in developing countries.[8]

Because microbial protein can be produced using hydrocarbons or their derivatives as substrates, the products must be carefully controlled.[9] Such protein products are now on the feed market as a result of several years of nutrition and toxicology feeding tests with a variety of domestic animals. However, some years of testing lie ahead before there will be consensus on the direct use of microbial protein for human consumption. Public opinion should be considered and people should be better informed about the potential of microbial protein.[10] Microorganisms have several advantages over plants and animals as potential food or feed sources. Both microorganisms and plants are capable of producing protein from inorganic nitrogen, but plants need an entire season to grow whereas microorganisms double their cell mass within hours. Furthermore, microorganisms produce high amounts of protein and can be modified genetically to produce cells of a desired nutritional composition. Production of microorganisms, as well, can be based on waste raw materials, which are available in large quantities at low cost.[11]

## General Characteristics of Microorganisms for Protein Production

Microorganisms suitable for protein production can be divided into four categories: bacteria, yeasts, fungi, and algae. To be considered for protein production, the microorganisms should possess certain characteristics as described in Table 2.

### *Bacteria*

Because bacteria have high growth rates (doubling times of 20–30 minutes) compared with yeast (2–3 hours) and algae and fungi (about 16 hours), they are of particular interest as a source of microbial protein. Bacteria have a high protein content. Their protein quality, in terms of amino acid profile, is also high because they have a higher concentration of sulfur-containing amino acids and lysine. Certain actinomycetes species have growth rates similar to those of bacteria and are therefore also of interest for protein production. Bacteria do, however, have some disadvantages such as very small cell size, which makes separation energy demanding, and a high nucleic acid content, which makes them unsuitable for human food.

A wide range of bacterial species have been considered for protein production. Important properties are specific growth rates, cell mass yield on substrate, pH and temperature tolerance, aeration requirements, and genetic stability.[12] Carbon source and nitrogen concentration are important factors affecting bacterial growth. Therefore, carbon and nitrogen ratios in growth media should be maintained at around 10:1 or less. Ammonia or ammonium salts are suitable nitrogen sources. Generally, natural water supplies provide adequate amounts of mineral salts, but supplements are sometimes required. Such additions should be carefully chosen to avoid corrosion problems.[13]

During growth, pH may be controlled in the range of 5 to 7. Temperature tolerance can also be an important characteristic of bacterial strains. For aerobic strains, oxygen transfer to growing cells is particularly important. Avoiding contamination is important in mass cultivation of all microorganisms. This criterion is easier to satisfy for fast-growing bacteria compared with other, slower-growing microorganisms.[14]

### *Yeasts*

Most experience in the manufacture and use of microbial protein has been with yeasts. Three types of yeast products are available: baker's yeast, brewer's yeast, and *Torula* yeast. In 1941, the British Royal Society coined the term "food yeast" as opposed to "fodder yeasts." Yeasts are known for their low content of sulfur amino acids but are good sources of the B vitamins and also of small amounts of vitamin E and provitamin D. Compared with bacteria, yeasts have a lower nucleic acid content, greater size, lower toxic potential, and greater acceptance as a protein source by consumers.

**Table 2.** Desirable Characteristics for Microorganisms to be Considered as a Source of Microbial Protein

| |
|---|
| **Physiologic** |
| High growth rate |
| Capable of growing on simple media, i.e., no requirements of expensive additives |
| Generation of high yields on the chosen carbon substrate |
| Ability to grow at high cell densities |
| Stable growth in continuous culture |
| Ability to use ammonia as nitrogen source |
| **Other Characteristics** |
| The protein, fat, and carbohydrate content should be of high quality |
| High digestibility of the product |
| High nutrient content |
| Low nucleic acid content |
| Absence of toxicity |
| Good taste |
| Easy recovery |
| Amenability to further processing, i.e., drying without change in color, texture, flavor, etc. |

The technology for yeast production was developed during the 19th century. The growth on various substrates of the genus *Saccharomyces* has been widely used for the production of single-cell protein. Substrates and nitrogen concentrations for yeast growth must be adjusted to provide carbon:nitrogen ratios in the range of 7:1 to 10:1. Anhydrous ammonia combined with phosphoric acid is a source of nitrogen and phosphorus and controls the pH level in the growth medium. High specific growth rates and yields in the pH range of 3.5 to 4.5 are typical, and the yeast product is always free of bacterial contamination. Usually heat is generated by yeasts growing on sugars, alcohols, or hydrocarbons.[15]

### *Fungi*

The use of fungi as food is not a new concept. Mushrooms have been used to flavor food for centuries. However, mass cultivation of fungal mycelium as a protein source is relatively new. Recent research has shown that growth rates of fungi are usually slower than those of yeasts and bacteria, fungi have a lower protein content than do yeasts and bacteria, fungal protein is often deficient in sulfur-containing amino acids, fungal cell wall digest-

ibility is a problem for monogastric animals, and fungi are somewhat of an unknown entity with regard to nutrition and toxicology. Fungi also have certain advantages: they produce a range of polysaccharide hydrolyzing enzymes that allow them to grow on complex and polymeric raw materials such as lignocellulosics and starch; fungal mycelium can be recovered by simple filtration, which offers a significant reduction in capital and processing costs compared with recovery by centrifugation of yeasts and bacteria; and because of slow growth rate, fungi generally have a lower nucleic acid content than bacteria.

Numerous species of molds and higher fungi have been used for the production of protein. During World War II, the biomass from a filamentous fungus grown on mild whey was used in Germany to supplement human food.[4] In general, concentrations of carbohydrate substrates used for this production are in the range of 1–10%. The carbon:nitrogen ratio can sometimes be as high as 20:1. Anhydrous ammonia or ammonium salts are generally used as nitrogen sources, and phosphoric acid is used as a phosphorus source and at the same time for pH adjustment. Usually, fungi are grown in batch or in semicontinuous cultures. Mineral nutrients such as potassium, sulfur, manganese, calcium, iron, magnesium, zinc, copper, and cobalt are required for the growth of most fungi, and a pH range of 3.0–7.0 is usually suitable. Their oxygen requirements vary greatly, and most fungi grow best at a temperature range of 25–36 °C.

### *Microalgae*

Algae can be grown photosynthetically and autotrophically using either artificial light or sunlight, or even heterotrophically in the dark with organic carbons as energy sources. The potential merits of algae as a protein source are related to their ability to multiply with carbon dioxide as the only carbon source. Some genera of Cyanophyta also have the advantage that they can use atmospheric nitrogen. When algae are growing in natural water bodies such as ponds and lakes, contamination by bacteria, fungi, yeast, and protozoa is common.[16,17] Algae are easy to harvest, but their multiplication is very slow, and the high investment costs for production in shallow artificial ponds yield low profitability.

Algae are a traditional food complement for some people living in Mexico (*Spirulina platensis*) and in Chad (*Spirulina maxima*). Algae have a low content of sulfur-containing amino acids. Because algae proteins have a high lysine content, they are suitable as supplements in cereals.

The interest in mass culture of microalgae began with the introduction of dense suspensions of *Chlorella* as a suitable tool for research by Warburg on photosynthesis.[18] It was then recognized that certain microalgae can increase their biomass several times per day and that their dry matter may contain more than 50% of crude protein.

Mass production of algae for protein production purposes was first tested in Germany in 1942,[19] but wide interest in this field began in the early 1950s and progressed thereafter.[20] Among phototrophic algae, several genera have been thoroughly investigated as sources of biomass for food and feed. These include *Chlorella, Spirulina, Scenedesmus, Dunaliella, Micractinium, Oscillatoria, Chlamydomonas,* and *Euglena.* Feeding experiments with pigs and hens have shown that the nutrient value of cell-free algal protein is lower than that of casein, owing to deficiency in sulfur-containing amino acids.[21] In general, cell walls of both green and blue-green algae are not readily digested by monogastric animals and humans, whereas ruminants generally utilize algae more efficiently.[22] Therefore, microalgal biomass protein production holds more potential for animal feed than for human food products.

## Potential Substrates for Protein Production

Utilizing the high productivity potential of microorganisms for protein production depends on the availability of inexpensive substrates, since the major single cost factor (30–50%) is the carbon substrate.[23]

The traditional carbon sources used for production of microorganisms have been carbohydrate-containing by-products from agriculture or industry. A breakthrough in fermentation processes for large-scale production of biomass was the use of petroleum-based raw materials such as gas/oil, n-paraffins, and synthetic methanol and ethanol as substrates. The oil crisis in the 1970s resulted in more expensive petrochemical raw materials. The fermentation industry therefore returned to renewable resources. Substrates from renewable resources include simple sugars, starch, hemicelluloses, and cellulose, which are readily available at low costs in a wide range of geographic regions. Parallel to efforts for protein production, research and development programs to use microorganisms for purification of waste waters from agriculture, forest industries, and food processing industries have been successfully introduced.

## Microbial Protein Production with Carbon Dioxide and Hydrogen as Substrates

Carbon dioxide ($CO_2$) is the simplest carbon source for biomass production. Because carbon dioxide is

the most oxidized carbon source, it cannot be used directly as an energy source. Its carbon must be reduced before it can be assimilated. Energy for this reduction is supplied as light energy and converted to chemical energy during photosynthesis.

Photosynthetic bacteria and algae have been used for protein production from $CO_2$. The reactions involved in the conversion of $CO_2$ to cell material can be summarized as follows:

$$6CO_2 + 18\ ATP + 12\ NADPH_2\ Fructose\text{-}6\text{-}P + 18\ ADP + 12\ NADP + 17\ Pi.$$

In microorganisms, photosynthesis occurs in green algae, cyanobacteria, and a few other species of bacteria:

$$CO_2 + H_2O + light\ CH_2O + O_2 + Chemical\ energy.$$

Bacterial photosynthetic processes are essentially anaerobic. Molecular hydrogen, sulfur compounds, and some organic compounds are electron donors:

$$CO_2 + 2H_2A + light\ (CH_2O) + 2\ A + H_2O.$$

Production of protein by photosynthetic bacteria is considered for only a limited number of species.[20,24] *Rhodopseudomonas capsulata* has been grown in Japan with industrial waste or sewage as substrate. The bacterium grows in mixed cultures with aerobic, heterotrophic, and nitrogen-fixing bacteria, yielding 1–2 g/L biomass.[25] The heterotrophic bacteria degrade organic materials in sewage and industrial wastes, thereby releasing $CO_2$.

*Rhodopseudomonas gelatinosa* has been used in a semicontinuous process with an average biomass productivity of 10 kg/m$^3$/day and total protein production capacity of 5 tonnes/day.[26] The bacterium was grown on previously hydrolyzed wheat bran (30% solid) with a suitable amount of sunlight or incandescent illumination at pH 7.0–7.2 at a temperature of 37 °C. The product was found to contain 65% protein with a significant content of essential amino acids.

Another method for microbial protein production from $CO_2$ is based on chemolithotrophic bacteria, which use molecular hydrogen as the electron donor. These bacteria belong to the genera *Hydrogenomonas* and *Alcaligenes*. These organisms require nitrogen in the form of ammonia, nitrate, or urea in addition to hydrogen, $CO_2$, and $O_2$.

Controlled large-scale microalgal cultures were developed in Germany during the 1940s.[24] In 1953, *Chlorella* algae were cultivated at the University of California, Richmond, at a 10,000-liter scale, and in 1955 a 2 million–liter pilot plant was operated for 2 years at the same place.[27] The different systems for protein production can be distinguished, depending on the substrate, raw materials, and the intended use of the protein product:[19]

1. Growing a selected algal culture in fresh water, mineral nutrients and additional carbon sources for using the protein product as a food supplement.
2. Treatment of sewage or industrial wastewater without the addition of external nutrients and the subsequent use of the biomass as animal feed.

The concentration of biomass obtained is often limited by low amounts of the carbon source in the culture medium.[27,28] Addition of $CO_2$, either in pure form or as a waste gas from combustion engines or fermentation processes, or of waste organic materials (molasses, cow manure, sewage, industrial wastes), would result in higher productivity.[29,30] However, typical cell densities obtained are 1–2 g/L and productivities obtained are between 10 and 30 g/m$^2$/day. An average yield of 20 g/m$^2$/day is equivalent to a yield of 70 tonnes of algal dry matter, or 35 tonnes of algal protein per hectare per year.[19] These values are much higher than those obtained with conventional agricultural crops, i.e., wheat (360 kg protein), rice (600 kg protein), and potato (800 kg protein) per hectare per year.

Unfortunately, harvesting algae is difficult. Large-scale production is therefore limited both by low biomass concentration and by high recovery costs. Among the various methods used for concentrating algae from pond effluent are flocculation, sedimentation, filtration, flotation, sand filtration, centrifugation, and coagulation.[27] Of these, the last two methods are the most frequently applied.

The Technion-Sherman Environmental Engineering Research Center, Haifa, Israel, developed the High Rate Algal Pond (HRAP) process with the idea of mass cultivation of algae in conjunction with sewage treatment.[31,32] The symbiotic cooperation between algae and bacteria, which is the basis for this process, involves sewage decomposition by bacteria releasing $CO_2$ while algae, in the presence of solar light influx, use the $CO_2$ and other nutrients (ammonium and phosphates) to synthesize cell material. The algal concentration fluctuates from 0.1 g/L in winter to 0.5 g/L in summer. The productivity values were about 10 g/m$^2$/day during the winter and about 30 g/m$^2$/day in the summer. The final algal biomass product contained 57.4% crude protein and a metabolizable energy of 2778 kcal/kg. The major advantage of the process is that it is a nonaseptic process that does not require additional nutrients, since municipal waste contains the necessary nutrients for algal growth. The major disadvantages of the HRAP process are the large area required and the limited applicability of the process to places with appropriate climatic conditions.[24]

Recently, only three microalgae—*Chlorella, Spirulina,* and *Dunaliella*—have been cultured commercially for which feasible production technology is available. The potential use of *Chlorella* as single-cell protein was realized by the Taiwanese, and they were the first to start large-scale production of microalgae in the 1960s.[33] The production of microalgae approached 1000 tonnes by late 1970s.[34] The main production of *Chlorella* takes place in Taiwan, with only 10–15% of the total production in Japan. *Chlorella* products are available to the health food market as biomass in the form of dry powder or compressed pills and extracts. Unfortunately, *Chlorella* production technology has not developed significantly during the last decade,[17] possibly because of competition from another microalga, *Spirulina*, which is more amenable for cost-effective technology and has a variety of applications.

In the early 1980s *Spirulina* became the candidate of choice for mass cultivation under outdoor environments because it grows in simple and highly alkaline media at pH levels between 9.5 and 11.[17,35,36] This allows for *Spirulina* production without contamination of other microorganisms.

*Spirulina* is cultivated in many countries worldwide. The biomass produced is sold mainly to the health food market in the form of either powder or pills. The powder is a supplement in a variety of food products. The protein, mainly phycocyanin extracted from *Spirulina*, is marketed as an ingredient in cosmetics, food color, creams, and confectionaries.[37] The production cost of feed grade *Spirulina* ranges from $6 to $8 per kilogram in most parts of the world, but the production costs in India are reported to be much lower.[17] Production costs can be even lower if *Spirulina* is cultivated in effluents from food industries. Many laboratories in India are actively engaged in perfecting the technology of biomass production with various microalgae. However, the major emphasis is on the application of algae as biofertilizer.[17]

*Dunaliella*, another microalga of interest, was first isolated from salt lakes and other salty habitats and from the Dead Sea in Israel.[38] The ability of the alga to grow under extreme salt concentrations (6–12%), where other algae or predators cannot thrive, facilitates its mass cultivation. Like *Spirulina*, *Dunaliella* is grown in large raceway ponds stirred with paddle wheels or in large lagoons. The biomass production in lagoons, however, is slow.

In an attempt to increase the *Dunaliella* production, a photobioreactor, made up of polythene tubes (5 km in length and 1 dm in diameter), has been constructed at a height of 2 m above ground on a fence-type support.[39] The flow is generated by airlift-type pumps. The system has several advan tages over open raceway pond cultivation, particu larly in maintaining consistency in the quality of th product.

Recently, *Dunaliella*, rich in carotene and use as provitamin A in food, feed, and pharmaceutic industries,[40] has picked up the largest market in th microalgal field. The cost of *Dunaliella* powder va ies from $50 to $80 per kilogram dry cellmass, de pending on its carotene contents. With increasin demand for ß-carotene, one can foresee a severa fold increase in the production of *Dunaliella* bio mass in the coming years.

## Nutrition, Safety, and Economic Aspects

### *Nutritive Value*

The nutritive value of a protein product of a give species of organism varies with the substrate use and the growth conditions. Although the principa nutritive value of the product is the protein conte and its composition of essential amino acids, othe components such as carbohydrates, minerals, lip ids, and nucleic acids are also present. Microbia protein products compare well with other high-qua ity protein sources such as soybean, with bacteria protein products generally containing more tha 65% true protein. Yeast products have 40–55% tru protein, and fungal and microalgal products 35–55 and 30–40% true protein, respectively.

For an adequate caloric input, the nutritiona protein quality depends on the composition of i amino acids. The amino acid composition of diffe ent microbial protein products are compared wit the established acceptable values set by by the Foo and Agriculture Organization of the United Nation All of the essential amino acids are present in m crobial products, although the content of sulfur-con taining amino acids such as methionine and cystein is somewhat low.

Total lipid content in the different microbial proc ucts varies from 1% to 15% and includes triglyce ides, phospholipids, and sterols. Microalgae have th highest lipid content. Although microalgae contai relatively high concentrations of unsaturated fatt acids, alkane-grown yeasts have high contents c uneven-numbered cyclopropane and branched-chai fatty acids (unsaturated). The cell walls represen between 0.5% and 10% of the cell dry matter, whic is expressed as the crude fiber fraction of the cel In yeasts, glucans and mannans are the main com ponents of the cell wall, with the remainder bein proteins and lipids. Carbohydrate contents of mi crobial products vary between 3.4% and 40% c cell dry weight. Although glycogen is found as a

**Table 3.** Vitamin Content (mg/kg) of Selected Microalgae and Protein Products[41,42]

| Organism | Vitamin A | Thiamin | Riboflavin | Nicotinate | Biotin | Vitamin C |
|---|---|---|---|---|---|---|
| *Spirulina maxima* | 225 | 14 | 28.5 | — | — | 103 |
| *Scendesmus obliquus* | 230 | 8 | 36.6 | 120 | 0.2 | 20 |
| *Chlorella prenoidosa* | 480 | 10 | 36 | 240 | 0.15 | — |
| Baker's yeast | — | 20–40 | 60–85 | 200–700 | 0.6–1.8 | — |
| Brewer's yeast | — | 104–250 | 25–80 | 300–627 | 1.1 | — |
| *Candida utilis* | — | 35–38 | 54–62 | 511–600 | 2.3 | — |
| *Hansenula* sp. | — | 9 | 54 | 590 | 1.7 | — |
| *Candida lipolytica* | — | 4 | 180 | 430 | 2.3 | — |

energy storage material in yeast, bacteria, and fungi, starch is the corresponding material in microalgae. Total minerals (ash content) vary from 4% to 12% of dry weight, and about 20% of the ash content is phosphorus salts.

Yeasts represent one of the richest sources of vitamins, particularly of the B-complex group (see Table 3). Biomass from microalgae is also a valuable source of nearly all important vitamins, which improves the nutritional value of microalgal protein products. The contents of vitamin A, C, and B-12 are particularly important.[41]

The most important measure of the nutritional value of microbial protein products is their performance in feeding studies. Protein efficiency ratio (PER), biological value (BV), net protein utilization (NPU), and protein digestibility as measured in rats are measures of the nutritional value of microbial protein products for feed/food applications. Metabolizable energy, protein digestibility, and feed conversion ratio (weight of ration consumed/weight gain) have been used commonly as indications of performance of microbial proteins for chicken and swine.[24,43–45] In general, microbial protein products (in the range of 5–15% of the total rations) have a nutritional value comparable to that of conventional protein sources (see Table 4), and would therefore be particularly useful for fortifying feed/food products.[24,45]

### *Safety*

Before any microbial protein product can be used as animal feed or human food, it is essential to determine the toxicologic status of the product. The potential toxicity of products can be considered through knowledge of the nature of the organism, the types of metabolites it produces, and the residues present as a result of the fermentation process used. All of the organisms to be used for protein production must be known to be nonpathogenic, but the possibility of contamination by pathogenic microorganisms must be considered. Although the downstream processes of microbial protein production are designed to destroy most of the viable microorganisms present, some could possibly survive.[24,25]

All types of microbial protein products contain high amounts of nucleic acids (up to 16% of dry weight). Consumption of products high in purines results in a higher plasma level of uric acid, and crystals of urate may form in tissues and joints, causing gout arthritis.[47,48] Potentially serious negative effects of consuming pyrimidine bases have also been observed in humans, where large amounts of nucleic acid ingestion resulted in fatty liver and liver degeneration.[49] A human intake of 2 g of nucleic acids per day from microbial protein products is acceptable as an addition to the usual diets of adults.[50] Because the nucleic acid content, mostly RNA, of microbial protein products is very high, attempts have been

**Table 4.** Nutritional Value of Selected Microbial Protein Products[41,43,46]

| Organism | Biological Value | Digestibility | Net Protein Utilization |
|---|---|---|---|
| *Spirulina* sp. | 77.6 | 83.9 | 65.0 |
| *Chlorella* sp. | 71.6 | 79.9 | 57.1 |
| *Scendesmus obliquus* | 81.3 | 82.8 | 67.3 |
| *Methylomonas clara* | 48.0 | 85.0 | — |
| *Acinetobacter cerificans* | 67.0 | 83.4 | — |
| *Cellulomonas* sp. | 62.0 | 90.0 | — |
| *Candida utilis* | 70.0 | 83.0 | — |
| *Pichia* sp. | 51.0 | 83.0 | — |
| *Fusarium graminearum* | 65.0 | 78.0 | — |
| *Paecilomyces variotii* | 54.0 | 81.0 | — |
| Casein | 87.7 | 95.1 | 83.4 |
| Egg | 94.7 | 94.2 | 89.1 |

made to reduce this level in the final product. Several chemical (base-catalyzed hydrolysis by $NH_4OH$, KOH, or NaOH) and enzymatic (exogenous RNAase or endogenous RNAase/heat shock) methods have been successfully employed to reduce RNA levels to 1–2% in the final product.[24,25,40,48]

No problems have been encountered with microbial protein for animal feed processed under well-defined and well-maintained conditions. This includes rats, dogs, swine, and monkeys tested with proportionally much larger doses than those fed to humans.[46] However, no amount of testing with experimental animals including primates can determine whether a given product can cause allergic symptoms in humans.[42] Essentially all protein-containing foods are capable of causing an allergic response in some individuals, although the frequency and nature of such responses may vary widely with the specific food and the population.[51] Although allergic reactions have been found to be common with some microbial protein products,[42,52,53] all tolerance trials indicate that the original cells did not provoke allergic responses. They did so only when they were processed to reduce their RNA content.[54]

The assurances necessary for the approval of microbial or any other novel protein sources are given in Table 5. The Protein Advisory Group–United Nations University (PAG-UNU)[55–57] emphasizes the requirements for the toxicologic safety of the methods for production, the composition of microorganisms and their metabolites, the effects on laboratory animals in limited feeding, and the evaluation of weaning foods that use novel protein sources. If the results are favorable, they must be followed by multiple feeding studies with rodents and other experimental animals, both short and long term.

### *Economic Considerations*

It is difficult to obtain a realistic economic basis for assessing the relative merits of alternative microbial protein production processes and the advantage of industrial protein production over agricultural protein production.[58,59] Comparisons of alternative routes and technologies tend to be unreliable because inflation on the costs quoted is frequently unqualified and because some cost evaluation exercises refer to commercial technology whereas others refer only to technology in some state of development.[24] Moreover, economic analyses are liable to become quickly outdated because of unpredicted changes in production plant construction costs and interest charges, energy costs, and feedstock prices. Comparative production costs of microbial biomass protein products are presented in Table 6.

**Table 5.** Required Safety Assurance for the Approval of Novel Protein Sources

| |
|---|
| **Animal Feed Purposes** |
| Safety of species |
| Safety of substrates |
| Safety of products |
| Adequate nutritional value |
| **Additional Assurance for Human Food Purposes** |
| Lack of carcinogenicity/mutagenicity |
| Minimal allergenicity |
| Favorable organoleptic characteristic |
| Cultural acceptability |

Capital costs for microbial biomass production depend on the costs of land, plant construction, and equipment required for storing, processing, and handling substrates, sterilization, product separation, recovery, and drying. Manufacturing costs are highly dependent on the costs of carbon and energy sources and may range from 15% to 60% depending on the year of the estimate and the nature of the raw material.[43] Utility costs include water, electricity, labor, and maintenance. Working capital, depreciation, taxes, and insurance must also be taken into account.

## Conclusion

There has been a great deal of interest since the 1960s in the production of microbial biomass to provide an additional source of protein to supplement conventional animal feeds and human foods. This interest was generated mainly because of growing awareness of the food needs of the world's expanding population, especially in developing countries. The diets of people in these countries are generally low in calories and unbalanced in other nutrients. Owing to the lack of variety in the diet, and particularly to the shortage of foods such as milk, eggs,

**Table 6.** Relative Production Costs for Microbial Protein Processes[23,24,60]

| Process | Relative Cost (%) | | |
|---|---|---|---|
| | Raw Materials and Chemicals | Utilities and Labor | Miscellaneous |
| Algae—$CO_2$ | 17 | 29 | 54 |
| Bacteria—methanol | 74 | 20 | 6 |
| Yeast—ethanol | 74 | 17 | 9 |
| Fungi—sulfite liquor | 51 | 36 | 13 |
| Fungi—cellulosic wastes | 15 | 34 | 51 |

fish, and meat, these diets fail to provide adequate quantities of vitamins and high-quality protein.

Microorganisms have been an important component of human food for thousands of years. The use of protein- and vitamin-containing yeasts and other microbial biomass directly for food instead of for baking and brewing is a 20th-century concept.[7] Microbial protein products can be produced with nonpathogenic yeasts, bacteria, filamentous fungi, and microalgae. They are good protein supplements to cereal diets for both animals and humans, as well as excellent natural resources of B vitamins. Exhaustive toxicologic testing has demonstrated that they are safe for animal feeding and that some of them can be produced in a manner suitable for human food. Microbial biomass protein products are higher in protein content than are cereals and legumes and, for human consumption at nutrient requirement levels, are comparable to legumes in quality.

Microbial biomass products produced from substrates of natural origin are generally regarded as safe and need to be evaluated simply for their ability to support efficient animal growth. Those derived from hydrocarbon feedstocks, however, need to be evaluated for safety by a series of toxicologic tests. The major areas of concern relate to organism pathogenicity, residual substrates, polycyclic hydrocarbons, unusual cellular metabolites, and allergic reactions by humans. The evidence provided to support biomass protein products produced on an industrial scale indicates no major problems that would preclude their use.

Although large-scale processes for manufacturing microbial protein products are technologically feasible, only a few processes, e.g., Rank Hovis McDougall's Mycoprotein, British Petroleum's Toprina, Imperial Chemical Industry's Pruteen, and Pekilo from Finland, among others, have been operated commercially at limited capacity. However, Marlow Foods, a joint venture between ICI and RHM, has been exceptionally successful in developing a market for Quorn® mycoprotein products in the United Kingdom, Belgium, Germany, and the Netherlands, and products may be launched in the United States (following Food and Drug Administration approval).

The main obstacle to the development of biomass protein products is the capital costs of the process. The costs incurred in producing a microbial biomass protein product must compete with proteins from crop plant (soybean) or animal product prices. However, it should be noted that economic circumstances often change, so that a process that is not economically attractive today may become so in the near future. The use in the fermentation industry of lignocellulosic raw materials presents tremendous potential for the development of modified and more economical microbial protein processes. Lignocellulosics are in abundance as renewable resources from agriculture, food processing, and forestry and are available in the form of either crop residues or by-product effluent wastes. An economic incentive also exists for the disposal of these wastes, which otherwise can cause a serious pollution problem.

Thus, it is expected that changes in the world's food and feed situation, together with current research and development efforts, might significantly improve the interest and economic viability for microbial protein production processes that could help solve the world's acute protein shortage.


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