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Single-Cell Proteins

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Summary. Both photosynthetic and nonphotosynthetic microorganisms, grown on various carbon and energy sources, are used in fermentation processes for the production of single-cell proteins. Commercial-scale production has been limited to two algal processes, one bacterial process, and several yeast and fungal processes. High capital and operating costs and the need for extensive nutritional and toxicological assessments have limited the development and commercialization of new processes. Any increase in commercial-scale production appears to be limited to those regions of the world where low-cost carbon and energy sources are available and conventional animal feedstuff proteins, such as soybean meal or fish meal, are in short supply.

The term "single-cell proteins" (SCP) refers to the dried cells of microorganisms such as algae, actinomycetes, bacteria, yeasts, molds, and higher fungi grown in large-scale culture systems for use as protein sources in human foods or animal feeds. Although these microorganisms are grown primarily for their protein contents in SCP production processes, microbial cells contain carbohydrates, lipids, vitamins, minerals, and nonprotein nitrogen materials such as nucleic acids.

The large-scale cultivation of microorganisms for use as a food source for humans and for animal feeds is an example of an early and progressing application of modern biotechnology. Microorganisms have been a component of human foods since ancient times. Examples include yeast as a leavening agent in bread-making; lactic acid bacteria in making fermented milks, cheeses, and sausages; and molds in making a variety of Oriental fermented foods (*1*). Algae of the genus *Spirulina* were harvested from alkaline ponds by the ancient Aztecs in Mexico and consumed as a source of protein. Dried *Spirulina* cells are eaten at the present time by the people in the Lake Chad region of Africa (*2*).

Present-day technology for SCP production began in 1879 in Great Britain with the introduction of aeration of the vats used for producing bakers' yeast. About 1900 in the United States, centrifugation was introduced for separating yeast cells from the growth medium (*1*). The first purposeful SCP production originated in Germany during World War I when bakers' yeast, *Saccharomyces cerevisiae*, was grown—with molasses as the carbon and energy source and ammonium salts as the nitrogen source—for consumption as a protein supplement. Also, incremental feeding of the carbon and nitrogen sources during growth was introduced during this period. In Germany during World War II, *Candida utilis* (Torula yeast) was cultivated on sulfite waste liquor from pulp and paper manufacture and wood sugar derived from the acid hydrolysis of wood and used as a protein source for humans and animals (*3*). During this period, the development of the Waldhof fermentor represented a significant advance in technology for mass cultivation of microbial cells. This fermentor provides both aeration and agitation by use of a wheel-type hollow-bladed impeller (*4*). After World War II, Torula yeast production was introduced into the United States and has continued until the present time. Torula yeast has been produced in many countries including Switzerland, Taiwan, and the U.S.S.R.

In recent years, technological improvements in microbial cell production for food and feed include the introduction of continuous processes, the development of airlift tower fermentors, and the development of novel methods for flocculating microbial cells to reduce centrifugation costs (*5*).

Raw Materials: Sources and Treatment

Many raw materials have been considered as carbon and energy sources for SCP production (Table 1). In many cases, raw materials must first be treated by physical, chemical, or enzymatic methods before they can be utilized as carbon and energy sources by microorganisms (*6*).

Sources of cellulose, such as wood and straw, are made up of a lignin-hemicellulose-cellulose (LHC) complex that cannot be readily hydrolyzed by enzymes or acids to liberate fermentable sugars. Lignin is a complex polyphenolic structure that protects cellulose and hemicellulose from acid or enzyme hydrolysis. In addition, the highly crystalline nature of cellulose gives a protective effect. Common physical methods for preliminary treatment of lignocellulosic materials include ball milling, two-roll milling, and grinding (*6*). Recently, explosive depressurization processes such as the Iotech process developed in Canada have been developed for facilitating the breakdown of the LHC complex and aiding in separating the lignin, hemicellulose, and cellulose components (*7*). Cellulose produced in this way has not yet been used as a substrate for SCP production. After cellulose is separated from the LHC complex, it is more susceptible to acid or enzyme hydrolysis to yield hexose sugars such as glucose and cello-

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biose. In the same manner, hemicellulose can be hydrolyzed by acids or enzymes to xylose and arabinose (*8, 9*).

Microbial cellulases have been investigated extensively for their utility in the hydrolysis of cellulose. *Trichoderma reesei* appears to be the most promising cellulolytic microorganism identified to date (*10*). The extent of hydrolysis of cellulosic materials such as rice or wheat straw by cellulases varies widely, depending on the raw material and preliminary treatment, enzyme concentration, and time for hydrolysis (*11*).

Starchy materials, such as potato processing waste, must be converted to mono- and disaccharides to be suitable as substrates for SCP production. The Swedish Sugar Corporation developed the Symba process for treatment of wastes that contain starch, such as those from potato and rice processing. Two organisms used are *Saccharomycopsis fibuligera*, which produces α- and β-amylases for hydrolysis of starch to glucose and maltose, and *C. utilis* for utilizing these sugars as a substrate for growth (*12*). This process was operated on a pilot plant scale to produce 40 to 100 kilograms of dry yeast per day, but its usefulness is limited by the intermittent availability of the waste stream from potato processing operations. Alternatively, starch can be hydrolyzed by a combination of α-amylase and amyloglucosidase (glucoamylase) as used in converting starchy materials for ethanol production by fermentation.

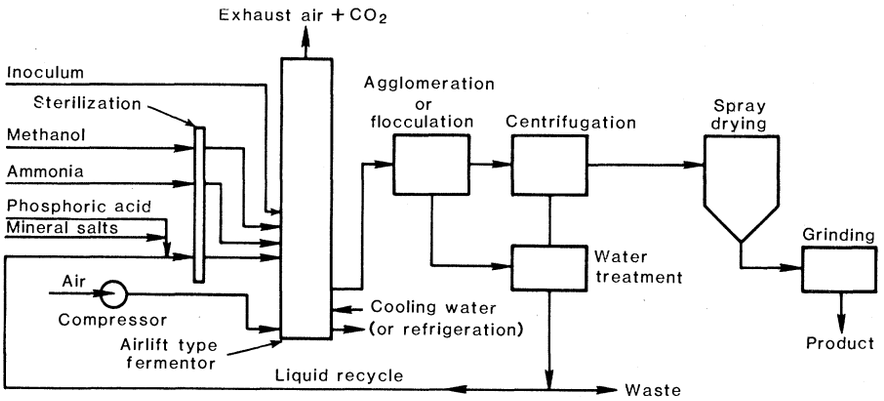

Fig. 1. Schematic diagram of a typical process for making single-cell protein from methanol (*5*).

Only a few microorganisms, such as *Kluyveromyces fragilis* (*13*) and *Penicillium cyclopium* (*14*) can use lactose as a carbon and energy source for SCP production. An immobilized enzyme process has been developed by Corning Glass Works and Kroger Company for converting lactose in cheese whey to a glucose-galactose that can be used as a carbon and energy source for growing bakers' yeast (*S. cerevisiae*) (*15*).

Sulfite waste liquor from paper mill operations contains concentrations of sulfur dioxide or sulfite that inhibit microbial growth. Treatment with lime, which results in the precipitation of calcium sulfite, and steam stripping in a tower are effective methods for reducing inhibitory concentrations of sulfur compounds. These methods have been used successfully in the production of Torula yeast at Rhinelander Paper Company in the United States (*16*) and in the Pekilo process in Finland (*16, 17*).

In the development of SCP processes based on hydrocarbons, British Petroleum evaluated both gas oil containing 25 percent C_{15} to C_{30} *n*-alkanes (boiling range, 300° to 380°C) and purified *n*-alkanes prepared by a molecular sieve process (97.5 to 99 percent C_{10} to C_{23}, with a boiling range of 175° to 300°C) as substrates for *Candida* sp. (*18*). The use of molecular sieves to purify *n*-alkanes in the British Petroleum process minimized the need for a complicated solvent clean-

Table 1. Selected raw materials used as carbon and energy sources in single-cell protein processes.

Raw material	Process type and scale*	Organism	Producer or developer	Reference
	Algal, 2 metric tons per day†	*Chlorella* sp.	Taiwan Chlorella Manufacture Co. Ltd., Taipei	*28*
CO_2	Photosynthetic			
Cane syrup, molasses (sucrose)	Nonphotosynthetic			
CO_2 or $NaHCO_3$-Na_2CO_3	Algal, 320 metric tons per year;‡ photosynthetic	*Spirulina maxima*	Sosa Texcoco, S.A., Mexico City	*25*
Methanol	Bacterial			
	70,000 metric tons per year	*Methylophilus methylotrophus*	Imperial Chemical Industries, Billingham	*31*
	1000 metric tons per year	*Methylomonas clara*	Hoechst-Uhde, Frankfurt, West Germany	*33*
Ethanol	Yeast, 7500 short tons per year	*Candida utilis* (Torula)	Pure Culture Products, Hutchinson, Minnesota	*19*
n-Alkanes, wood hydrolyzates	Yeast (several plants), 20,000 to 40,000 metric tons per year	*Candida* sp.	All-Union Research Institute of Protein Biosynthesis, U.S.S.R.	*57*
Sulfite waste liquor	Yeast, 15 short tons per day	*Candida utilis*	Rhinelander Paper Corp., Rhinelander, Wisconsin	*16*
	Mold, 10,000 metric tons per year	*Paecilomyces varioti*	Pekilo Process, Finnish Pulp and Paper Research Institute, Jamsankoski, Finland	*17*
Glucose	Mold, 50 to 100 metric tons per year	*Fusarium graminearum*	Rank Hovis MacDougall Research Limited, High Wycombe, U.K.	*58*
Cheese whey (lactose)	Yeast, 5000 short tons per year	*Kluyveromyces fragilis*	Amber Laboratories, Juneau, Wisconsin	*13*
	Mold, 300 metric tons per year	*Penicillium cyclopium*	Heurty, S. A., France	*14*

*Plant capacity, metric tons (1000 kilograms), or short tons (2000 pounds) per unit of time indicated. †Total pond area, 83,400 square meters. ‡Pond area, 900 hectares.

up of the yeast produced to decrease the amounts of residual hydrocarbons. However, this process was abandoned because it was not economically feasible as a result of increased costs of the hydrocarbon feedstocks (*18*).

Ethanol has been used for the production of *C. utilis* by Pure Culture Products, Inc. in the United States (*19*) and in pilot plant studies in Japan (*20*) and Czechoslovakia (*21*). Synthetic ethanol may contain the impurities propanol, 2-methyl-2-propanol, and 2-butanol (crotonaldehyde); these are not present in ethanol produced by yeast fermentation. 2-Butanol inhibits the growth of *C. utilis*, lengthens the lag phase, decreases cell yield, and decreases the crude protein content of the cells in batch growth systems. However, concentrations in the range of 1 gram per milliliter are tolerated by this organism in continuous culture systems (*22*).

In most SCP processes, the carbon substrate is present in relatively low concentrations, either because of its solubility or because the amount that can be tolerated by a given microorganism is limited. In algal processes, the amount of carbon dioxide in air (0.03 percent) is inadequate for growth, and additional CO_2 must be supplied from sources such as carbonates or bicarbonates in alkaline waters or from natural deposits of carbonaceous minerals, from combustion gas, or from decomposition of organic matter in sewage or industrial waste. When CO_2 is supplied to algae in the gaseous form, such as in combustion gases, concentrations have ranged from 0.5 percent to 5 percent in air (*5*).

For nonphotosynthetic microorganisms grown in batch cultures, carbon and energy source concentrations generally range from 1 to 5 percent when soluble carbohydrates are used. Continuous processes are usually used for growing microorganisms on hydrocarbons, methanol, or ethanol. In these processes, concentrations of the carbon and energy source are generally less than 1 percent. The rate of feeding the substrate to the fermentor is adjusted so that the amount supplied will meet the demand of the growing organism, and losses by evaporation will be minimized.

In addition to the carbon and energy

Table 2. Operating characteristics of selected single-cell protein processes.

Item	Process			
	Algal *Spirulina maxima*	Bacterial *Methylophilus methylotrophus* (methanol)	Yeast *Candida utilis* (ethanol)	Mold *Paecilomyces varioti* (sulfite waste liquor)
Type of process	Batch or semicontinuous	Continuous	Continuous or batch	Continuous
Sterility	Nonaseptic	Aseptic	Aseptic	Nonaseptic
Fermentor	Ponds	Airlift	Agitated	Agitated
Feedstock utilization	Partially or fully utilized	Fully utilized	Fully utilized	Partially utilized
Temperature (°C)	Ambient	35 to 42	30 to 40	38 to 39
*p*H	9 to 11	6.0 to 7.0	4.6	4.5 to 4.7
Product recovery	Filtration	Agglomeration and centrifugation	Centrifugation	Filtration

Table 3. Growth characteristics and protein contents (nitrogen content × 6.25) (percent) of selected microorganisms of interest for single-cell protein production. N.D., no data.

Organism	Carbon and energy source	Specific growth rate (μ)* or dilution rate (D)†	Output	Crude protein (%)	Reference
		Photosynthetic			
Algae					
Scenedesmus acutus	CO_2, sunlight	N.D.	20‡	55	*24*
Spirulina maxima	CO_2, HCO_3, CO_3^{2-}, sunlight	N.D.	15‡	62	*25*
Bacteria					
Rhodopseudomonas capsulata	Industrial wastes, sunlight	N.D.	1.2 to 2.0§	61	*29*
		Nonphotosynthetic			
Bacteria					
Cellulomonas sp.	Bagasse	0.20 to 0.29*	0.44 to 0.50‖	87	*35*
Alcaligenes sp.					
Methylococcus capsulatus	Methane	0.14*	1.00 to 1.03‖	N.D.	*59*
Methylophilus methylotrophus	Methanol	0.38 to 0.50*	0.50‖	72	*31*
Yeasts					
Candida lipolytica	*n*-Alkanes	0.16†	0.88‖	65	*18*
Candida utilis	Ethanol, sulfite waste liquor	0.50*	0.70‖	50 to 55	*19*
		0.30*	0.50‖		*16*
Kluyveromyces fragilis	Cheese whey (lactose)	0.66*	0.55‖	45 to 54	*13*
Saccharomyces cerevisiae	Molasses	0.25*	0.50‖	53	*4*
Molds and higher fungi					
Cephalosporium eichhorniae	Cassava starch	N.D.	0.45‖	48 to 50	*37*
Chaetomium cellulolyticum	Agriculture and forestry wastes	0.24*	N.D.	45	*39*
Paecilomyces varioti	Sulfite waste liquor	0.20*	0.55‖	55	*38*
Penicillium cyclopium	Cheese whey (lactose)	0.20*	0.68‖	47.5	*14*
Scytalidium acidophilum	Acid-hydrolyzed waste paper	N.D.	0.43 to 0.46‖	44 to 47	*38*

*$\mu = dX/X\,dt$, where μ is specific growth rate (hour^{-1}), X is cell concentration (grams per liter), and t is time (hours). †$D = P/X$, where D is dilution rate (in continuous processes) (hour^{-1}), P is productivity (grams per liter per hour), and X is cell concentration (grams per liter). ‡Productivity (area basis) in units of grams per square meter per day. §Cell concentration X in grams per liter. ‖Yield Y in grams per gram substrate utilized; $Y = dX/dS$, where S is the substrate concentration (grams per liter).

source, microorganisms require sources of nitrogen, phosphorus, and mineral nutrients, and may require supplemental nutrients such as vitamins. Suitable nitrogen sources for SCP production are ammonia, ammonium salts, nitrates, urea, and organic nitrogen sources such as protein hydrolyzates. It is important to adjust the supply of the nitrogen source so that a ratio of 10:1 or less for carbon to nitrogen can be maintained in the medium during growth to minimize the accumulation of lipids or storage substances, such as poly-β-hydroxybutyrate, and to favor high protein contents in the cell.

The phosphorus source for SCP production is usually supplied as either phosphoric acid or soluble phosphates; a food- or feed-grade source of phosphorus, which is low in arsenic, fluoride, or heavy metals, should be used. Natural water supplies may provide sufficient quantities of mineral nutrients, such as iron, magnesium, manganese, calcium, sodium, and potassium salts, but in most cases, the water supply must be supplemented to make up for any deficiencies. To avoid corrosion problems in fermentation equipment, mineral salts should be added as sulfates or hydroxides rather than chlorides (*5*).

It is apparent that large quantities of process water are required for SCP production, including medium preparation, cell washing, cleanup, and steam generation. For example, an estimated water requirement for producing bacterial SCP from methanol is in the range of 45.5 million liters per 100,000 metric tons per year of production (*23*).

Process Characteristics

The type of process (batch or continuous), growth rate, sterility requirements, type of fermentor or bioreactor, extent of feedstock utilization and yield, temperature, *p*H, and methods used for product recovery all are important factors in determining the economic viability of SCP process (Table 2). The growth characteristics and protein contents for selective microorganisms for SCP production, including cell yields and crude protein contents, are summarized in Table 3.

Photosynthetic organisms. Both algae and photosynthetic bacteria have been used for SCP production. The photosynthetic production of SCP by *Chlorella* species can be represented by the following typical equation (*24*):

$$6.14\ CO_2 + 3.65\ H_2O + NH_3 \xrightarrow{\text{light}} C_{6.14}H_{10.3}O_{2.24}N + 6.85\ O_2$$

The composition of the cell product will vary with different algal species. Algae can be grown either in batch tanks or semicontinuous ponds operated on a fill and draw principle (Table 2). Algal production is often carried out under nonsterile mixed-culture conditions where the organism that is desired usually predominates over other competing species. *Spirulina maxima* grows best in highly alkaline waters having a *p*H in the range 9 to 11 as is the case in Lake Texcoco in Mexico (*25*). In the Indian–West German process, *Scenedesmus acutus* is grown in pure culture at a *p*H of 7 to 8 (*24*). Mixed algal cultures develop in the experimental pilot plant systems at the University of California (*26*) and in Israel (*27*).

In Japan and Taiwan, *Chlorella* species have been grown either photosynthetically or in nonphotosynthetic heterotrophic systems, with carbon sources such as sugar syrups or molasses at *p*H 6 to 7 (*28*). Dried algae and algae tablets are sold as health foods in both of these countries.

Important factors in large-scale photosynthetic algal cultivation systems include lack of cloud cover and minimal diurnal variations in light intensity with temperatures above 20°C for most of the year. Large pond areas must be used because cell densities seldom exceed 1 to 2 grams per liter (dry weight) as compared with 30 to 40 grams per liter with yeasts and some bacteria. Growth occurs in the top 20- to 30-centimeter layer in open ponds. At present, algal productivities of 20 to 25 grams per square meter per day are attained in controlled culture systems. Productivities of 30 to 40 grams per square meter per day are possible under optimum growth conditions (*24*). The culture system must be agitated either mechanically or by recirculation to prevent settling of the algae and to prevent thermal stratification and depletion of nutrients at the surface. Harvesting algae is a particularly difficult problem in view of the low cell densities and large volumes of water that must be handled. The use of microstrainers and other filtration methods is probably the best approach (*25*).

Photosynthetic bacteria such as *Rhodopseudomonas capsulata* have been grown in Japan, with sewage or industrial waste as substrates. Generally, these organisms grow in mixed culture with aerobic, heterotrophic, and nitrogen-fixing bacteria. Again, culture densities are low, on the order of 1 to 2 grams per liter (dry weight) (*29*).

Nonphotosynthetic organisms. Actinomycetes, nonphotosynthetic bacteria, molds, yeasts, and higher fungi all require aerobic conditions for growth in SCP processes. Substrate and oxygen transfer to and across the cell surface are limiting factors in aerobic growth. Since oxygen has a low solubility in water, a fermentor design that gives maximum oxygen transfer rates with minimum power requirements should be selected.

Heat is produced as a result of microbial growth. For yeasts such as *Candida* species, this amounts to approximately 0.46 kilojoule per millimole of oxygen consumed (*30*) or 14,410 kilojoules per gram of yeast solids if 1 gram of oxygen is required for each gram of yeast solids produced (*4*). Consequently, heat removal is a significant factor in SCP production.

Many species of bacteria have been investigated for use in SCP processes because of their short generation times (20 to 30 minutes) and their ability to use a variety of raw materials ranging from carbohydrates to gaseous and liquid hydrocarbons and petrochemicals. Figure 1 shows a schematic diagram for bacterial SCP production from methanol.

An example of a commercial-scale SCP process based on methanol now in operation is that developed by Imperial Chemical Industries, Ltd., in the United Kingdom for growing *Methylophilus methylotrophus*. The conversion of methanol to the SCP product is represented by the equation (*31*)

$$1.72\ CH_3OH + 0.23\ NH_3 + 1.51\ O_2 \rightarrow 1.0\ CH_{1.68}O_{0.36}N_{0.22} + 0.72\ CO_2 + 2.94\ H_2O$$

This process has been operated intermittently over the past year at 6000 metric tons per month. The organism is grown continuously under aseptic conditions (Table 2). A special "pressure cycle" airlift type fermentor is used (*30*). The process is operated at 35° to 42°C to minimize cooling cost, since the growth of these organisms is highly exothermic. An unusual feature of the process is the initial separation of the cells from the production medium by agglomeration in the aqueous growth medium so that a higher solids slurry can be fed to the centrifuges than is normally practiced in most SCP processes. Purified CO_2 is sold as a by-product. Relatively high specific growth rates of 0.50 $hour^{-1}$ and cell yields exceeding 0.50 gram per gram of substrate (*31, 32*) (Table 3) are reported for this process.

Hoechst-Uhde in West Germany has developed a pilot plant process for producing *Methylomonas clara* with methanol as the carbon and energy source

Table 4. Typical 1982 selling prices of selected microbial plant and animal protein products. Selling prices were obtained from trade sources.

Product	Protein content (%)	1982 selling price (U.S. dollars per kilogram)
	Food-grade products	
Candida utilis (Torula yeast)	50 to 55	1.87 to 2.24
Kluyveromyces fragilis	45 to 50	2.09 to 2.29
Soy protein concentrate	72	0.88 to 1.03
Soy protein isolate	92	2.59 to 2.68
Dried skim milk	37	1.16 to 1.21
	Feed-grade products	
Saccharomyces cerevisiae	45 to 50	0.48 to 0.66
Soybean meal	44	0.19 to 0.20
Meat and bone meal	50	0.19 to 0.21
Fish meal	65	0.23 to 0.40

(Table 1) (*33*). Their interest has been on the potential for producing a 90 percent protein concentrate and nucleic acid by-products. Shell Research Ltd. constructed a pilot plant for producing *Methylococcus capsulatus* or a mixed culture of methane-utilizing bacteria from methane (*34*). However, this process was not developed to a commercial scale for economic reasons. Research was also conducted at Louisiana State University on the use of bagasse, a by-product of sugar mill operations, as a substrate for the growth of cellulolytic bacteria. A mixed culture of *Cellulomonas* and *Alcaligenes* species grew on a medium containing bagasse but the economics of this process were not sufficiently attractive for further development (*35*).

As mentioned earlier, yeasts were the first microorganisms to be grown on a large scale. At the present time, *C. utilis* (Torula yeast) is being produced from ethanol by Pure Culture Products at Hutchinson, Minnesota (*19*). This organism can be grown in either continuous or batch culture systems. In the Pure Culture Products process, a conventional baffled, agitated, and aerated fermentor system is used with aseptic conditions. The substrate is fed at a rate adjusted for full utilization by the yeast (Table 2). *Candida utilis* is also produced from sulfite waste liquor from paper mill operations. Operating conditions and growth rates are similar to those in the ethanol-based process (*16*).

Approximately 14.6×10^9 kilograms of cheese whey are produced in the United States and in Western Europe each year. The yeast *K. fragilis* utilizes lactose readily and has been used to produce an SCP product from whey at Amber Laboratories, Juneau, Wisconsin (Table 1) (*13*). Again, operating conditions and yields are similar to those obtained with *C. utilis* and bakers' yeast (Table 3). Molds and higher fungi are capable of utilizing diverse carbohydrate materials, including agricultural and food processing wastes. Large-scale systems for producing these microorganisms are similar to those used for yeast SCP production.

The largest mold-SCP process now being operated is the Pekilo process developed in Finland at the Finnish Pulp and Paper Institute (*17*) (Tables 1 and 2). This process is operated continuously. Residual SO_2 is stripped from the sulfite liquor feedstock. Sterile air, ammonia, and feedstock are supplied to two baffled, agitated-aerated fermentors that produce 15 to 16.5 tons (dry weight) of *Paecilomyces varioti* mycelium in 24 hours. The mycelial product has the advantage over bacterial and yeast cells that it can be recovered on drum filters in a process that is less costly than centrifugation. The growth rate is slower than rates observed with yeasts or bacteria, but the yield (grams of dry cell product per gram of substrate) and the protein content (55 percent) are similar to those of yeast (*5*) (Table 3).

In the United Kingdom, Rank Hovis MacDougall has grown *Fusarium graminearum* continuously in 1300-liter fermentors, with glucose as the substrate and ammonia as the nitrogen source. The specific growth rate is approximately 0.18 $hour^{-1}$ and the cells contain 45 percent protein (*36*).

Examples of SCP processes under development are the utilization of cassava starch by *Cephalosporium eichhorniae* (*37*), cheese whey by *Penicillium cyclopium* (*14*), and acid-hydrolyzed waste paper by *Scytalidium acidophilum* (*38*) (Table 3). However, none of these processes have reached even the pilot plant stage.

The so-called "solid substrate" fungal processes are being altered to upgrade the value of agricultural, forestry, and food processing wastes as animal feeds. In these processes, which are analogous to composting, water is removed from the substrate until the moisture content is 50 to 80 percent. Nitrogen and phosphorus are supplied by addition of commercial fertilizer. The waste is inoculated, aerated, and the product is then recovered and dried.

The Waterloo process, developed at the University of Waterloo in Canada, is based on the cellulolytic fungus *Chaetomium cellulyticum* and can be operated either in a conventional aerated fermentation system or in a solid substrate system, depending on the substrate (*39*). In the solid substrate process, materials such as corn stover or Kraft paper mill clarifier sludge are subjected to an initial thermal or chemical treatment, followed by aerobic fermentation, separation of the fungal mycelium, and drying. The final product contains up to 45 percent protein. This process has been operated only on a small pilot plant scale.

Tate and Lyle in the United Kingdom have investigated the use of *Aspergillus niger* in solid substrate systems for recovering fruit and vegetable processing wastes. The yield of the fungal product on carrot wastes was 0.11 gram per gram of substrate, and the crude protein content was increased from 9 percent in the starting material to 29 percent in the finished product (*40*). It is doubtful that this process will be economically feasible for treating fruit and vegetable wastes that are available only during a short growing season.

Economics

Among the factors affecting the economic viability of SCP processes are the capital costs of facilities, including working capital; the site location, taking into account the availability of raw materials and the size and proximity of markets; and manufacturing costs, including costs of raw materials (carbon and energy source, nitrogen source, and mineral nutrients), energy, water supply, waste treatment, labor, and maintenance, as well as depreciation and the desired profit margin.

The highest capital costs for facilities are incurred in processes that must be operated under aseptic conditions to produce a food-grade product. Estimates published for various processes during 1975 and 1976 for producing feed-grade SCP from methanol were in the range of $660 to $1000 per metric ton of annual capacity for 50,000 to 100,000 metric ton

capacity plants (*5*). Current costs for food-grade SCP products would be much higher. Costs of raw materials range from 14 percent of manufacturing costs for agricultural and forestry wastes with solid substrate fermentations (*39*) to more than 50 percent for processes requiring methanol or ethanol (*41*). Increasing prices of methanol and ethanol in the future will make SCP processes based on these substrates economically unattractive.

Estimates of energy requirements for SCP processes vary widely. Typical values for total energy inputs are estimated to range from 185 to 190 megajoules per kilogram of protein for *Candida* species grown on ethanol to 30 megajoules per kilogram for *A. niger* grown on agricultural processes wastes, when land and labor requirements are taken into account (*42*).

Product Quality and Safety

Single-cell protein products can be used as (i) protein supplements in human foods, (ii) functional food ingredients to provide, for example, flavor, fat and water binding, dispersing action, whipping and foaming action, and extrusion and spinning characteristics, and (iii) protein supplements for livestock feeding.

Data given in Table 3 on crude protein contents determined by multiplying nitrogen contents by the factor 6.25 do not reflect the true value of these products in human and animal nutrition since amino acid profiles vary widely (*42*) and nonprotein nitrogen substances such as nucleic acids are included. Nucleic acid contents may range from 5 to 15 percent depending upon the organism and growth conditions used (*43*). These substances have no nutritional value for nonruminant animals, and intakes by humans must be limited to 2 grams of nucleic acid per day to avoid kidney stone formation or gout. They may be removed by acid, alkali, or enzyme treatment of the cells or by enhancing endogenous nucleases (*44*). Feeding studies with broiler chickens and swine have shown the importance of supplementing yeast SCP products with methionine or its hydroxy analog and adjusting arginine and lysine ratios (*45*). Best efficiencies of feed conversion with broiler chickens and swine are obtained when SCP products are used at 7 to 15 percent in the ration, but levels up to 25 percent have been used in broiler chicken rations supplemented with selenium at 0.3 part per million (*46*).

Table 5. Improvements in single-cell protein production.

Item	Example	Reference
Strain improvement	Mutants of *S. cerevisiae* forming enlarged cells for improved recovery	*50*
Genetically engineered cultures	Cloning of genes for higher amino acid contents in methanol-utilizing bacteria	*51*
	Improved NH_3 utilization by transfer of glutamic dehydrogenase gene from *Escherichia coli* to *Methylophilus methylotrophus*	*52*
Enzyme for degrading cell walls for protein concentrate production	*Rhizoctonia solani* 1,3-β-D-glucanase for degrading yeast cell walls	*53*
Extracellular production of proteins	Excretion of protein into medium by *Bacillus brevis*	*54*
Improved harvesting methods	Agglomeration, electrocoagulation	*55*
Automation of production	Computer control of Pekilo process	*56*

Currently, in the United States, Food and Drug Administration regulations permit the human food use of dried cells of *S. cerevisiae* (bakers' yeast), *C. utilis* (Torula yeast), *K. fragilis* (fragilis yeast), and bakers' yeast protein concentrate produced by extraction of protein from *S. cerevisiae* (*47*). In the United Kingdom, the Ministry of Agriculture, Fisheries, and Food has allowed test market studies on the food use of dried mycelium of *F. graminearum* developed by Rank Hovis MacDougall (*36*), and animal feed use of the Imperial Chemical Industries SCP product (dried cells of *M. methylotrophus*) (*31*). However, possible iron and zinc deficiencies in the Rank Hovis MacDougall product may require supplementation with these mineral nutrients. The Protein Advisory Group of the United Nations has developed guidelines for the production and evaluation of SCP products (*48*). In addition to short-term toxicological studies in rats, more extensive assessments of carcinogenicity, teratogenicity, and mutagenicity, including multigenerational feeding studies, may be required by government regulatory agencies.

Market Considerations

Establishing markets or maintaining existing markets for SCP products for animal feed applications depends on their price and feeding performance in broiler chicken, turkey, laying hen, or swine rations as compared with existing protein feedstuffs such as soybean meal and fish meal. The extensive livestock studies conducted by British Petroleum and Imperial Chemical Industries on their SCP products exemplify the demonstration of feeding performance needed to satisfy users and government regulatory agencies (*31, 45, 49*).

In human foods, flavor and texture, in addition to nutritional value of SCP products, are important determinants of acceptability. At the present time, the major market for food-grade SCP products is for functional uses in foods. For example, yeast protein autolyzates and hydrolyzates have been used as food flavoring for many years. Torula yeast products are being sold as functional food additives in processed meats and bakery products in the United States. Typical 1982 selling prices for selected microbial, plant, and animal protein products are presented in Table 4. Food-grade yeast products must provide special functional characteristics such as flavoring, in addition to nutrient content, to compete with functional soy protein products. Feed-grade SCP products must be competitive with established feedstuffs, such as soybean meal and fish meal, on price and feeding performance bases. In addition, any new SCP product will have to satisfy government regulatory agency requirements for safety in human or animal feeding.

Prospects

Table 5 summarizes some of the current research and development efforts that may lead to significant improvements in SCP processes. These range from strain improvement (*50–52*), including genetically engineered cultures such as the glutamic dehydrogenase recombinants developed by ICI in the United Kingdom, to improved methods for protein isolation (*53, 54*) and cell harvesting (*55*), process monitoring, and computer control of production (*56*). However, the impact of these developments on the future economic viability of SCP processes remains to be seen.

It is apparent that large-scale process-

es for manufacturing SCP products are technologically feasible, and selected processes are now being operated to a limited extent on a commercial scale worldwide. However, the introduction of new SCP products will be limited by economic, market, and regulatory factors rather than by technological considerations.

The future prospects for large-scale SCP production for human food appear to be limited to use as protein supplements and functional protein ingredients rather than as primary sources of protein in human diets. For animal-feed applications, SCP production will be limited to those areas where low-cost substrates such as waste carbohydrates are available and conventional protein feedstuffs such as soybean meal and fish meal are in short supply.

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