Protein Production by Micro-organisms

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1 Introduction

The concept of micro-organisms as components in human foods and animal feeds has long been established. Historically, micro-organisms have played an important role in the preparation of fermented foods and have frequently been consumed, in significant quantities, together with products that they have been used to prepare. As ingredients in animal feeds, they have found widespread application. In ruminants, of course, micro-organisms are produced in the rumen, which is effectively an *in situ* anaerobic fermenter, whilst in non-ruminants, micro-organisms are frequently added to mixed feeds as sources of vitamins and other growth factors.

The fundamental attractiveness of micro-organisms as a principal protein source in either mixed or compounded animal feeds stems from the fact that micro-organisms can be produced from raw materials that are themselves essentially inaccessible to mammalian digestion by using high rate aerobic cultivation (fermentation) processes rather than relying on either relatively slow and frequently weather disrupted traditional agricultural production routes or fisheries, essentially a form of hunting, to satisfy our protein requirements. High rate aerobic fermentation processes effectively add a new dimension to animal feed production.

Micro-organisms are an abundant natural resource. The versatility, complexity, and specificity of their metabolic reactions make them suitable for exploitation in various industrial processes. They can be grown on either moist or submerged surfaces and as suspensions in aqueous media. For growth, aerobic micro-organisms require oxygen, a carbon source, an energy source which is frequently the carbon source, a nitrogen source usually in the form of an ammonium salt, a nitrate, or urea, although some micro-organisms can fix atmospheric nitrogen, phosphate, sulphate, and a range of metal ions in either small or trace quantities. Many of the more fastidious micro-organisms also require biological growth factors such as amino-acids and vitamins, but such micro-organisms are unlikely to be of interest for protein production. Growing micro-organisms contain some 75w% intra-cellular water, and their protein content varies between 35 and 75w% on a dry weight basis. When micro-organisms are grown in aqueous suspension, it is unusual for their concentration to exceed 40 $g\,l^{-1}$ on a dry weight basis, although this concentration should not be regarded as an absolute maximum.

Prior to 1950, virtually all investigations concerned with production of

microbial protein were stimulated by acute food shortages during times of war, when normal supply and demand patterns, particularly for imported foods and feed-stuffs, were artificially disrupted and strategic arguments rather than strict economic criteria were applied. During the 1950's doubts were expressed concerning the future availability of adequate world-wide food supplies even in times of peace. The strength of such arguments resulted in the current epoch of microbial protein production processes.

The generic name for microbial protein, Single-Cell Protein (SCP), was proposed[1] and generally accepted in 1966. The adoption of this nomenclature effectively avoided the potentially unpleasant connotations, so frequently, but erroneously, associated with names such as bacterial, fungal, or microbial protein. SCP comprises, whole, dried, non-viable cells of algae, bacteria, yeasts, or fungi, and is a product destined for use as a major protein ingredient in animal feeds,[2] although longer term, some SCP products will probably be incorporated in certain processed human foods in a similar manner to the way in which soya bean protein is used today.

It has been known for many years that most, if not all, simple carbon compounds are susceptible to microbial attack, *i.e.*, they can either serve as carbon substrates for the growth of one or more species of micro-organisms or they can be co-oxidized by micro-organisms growing on another carbon substrate. Numerous carbonaceous feedstocks have been proposed and studied in order to determine their suitability for conversion into SCP. In order for any feedstock to offer reasonable potential for the manufacture of SCP on a commercial-scale, it must be relatively cheap, it must be such that micro-organisms assimilate most of its carbon into cellular material rather than oxidize it to carbon dioxide or other products, its physical properties must be such as to permit rapid transfer to the growing micro-organisms and it must not remain associated with the microbial cells at the conclusion of the production process.

A source of assimilable carbon is only one requirement for microbial growth. Generally micro-organisms obtain their energy from their carbon substrate, but this is not exclusively the case. The photosynthetic and the chemolithotrophic micro-organisms use carbon dioxide as their carbon substrate, whilst their requirement for energy is satisfied by light energy in the former case and by energy derived from inorganic chemical reactions in the latter.

Micro-organisms are able to grow on carbon compounds that are gases, vapours, water miscible liquids, water immiscible liquids, water soluble solids, and water insoluble solids, and compounds of all these types have been considered as potential carbon feedstocks for SCP manufacturing processes.

The major process routes for SCP production that have been subjected to study by industrial and commercial organizations include:

(*a*) The growth of yeasts and bacteria on waxy n-alkanes;

(*b*) The growth of yeasts and bacteria on methanol;

[1] N. S. Scrimshaw, in 'Single-Cell Protein', ed. R. I. Mateles and S. R. Tannenbaum, p. 3, M.I.T. Press, Cambridge, Mass., 1968.
[2] IUPAC, Techn. Rept. No. 12, 26 p. (1974).

(*c*) The growth of yeasts on ethanol;

(*d*) The growth of bacteria on methane and natural gas;

(*e*) The photosynthetic growth of algae on carbon dioxide;

(*f*) The growth of bacteria on carbon dioxide and hydrogen;

(*g*) The growth of fungi on carbohydrates and cellulose;

(*h*) The growth of yeasts and fungi on industrial waste liquors.

The current state of development of these several routes varies considerably. In some cases, commercial-scale production facilities have been erected, whilst in others, only small-scale laboratory experiments have been undertaken. In addition, there is some confusion, when the various potential production routes are discussed, as to what constitutes research on the one hand and development on the other. In this review an attempt will be made to assess both the status and potential of each of the routes mentioned above.

SCP technology, as we know it today, has been developed only during the past two decades. In the earlier stages emphasis was, almost exclusively, placed on hydrocarbon or hydrocarbon derived feedstocks, whilst more recently, as the prices of such feedstocks have escalated, the emphasis, particularly at the research level, has moved towards either renewable feedstocks or carbonaceous wastes.

The value attributed to any particular hydrocarbon feedstock is usually based on its equivalent fuel value together with some allowance for quality related and/or alternative use factors. The change of price of hydrocarbons since 1973 has seriously undermined the economics of several SCP manufacturing ventures based on liquid hydrocarbon feedstocks, whilst for ventures based either directly or indirectly on gaseous hydrocarbon feedstocks, it seems probable that, apart from one or two exceptions, production facilities will be located in major hydrocarbon exporting regions rather than in hydrocarbon importing regions. The reason for this is that whereas liquid hydrocarbon fractions tend to have, apart from a relatively small transport element, a world-wide price irrespective of geographical location, gaseous hydrocarbons have, prior to export, to be liquified, transported in a sophisticated manner, and stored under conditions where significant losses occur upon delivery, thus establishing a significant price difference between exporting and importing regions. The above situation has resulted in a general movement of interest in hydrocarbon based SCP technology from the USA, Western Europe, and Japan to the hydrocarbon exporting countries of the Middle East and North Africa.

All proposals for SCP manufacture are based on the use of continuous culture techniques for the growth of the micro-organisms. The basis for the continuous chemostat culture of micro-organisms was established as recently as 1950 by Monod.[3] Traditional microbiological production processes were based on either batch cultivation or semi-continuous fill and draw techniques, and, together with

[3] J. Monod, *Ann. Inst. Pasteur*, 1950, **79**, 390.

the normally low concentrations of product present in the fermentation broth, have tended to eliminate microbiological processes for the production of all but complex fine chemical and pharmaceutical products.

2 General Aspects of SCP Technology

The various routes proposed for SCP manufacture are usually presented and discussed in isolation with no mention whatsoever of alternative technologies. It is important to realize that the process steps involved in one route are very similar to those of other routes, with the possible exception of the route employing photosynthetic micro-organisms. Irrespective of the route employed, SCP production processes will be operated in the continuous mode. A typical flow diagram for the operations involved in SCP production is shown in Figure 1.

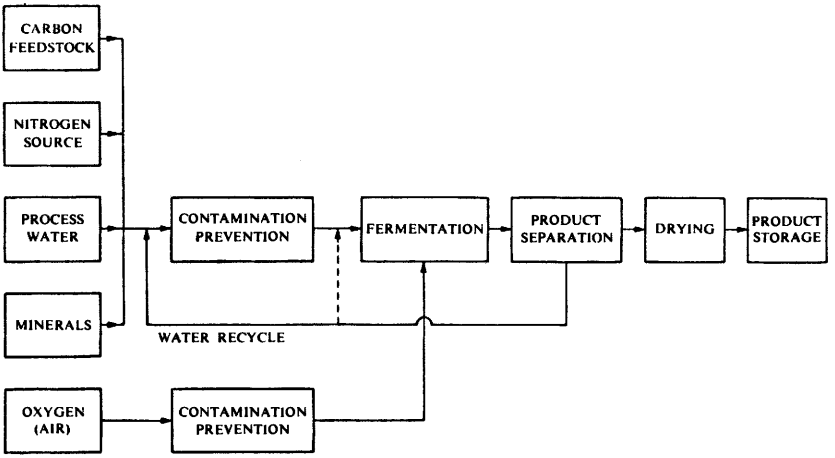


Figure 1 *Unit Processes for SCP Production*

The detailed techniques for achieving each particular process operation will vary as a result of the feedstock and the production micro-organism employed, and varying judgments with respect to efficiency.

Virtually all potential production systems for SCP use submerged culture, where the micro-organisms grow as an evenly distributed aqueous suspension rather than attached to a solid surface or at an interface. The only exception is photosynthetic algae production in poorly mixed natural water bodies where light penetration problems dictate that growth occurs only in the liquid layers close to the surface.

Most proposed production ventures for SCP manufacture are based on the economy of scale concept. Few potential manufacturers consider anything less than 30 000 tonnes per annum to be a viable commercial venture, and the current design production capacity for actual and proposed plants is 100 000 tonnes per annum. Whilst it is presently feasible to consider a single fermenter for pro-

duction capacities up to 75 000 tonnes per annum, most of the 100 000 tonnes per annum plants that have been proposed have two or more fermenters, a feature that allows greater flexibility in the overall operation of the process.

Although the cells that comprise SCP contain some 45—50w% carbon, from the nutritional viewpoint protein is more important as a source of nitrogen rather than as a source of carbon, and SCP can conveniently take up a position in a modified nitrogen cycle[4] as shown in Figure 2. The candidate nitrogen

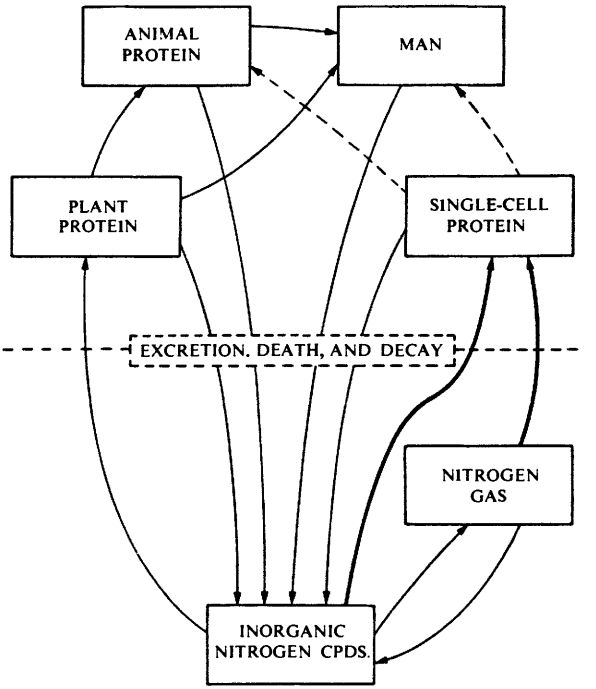


Figure 2 *Nitrogen Cycle Modified to include SCP*[4]

sources for SCP manufacture are ammonia, ammonium salts, nitrates, urea, and atmospheric nitrogen. Both the unit cost of the nitrogen source and the efficiency with which it can be incorporated into protein by micro-organisms dictate the source selected. Most micro-organisms can use several nitrogen sources, although the fixation of atmospheric nitrogen is an uncommon and energy demanding property.

The basic characteristics, irrespective of the particular route, that potential production cultures have to exhibit include:

(i) A high yield coefficient;

(ii) A high growth rate;

[4] J. R. Norris, *Advancement of Science*, 1968, **25**(12), 143.

(iii) The capability to grow at high cell densities;

(iv) A high affinity for the carbon substrate;

(v) Stable growth in continuous culture;

(vi) Resistance to contamination;

(vii) Thermo-tolerance.

These requirements can be satisfied by either pure cultures or mixed cultures of micro-organisms. However, when mixed cultures are employed, it is important that they have been reconstituted from their component micro-organisms and that the characteristics of the component micro-organisms, as well as of the culture as a whole, are defined.

Without doubt the yield coefficient is the most important physiological factor describing the efficiency of microbial growth and cell production. The yield coefficient is defined as the weight of dry cells produced per unit weight of carbon substrate utilized, whilst conversion is defined as the weight of dry cells produced per unit weight of carbon substrate supplied, and is not necessarily identical with the yield coefficient particularly when gaseous, water immiscible, or water insoluble carbon substrates are employed.

When micro-organisms grow on a particular carbon energy substrate, part of the substrate is oxidized to carbon in order to provide energy for the assimilation of the remaining carbon into cellular carbon. Under some growth conditions, extra-cellular carbon compounds are also produced, but from the viewpoint of cell production, this diversion of substrate to alternative products can only be considered as undesirable. Further, the composition of the cells, particularly their protein content, is also affected by growth conditions. In a continuous flow fermenter, operated as a chemostat, cell growth is limited by a single nutrient, and in order to enhance the protein content of the cells, growth limitation by the carbon substrate is preferred.[5] Under operating conditions where other nutrients limit growth, in the presence of excess of carbon substrate, large quantities of storage materials, particularly carbohydrates and fats, can be deposited inside the cells.

Two main approaches with respect to the assessment of the efficiency of carbon substrate utilization are in general use. The first of these is the thermochemical approach first proposed by Baas-Becking and Parks[6] and the second is the ATP yield concept approach, proposed originally for anaerobic microbial growth by Bauchop and Elsden.[7]

3 Yeast and Bacterial Growth on n-Alkanes

The concept of producing animal feed protein from fossil fuels stimulated wide interest when it was first discussed as a commercially interesting process.[8] How-

[5] D. E. F. Harrison, H. H. Topiwala, and G. Hamer, Proc. 4th Internat. Ferment. Symp., Kyoto, 1972, 491.
[6] L. G. M. Baas-Becking and G. S. Parks, *Physiol. Revs.*, 1927, **7**, 85.
[7] T. Bauchop and S. R. Elsden, *J. Gen. Microbiol.*, 1960, **23**, 457.
[8] A. Champagnat, C. Vernet, B. Lainé, and J. Filosa, *Nature*, 1963, **197**, 13.

ever, it had been known since 1895 that micro-organisms were capable of utilizing n-alkanes as their sole carbon energy substrate.[9] Prior to the recognition of the potential offered by hydrocarbon substrates, very few microbial growth studies concerning growth on waxy n-alkanes were performed. An exception was the work of Just *et al.*,[10,11] who examined various fractions of n-alkanes produced by Fischer–Tropsch synthesis.

In 1963, Champagnat *et al.*[12] discussed the process for microbiologically dewaxing gas oil such that the wax removed was converted into protein suitable for use as an animal feed ingredient. The process was, of course, subject to both technical and economic uncertainties, and under the former heading, potentially the most difficult problem was the question of carry-over of unused components from the feedstock into the product, particularly as the waxy n-alkanes represent less than 25% of the unwaxed gas oil used as the feedstock. Subsequently, the development of the British Petroleum Company's process was diversified such that in addition to concomitant dewaxing and protein production, a route utilizing a purified waxy n-alkane fraction for protein production received equal development effort.[13] In fact, the two processes that were developed were, not surprisingly, similar, although for the gas oil based route solvent extraction of the product was incorporated[13] and whereas the gas oil based process was operated non-aseptically, the n-alkane based process was designed for aseptic operation.[14] Not only did solvent extraction introduce an additional process step, but it removed lipids from the product, hence adversely affecting the nutritional value.[15] The question of fully aseptic operation of a massive protein production plant is a problem that can only be solved at a prohibitive cost, and hence, fully aseptic operation is unlikely to be commercially viable. There is, of course, no reason why animal feed protein should be produced aseptically, provided the process eliminates potential pathogens. In fact, SCP produced under non-aseptic conditions, but with reasonable precautions such that it meets recommended product quality specifications,[2] will contain a lower level of microbial contamination than virtually all other conventional animal feed protein ingredients, irrespective of either their source or method of production.

The British Petroleum Company's development has, to some extent, suffered from being the pioneer SCP process in the present era of microbiological protein production. Not only has the product been subjected to ever more stringent and costly tests to meet unnecessarily rigorous product registration requirements, other organizations have largely copied the waxy n-alkane technology but rarely

[9] M. Miyoshi, *Jahrb. Wiss. Bot.*, 1895, **28**, 269.
[10] F. Just, W. Schnabel, and S. Ullmann, *Die Brauerei Wiss. Beil.*, 1951, **4**, 57.
[11] F. Just, W. Schnabel, and S. Ullmann, *Die Brauerei Wiss. Beil.*, 1951, **4**, 71.
[12] A. Champagnat, C. Vernet, B. Lainé, and J. Filosa, Proc. 6th World Petroleum Congress, Frankfurt, 1963, **4**, 259.
[13] G. H. Evans, in 'Single-Cell Protein', ed. R. I. Mateles and S. R. Tannenbaum, p. 243, M.I.T. Press, Cambridge, Mass., 1968.
[14] D. A. B. Llewelyn, in Proc. Conf. on Microbiology, ed. P. Hepple, p. 63, Inst. Petroleum, London, 1968.
[15] B. Lainé, C. Vernet, and G. Evans, Proc. 7th World Petroleum Congress, Mexico, 1967, **8**, 197.

improved it. Amongst these similar developments are the processes of the Gulf Co.,[16] the Japanese companies, Kanegafuchi Chemical, whose process was subsequently licensed to Liquichimica in Italy[17] and Dainippon Ink and Chemicals, whose process was subsequently licensed to Roniprot in Romania,[17] those of the French Institute of Petroleum[18] and Uhde GmbH,[19] both of whom are still seeking potential licencees, and processes developed in the USSR.[20] Detailed technical information concerning these subsequently developed processes is sparse. Alternative technology using bacteria rather than yeasts has been investigated by Exxon in conjunction with Nestlé,[21] but has not been commercialized.

n-Alkane utilization by yeasts has been extensively reviewed by Shennan and Levi,[22] who have provided a fully comprehensive bibliography. The yeasts that have attracted the most commercial interest as potential production cultures for SCP include *Candida lipolytica* (*Endomycopsis lipolytica*), *Candida tropicalis*, and *Torulopsis candida*. Their main disadvantage as production cultures is that most strains have temperature optima within the range 26 to 34 °C and thus, processes based upon them can be expected to present severe cooling problems.

The mechanisms and routes for microbial hydrocarbon oxidation have been reviewed by McKenna and Kallio[23] and Klug and Markovetz.[24] Three mechanisms have been proposed for the initial oxidation of the n-alkane molecule.[25] The first and most widely supported of these is a methyl group hydroxylation mechanism involving the direct incorporation of oxygen, catalysed by a mixed function oxygenase:

$$RCH_2CH_3 + O_2 + NAD(P)H + H^+ \rightarrow RCH_2CH_2OH + NAD(P) + H_2O$$

The second mechanism involves a hydroperoxidation reduction reaction:

$$RCH_2CH_3 + O_2 \rightarrow RCH_2CH_2OOH$$
$$\xrightarrow{NAD(P)H} RCH_2CH_2OH + NAD(P) + H_2O$$

The third mechanism involves dehydrogenation with no oxygen requirement:

$$RCH_2CH_3 + NAD(P)^+ \rightarrow RCH{=}CH_2 + NAD(P)H + H^+$$
$$RCH{=}CH_2 + H_2O \rightarrow RCH_2CH_2OH$$

The subsequent oxidative pathways are essentially conventional.

[16] P. G. Cooper, R. S. Silver, and J. P. Boyle, in SCP II, ed. S. R. Tannenbaum and D. I. C. Wang, p. 454, M.I.T. Press, Cambridge, Mass., 1975.
[17] J. C. Senez, in 'Proc. Regional Seminar on Microbial Conversion Systems for Food and Fodder Production and Waste Management', ed. T. G. Overmire, p. 83, KISR, Kuwait, 1977.
[18] D. Ballerini, *Rev. Inst. Franç. Pétrole*, 1978, **33**, 101.
[19] J. W. Birckenstaedt, U. Faust, and W. Sambeth, *Process Biochem.*, 1977, **12**(9), 7.
[20] S. V. Chepigo, I. D. Boiko, A. D., Gololobov, A. P. Kryuchkova, G. I. Vorobyeva, P. N. Fisher, V. K., Pokrovski, and N. I. Korotchenko, Proc. 7th World Petroleum Congress, Mexico, 1967, **8**, 205.
[21] J. G. McNab and L. R. Rey, Paper to Am. Assoc. for Advancement of Science, Washington, D.C., December 1966.
[22] J. L. Shennan and J. D. Levi, *Prog. Indust. Microbiol.*, 1974, **13**, 1.
[23] E. J. McKenna and R. E. Kallio, *Ann. Rev. Microbiol.*, 1965, **19**, 183.
[24] M. J. Klug and A. J. Markovetz, *Adv. Microbial Physiol.*, 1971, **5**, 1.
[25] W. R. Finnerty, *Trends Biochem. Sci.*, 1977, **2**, 73.

From the economic viewpoint, the yield coefficient for yeasts growing on n-alkanes is of paramount importance. It has been reported that for n-alkane chain lengths from C_{12} to C_{18} the yield coefficient is 1.0, but for chain lengths below this there is a marked and progressive reduction.[26] This, of course, applies to yeasts isolated for growth on the C_{12} to C_{18} range of n-alkanes, and is not necessarily the case for micro-organisms isolated for their capability to grown on short chain n-alkanes.

The present status with respect to the commercialization of SCP production from n-alkanes in Western Europe is that the British Petroleum Co. have constructed three plants; a 16 000 tpa plant at Lavera, France, using the gas oil process, where production has been discontinued, a 4000 tpa demonstration plant at Grangemouth, Scotland, using the purified n-alkane process, and a 100 000 tpa plant at Sarrouch in Sardinia (jointly with ANIC) based on purified n-alkane technology where production has also been discontinued, but not for technological reasons. A 100 000 tpa plant has also been built by Liquichimica, using Kanegafuchi purified n-alkane technology, in Reggio di Calabria, Italy, but production has not commenced.

4 Yeast and Bacterial Growth on Methanol

Until relatively recently, methanol received little more than cursory attention as a growth substrate for micro-organisms, although during the last 50 years, several bacteria capable of growth on methanol as their sole carbon energy substrate have been identified. These include *Pseudomonas extorquens*,[27] *Protaminobacter ruber*,[28] *Hyphomicrobium vulgare*,[29] and a range of other *Pseudomonas* spp.[30,31,32] which have been subjected to extensive biochemical investigations. Quayle[33] has comprehensively reviewed most of these biochemically oriented studies. In contrast, remarkably few studies have been directed towards the growth characteristics of methanol-utilizing bacteria.

Largely as a result of the stimulus created by the potential of SCP production from methanol, several yeasts capable of growth on methanol have been isolated during the past ten years. These include yeasts classified in the genera *Kloeckera*,[34] *Pichia*,[35] *Hansenula*,[36] *Candida*,[37] and *Torulopsis*.[38] Methanol utilization has also

[26] J. A. Gosling, in 'Proc. Regional Seminar on Microbial Conversion Systems for Food and Fodder Production and Waste Management', ed. T. G. Overmire, p. 97, KISR, Kuwait, 1977.
[27] K. Bassalik, *Jahrb. Wiss. Bot.*, 1914, **53**, 255.
[28] L. E. den Dooren de Jong, *Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg., Abt. II*, 1927, **71**, 193.
[29] W. Mevius, *Arch. Mikrobiol.*, 1953, **19**, 1.
[30] T. Kaneda and J. M. Roxburgh, *Canad. J. Microbiol.*, 1959, **5**, 87.
[31] D. Peel and J. R. Quayle, *Biochem. J.*, 1961, **81**, 465.
[32] C. Anthony and L. J. Zatman, *Biochem. J.*, 1964, **92**, 609.
[33] J. R. Quayle, *Adv. Microbiol. Physiol.*, 1972, **7**, 119.
[34] K. Ogata, N. Nishikawa, and M. Ohsugi, *Agric. Biol. Chem.*, 1969, **33**, 1519.
[35] W. Hazeu, J. C. de Bruyn, and P. Bos, *Arch. Mikrobiol.*, 1972, **87**, 185.
[36] D. W. Levine and C. L. Cooney, *Appl. Microbiol.*, 1973, **26**, 982.
[37] H. Sahm and F. Wagner, *Arch. Mikrobiol.*, 1972, **84**, 29.
[38] H. Asthana, A. E. Humphrey, and V. Moritz, *Biotechnol. Bioeng.*, 1971, **13**, 923.

been reported as a characteristic of three fungi.[39] The growth of yeasts on methanol has been the subject of a recent comprehensive review by Sahm.[40] All methanol-utilizing yeasts so far isolated have proved to be nutritionally fastidious, having a requirement for thiamin and biotin.

Probably the two most important factors that are encountered when micro-organisms are grown on methanol are the inhibitory nature of methanol to methanol-utilizing micro-organisms and the uncoupling of growth from the oxidation of methanol under conditions where significant methanol concentrations are present in the growth medium.[5]

The growth of micro-organisms on either methanol or other C_1 compounds requires that the micro-organisms are able to generate energy and reduction equivalents from the compounds, and that they possess the metabolic pathways for synthesis of a C_3 skeleton from which a central intermediary metabolite such as pyruvate or phosphopyruvate may be derived. Once synthesis of such central intermediary metabolites is accomplished, there is no evidence to suggest that the main pathways leading from the C_3 skeleton to the main groups of cell constituents will be different from the well established pathways of intermediary metabolism found in other micro-organisms.

According to Quayle,[41] there are three main pathways which accomplish net synthesis of a C_3 skeleton from C_1 compounds in bacteria. These are:

(i) The ribulose diphosphate or Calvin cycle for carbon dioxide fixation;

(ii) The ribulose monophosphate cycle for formaldehyde fixation of which three variants exist;[42]

(iii) The serine pathway for formaldehyde fixation of which two variants have been proposed.[42]

The ribulose monophosphate cycle and the serine pathway are represented schematically in Figures 3 and 4. Methanol-utilizing bacteria that use the

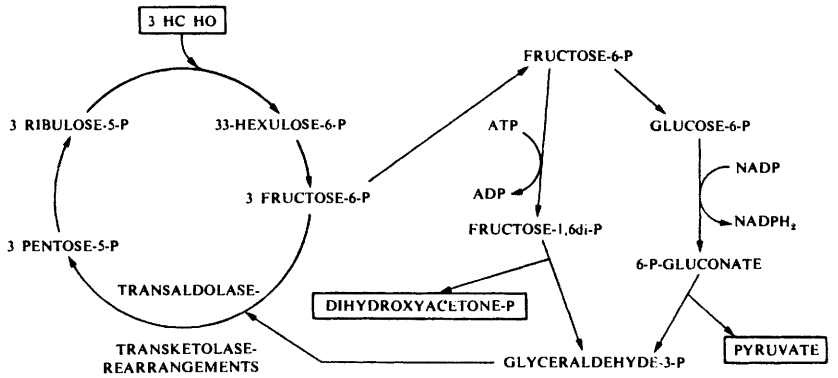


Figure 3 *Ribulose Monophosphate Cycle for Formaldehyde Fixation*[40]

[39] K. Sakaguchi, R. Kurane, and M. Murata, *Agric. Biol. Chem.*, 1975, **39**, 1695.
[40] H. Sahm, *Adv. Biochem. Eng.*, 1977, **6**, 77.
[41] J. R. Quayle, 'Proc. Internat. Symp. on Growth on C_1-Compounds', p. 59, Tokyo, 1974.
[42] J. P. van Dijken, Doctoral dissertation, Univ. of Groningen, pp. 122, 1976.

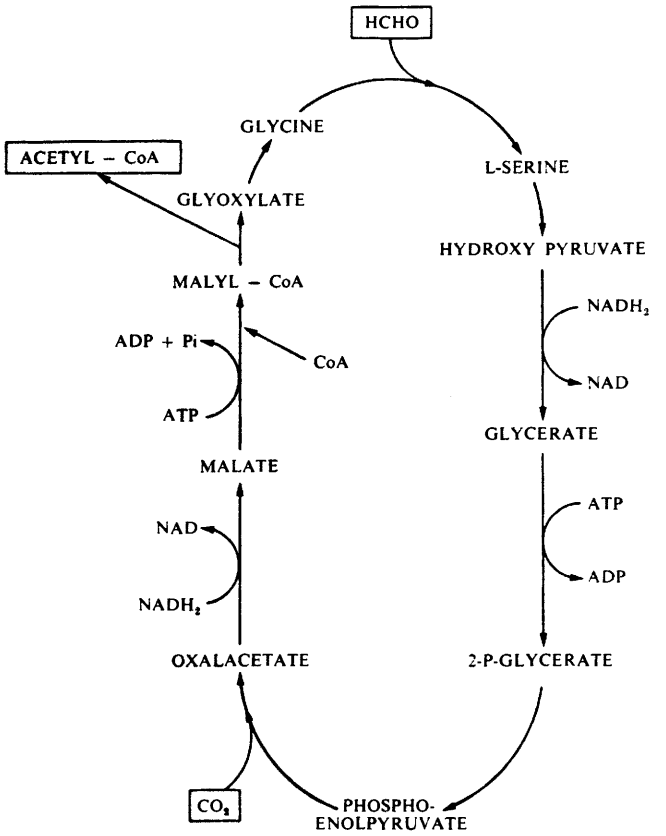


Figure 4 *Serine Pathway for Formaldehyde Fixation*[40]

ribulose monophosphate cycle include *Pseudomonas methylotropha*[43] and some of the non-methane-utilizing obligate methylotrophs[44] whilst the serine pathway is used by *Pseudomonas* AM1, *Pseudomonas extorquens*, and *Hyphomicrobium* spp.

It has been proposed[40] that methanol-utilizing yeasts oxidize methanol using the pathway represented schematically in Figure 5. There is also evidence that suggests that some yeasts use the dissimilatory ribulose monophosphate cycle for oxidation of formaldehyde to carbon dioxide.

For methanol-utilizing micro-organisms, yield coefficient data vary considerably for reasons mentioned earlier. In fact, it is invalid to compare yield coefficients determined in batch culture with those determined in methanol-limited

[43] D. Byron and J. C. Ousby, 'Proc. Internat. Symp. on Microbial Growth on C_1-Compounds', p. 23, Tokyo, 1974.
[44] T. E. Patt, M. O'Connor, G. C. Cole, R. Day, and R. S. Hanson, 'Proc. Conf. on Microbial Production and Utilization of Gases', p. 317, Göttingen, 1977.

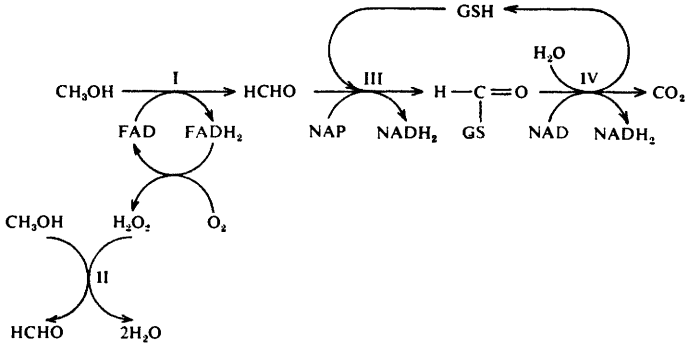


Figure 5 *Pathway for Methanol Oxidation by Yeasts.*[40]
(I = Alcohol Oxidase, II = Catalase, III = Formaldehyde Dehydrogenase, IV = Formate Dehydrogenase)

continuous culture. For the commercial production of SCP from methanol, it will be essential to achieve a yield coefficient in excess of 0.45. In the literature a yield coefficient of 0.54 has been reported by Battat *et al.*[45] for *Pseudomonas* C growing in continous culture at 35 °C, one of 0.54 by Harrison *et al.*[46] for a mixed bacterial culture growing in continuous culture at 42 °C and one of 0.45 by Asthana *et al.*[38] for *Torulopsis glabrata* growing at 30 °C. Other reported figures fall below 0.45.

The status of commercialization of SCP manufacturing processes from methanol is encouraging. The most advanced process is that of Imperial Chemical Industries,[47] who presently have a plant of between 54 000 and 70 000 tonnes of bacterial SCP per annum under construction at Billingham, England. Other processes that could potentially be commercialized in the relatively near future are those developed by the Hoechst–Uhde partnership[48] in West Germany, a bacterial route, the yeast routes of Mitsubishi Gas Chemical[49] in Japan and of the French Institute of Petroleum[50] in France, and perhaps the least discussed of all the developments, the Norprotein Group's bacterial route developed in Scandinavia. At present methanol based SCP production seems the most likely of the hydrocarbon or petrochemical based routes to gain widespread acceptance and application.

5 Yeast Growth on Ethanol

The widespread introduction of petrochemical routes for ethanol production, by

[45] E. Battat, I. Goldberg, and R. I. Mateles, *Appl. Microbiol.*, 1974, **23**, 906.
[46] D. E. F. Harrison, T. G. Wilkinson, S. W. Wren, and J. H. Harwood, in 'Continuous Culture 6: Applications and New Fields', ed. A. C. R. Dean, D. C. Ellwood, C. G. T. Evans, and J. Melling, p. 122, Ellis Horwood, Chichester, 1976.
[47] J. S. Gow, J. D. Littlehailes, S. R. L. Smith, and R. B. Walter, in 'SCP II', ed. S. R. Tannenbaum and D. I. C. Wang, p. 370, M.I.T. Press, Cambridge, Mass., 1975.
[48] U. Faust, P. Präve, and D. A. Sukatsch, *J. Ferment. Technol.*, 1977, **55**, 609.
[49] M. Kuraishi, H. Ohkouchi, N. Matsuda, and I. Terao, 'Paper to 2nd Internat. Symp. on Microbial Growth on C_1-Compounds', Pushino, USSR, July 1977.
[50] D. Ballerini, *Rev. Inst. Franç. Pétrole*, 1978, **33**, 11.

the hydration of ethylene, very largely eliminated the commercial production of ethanol by fermentation, although recently, as the prices of hydrocarbon feedstocks have soared, the reintroduction of commercial alcohol production by fermentation has occurred in the USA and Brazil, as a result of these two countries massive production of suitable carbohydrate feedstocks of agricultural origin.

A wide range of yeasts and bacteria are able to grow on ethanol as their sole carbon energy substrate, including yeasts that are approved as feed components and food additives, and further, the ethanol used as feedstock for yeast production can also satisfy food quality requirements. Thus, SCP production from ethanol offers a route which, even for a human food product, does not, for product registration purposes, require extensive testing of the product's nutritional and toxicological characteristics. Amoco Food Company in the USA have adopted this approach using *Candida utilis* as the production micro-organism. Some other organizations have investigated similar approaches.[51]

Several research papers concerning scientific aspects of yeast growth on ethanol have been published, including continuous culture studies of the growth of *Saccharomyces cerevisiae* under both steady-state[52] and transient-state conditions,[53] modelling and parameter estimation studies[54] and biomass production studies with *C. utilis*.[55]

Recently, it has been proposed[56] that synthetic ethanol should be used to supplement the utilizable carbon for yeast production from sulphite liquors, thus overcoming the limitation of fermenter productivity generally encountered with waste streams.

It seems unlikely that further processes for SCP production from ethanol will be developed. However, ethanol is an intermediate in the metabolic pathways for the breakdown of other potential feedstocks and hence, ethanol-utilizing yeasts and bacteria may still have a role to play in integrated SCP production processes in the future.

6 Bacterial Growth on Methane and Natural Gas

The first reports of microbial methane oxidation appeared more than 70 years ago. Virtually simultaneously, Kaserer[57] reported the existence of methane-utilizing micro-organisms, and Söhngen[58] described the isolation from nature of the bacterium *Bacillus methanicus*, subsequently described by Jensen[59] as *Methanomonas methanica*, discussed its taxonomy and reported the results of

[51] Y. Masuda, *Chem. Econ. Eng. Rev.*, 1974, **6**(11), 54.
[52] J. R. Mor and A. Fiechter, *Biotechnol. Bioeng.*, 1968, **10**, 159.
[53] J. R. Mor and A. Fiechter, *Biotechnol. Bioeng.*, 1968, **10**, 787.
[54] A. Prokop, J. Votruba, M. Sobotka, and J. Panoš, *Biotechnol. Bioeng.*, 1978, **20**, 1523.
[55] F. Machek, F. Štros, A. Prokop, and L. Adámek, in 'Continuous Culture 6: Applications and New Fields', ed. A. C. R. Dean, D. C. Ellwood, C. G. T. Evans, and J. Melling, p. 135, Ellis Horwood, Chichester, 1976.
[56] M. Rychtera, J. Barta, A. Fiechter, and A. A. Einsele, *Process Biochem.*, 1977, **12**(3), 26.
[57] H. Kaserer, *Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg., Abt. II*, 1905, **15**, 573.
[58] N. L. Söhngen, *Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg., Abt. II*, 1905, **15**, 513.
[59] O. Jensen, *Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg., Abt. II*, 1909, **22**, 305.

growth experiments where the bacterium utilized methane as its sole carbon energy source. Many of the laboratory techniques necessary for the cultivation and handling of methane-utilizing bacteria were not developed until relatively recently. In fact, only isolated papers, such as, for example, those by Aiyer[60] and Hutton and ZoBell[61] referred to studies of microbial methane utilization during the next fifty years. It was not until Foster and his co-workers started to publish the results of their studies with methane and other gaseous hydrocarbons in 1956 that any marked advances beyond the observations of Kaserer and Söhngen, were made. Foster[62] reviewed much of his own and his colleagues' work in his A. J. Kluyver Memorial Lecture. However, for comprehensive discussion of the early studies concerning microbial methane-oxidation, the reviews of Silverman[63] and Coty[64] should be consulted.

Foster's three major findings that were particularly applicable to SCP production from natural gas were:

(i) The definition of the route whereby methane is oxidized by bacteria to form biomass and carbon dioxide—$CH_4 \rightarrow CH_3OH \rightarrow HCHO \rightarrow HCOOH \rightarrow CO_2$ thus establishing methanol as an intermediate;[65]

(ii) The discovery that the methane-oxidizing bacterium, *Pseudomonas methanica*, was capable of co-oxidizing other gaseous alkanes;[66]

(iii) The isolation of the thermophilic methane-oxidizing bacterium, *Methylococcus capsulatus*.[67]

One other important discovery with respect to methane utilization was that the methane-utilizing bacterium, *Pseudomonas methanitrificans*, was able to fix dinitrogen.[68]

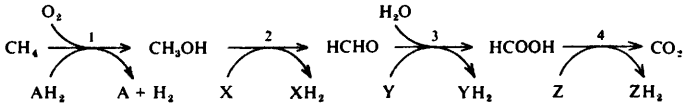
Occasional papers have suggested that micro-organisms other than bacteria are able to utilize methane for growth,[69,70] but no independently authenticated reports of methane utilization by micro-organisms other than bacteria exist.

The first reports suggesting methane as a potential carbon energy substrate for SCP production were published by Wolnak *et al.*[71] and Hamer *et al.*[72] and, in the same year, some important data concerning cell yields for bacteria grown on methane were published by Vary and Johnson.[73]

[60] P. A. S. Aiyer, *Mem. Dept. Agric., India, Chemical Ser.*, 1920, **5**, 173.
[61] W. E. Hutton and C. E. ZoBell, *J. Bacteriol.*, 1949, **58**, 463.
[62] J. W. Foster, *Antonie van Leeuwenhoek J. Microbiol. Serol.*, 1963, **28**, 241.
[63] M. P. Silverman, U.S. Dept. of the Interior Bureau of Mines Information Circular, IC 8246, pp. 37, 1964.
[64] V. F. Coty, *Biotechnol. Bioeng.*, 1967, **9**, 25.
[65] M. Dworkin and J. W. Foster, *J. Bacteriol.*, 1956, **72**, 646.
[66] E. R. Leadbetter and J. W. Foster, *Arch. Mikrobiol.*, 1958, **30**, 91.
[67] J. W. Foster and R. H. Davis, *J. Bacteriol.*, 1966, **91**, 1924.
[68] J. B. Davis, V. F. Coty, and J. P. Stanley, *J. Bacteriol.*, 1964, **88**, 468.
[69] L. Enebo, *Acta Chem. Scand.*, 1967, **21**, 625.
[70] J. E. Zajic, B. Volesky, and A. Wellman, *Canad. J. Microbiol.*, 1969, **15**, 1231.
[71] B. Wolnak, B. H. Andreen, J. A. Chisholm, and M. Saadeh, *Biotechnol. Bioeng.*, 1967, **9**, 57.
[72] G. Hamer, C.-G. Hedén, and C.-O. Carenberg, *Biotechnol. Bioeng.*, 1967, **9**, 499.
[73] P. S. Vary and M. J. Johnson, *Appl. Microbiol.*, 1967, **15**, 1473.

The growth of micro-organisms on methane requires the micro-organisms to generate both energy and reduction equivalents, and at the same time, they must also possess metabolic pathways for the synthesis of a C_3 skeleton from which a central intermediary metabolite such as either pyruvate or phosphopyruvate may be derived.

The currently accepted view on oxidation of methane by bacteria can be summarized by the following series of reactions:



The nature of the electron donor AH_2 and of the acceptors X and Y are uncertain, and the energy yield of the oxidation of methane to carbon dioxide is unknown, although it is now considered possible to estimate a maximum limit for the energy yield for this sequence of reactions.[33]

According to Quayle,[41] there are three main pathways which accomplish net synthesis of the C_3 skeleton in C_1-utilizing bacteria. These were listed earlier in the section dealing with methanol. Methane utilizing bacteria employ variants of the ribulose monophosphate cycle and the serine pathway in order to assimilate methane.

On the basis of energy requirements, it has been predicted[74] that a bacterium having the ribulose monophosphate cycle as its main path of carbon assimilation should show a higher yield on methane than one with the serine pathway. Proof of this prediction is, of course, extremely difficult, as it is most unlikely that optimal growth with maximum yield coefficients of species exhibiting the two alternatives will occur under similar growth conditions and at identical growth rates. Neither the yield coefficient data for methane-utilizing bacteria reviewed by Hamer and Norris[75] nor much of the subsequently published data could stand rigorous examination. Details of appropriate techniques for accurate yield coefficient measurements for methane utilizing bacteria have recently been published by Barnes *et al.*[76]

In most studies concerned with the production of SCP, a great deal of emphasis is placed on the carbon energy substrate, and very little emphasis is placed on the nitrogen substrate, even though nitrogen is such an important constituent of proteins. Most proposed routes for SCP production employ either ammonia or ammonium salts as the nitrogen source for growth. The versatility of methane-utilizing bacteria with respect to their nitrogen metabolism exceeds, by far, that of most other micro-organisms proposed for SCP production. Some methane-utilizing bacteria are able to utilize either ammonia or nitrate and fix di-nitrogen

[74] J. P. van Dijken and W. Harder, *Biotechnol. Bioeng.*, 1975, **17**, 15.
[75] G. Hamer, and J. R. Norris, 'Proc. 8th World Petroleum Congress, Moscow', 1971, **5**, 133.
[76] L. J. Barnes, J. W. Drozd, D. E. F. Harrison, and G. Hamer, 'Proc. Conf. on Microbial Production and Utilization of Gases', Göttingen, p. 301 (1977).

to supply their nitrogen requirements. Of course, in a production process the appropriate decision with respect to nitrogen source can be critical from the economic viewpoint. For a *Methylococcus* sp. growing in continuous culture on methane at 45 °C with ammonia as the nitrogen source, the yield coefficient was reported to be 0.85, whilst for di-nitrogen as nitrogen source, the yield coefficient was only 0.65, and the value in the case of nitrate was between the two.[77] Although the use of ammonia will enhance the yield coefficient and hence the economics of the process, ammonia is also a competitive inhibitor of methane oxidation in methane-utilizing bacteria[78] as are other hydrocarbons in natural gas and may, therefore, adversely affect culture stability.

Without doubt, the solution of the scientific and technological questions concerning the conversion of methane into SCP has been more demanding than the solution of similar problems for other potential production routes employing either hydrocarbon or petrochemical feedstocks and now that most of these problems are satisfactorily solved it is unfortunate that the methane route for SCP production is no longer being developed because of the short sightedness in the economic evaluation techniques used to assess its potential. On a world-wide, and in some cases on a national scale, the quantities of natural gas that are flared as waste are still considerable.

7 Photosynthetic Growth of Algae and Bacteria

The photosynthetic routes for SCP production have been extensively studied for many years. However, they have rarely been subjected to rigorous development studies oriented towards the establishment of economically optimized production systems. With photosynthetic systems, the emphasis has usually been placed on the efficiency of conversion of the light energy rather than on the maintenance of high algal concentrations which make the cultivation and harvesting operations more compatible.

The economic bulk production of biomass demands the use of solar energy rather than artificial light sources, and, although it is desirable to harness as much as possible of the available solar energy, this should not be done at the expense of reducing the algal concentration in the production system.

The physico-chemical factors affecting the metabolism and growth rate of photosynthetic algae have been reviewed by Soeder and Stengel.[79] In general, it is these factors, particularly light intensity, temperature, pH, salt content of the medium, and carbon dioxide concentration, that influence the growth process and, for high productivities, it is necessary to develop cultivation systems where such physico-chemical factors can be fully optimized.

The cultivation of algae in continuous culture was first examined by Myers and

[77] G. Hamer, in 'Proc. Regional Seminar on Microbial Conversion Systems for Food and Fodder Production and Waste Management', ed. T. G. Overmire, p. 109, KISR, Kuwait, 1977.

[78] J. W. Drozd, B. Khosrovi, J. Downs, M. L. Bailey, L. J. Barnes, and J. D. Linton, Paper to 7th Internat. Continuous Culture Symp., Prague, July 1978.

[79] C. Soeder and E. Stengel, in 'Algal Physiology and Biochemistry, Botanical Monographs', ed. W. D. P. Stewart, Vol. 10, p. 714, Univ. of California Press, Berkeley, 1974.

Clark.[80] Their growth characteristics have generally been considered to be markedly different from those of non-photosynthetic micro-organisms, particularly in continuous culture. It has been denied that the conventional Monod theory[3] is applicable to their growth,[81] but recently, Watts-Pirt and Pirt[82] have examined this question in detail and have challenged this view. When micro-organisms are grown in continuous culture under carbon energy substrate limitation, with all other nutrients in excess, the protein content of the cells is maximized and the production of storage products minimized, whilst under growth conditions where the carbon energy substrate is in excess and growth is limited by another nutrient, there is a tendency for intracellular storage products to be formed. For the photo-autotrophic growth of algae, the carbon source (carbon dioxide) and the energy source (light) are separate and, therefore, growth can occur under either carbon or energy limitation. Under energy limitation, with an excess of carbon dioxide available, storage products such as starch are produced in algae. By accounting for starch formation, Watts-Pirt and Pirt[82] have shown that for the continuous cultivation of *Chlorella vulgaris*, the Monod theory for growth in continuous culture describes the growth.

The three species of algae that have been subjected to the most extensive investigations with respect to their potential for protein production are *Chlorella*, *Scenedesmus*, and *Spirulina*. In fact, for many years, the mass culture of micro-algae was virtually limited to the cultivation of *Chlorella* spp. as witnessed in the volume edited by Burlew[83] and the review of Wassink.[84] Czechoslovak work[85] stimulated interest in *Scenedesmus* spp., whilst the interest in the cultivation of *Spirulina* spp.[86] results largely from the work undertaken at the French Institute of Petroleum and co-operating laboratories.

The photo-autotrophic production of algae on an industrial scale has developed only slowly during the past three decades, in spite of supposedly promising results obtained in both laboratory and pilot plant operations prior to 1950. Two major philosophies with respect to production systems have developed. In the first the algae are grown in a reactor designed specifically for algae production and in the second, the cultivation is performed in a natural or semi-natural water body where little possibility of rigorous environmental control exists. Because of the nature of photo-autotrophic growth, both systems have large free surface areas relative to their volumes, which tends to enhance both evaporation from and contamination of the system.

Unlike other SCP production processes, photo-autotrophic systems are affected by seasonal and diurnal changes in energy supply. The diurnal sequence subjects the growing culture to both cyclical and unsteady-state conditions, whilst

[80] J. Myers and L. B. Clark, *J. Gen. Physiol.*, 1944, **28**, 103.
[81] N. Nyholm, *Biotechnol. Bioeng.*, 1976, **18**, 1043.
[82] M. Watts-Pirt and S. J. Pirt, *J. Appl. Chem. Biotechnol.*, 1977, **27**, 643.
[83] J. S. Burlew, in 'Algal Cultivation from Laboratory to Pilot Plant', ed. J. S. Burlew, p. 3, Carnegie Instn. Publ. 600, Washington, D.C., 1963.
[84] E. C. Wassink, in 'Autotrophic Micro-organisms', Proc. 4th Symp. Soc. Gen. Microbiol., London, p. 247, Cambridge Univ. Press, Cambridge, 1954.
[85] I. Šetlík, Paper to IBP Meeting on Novel Protein Sources, Warsaw, August, 1966.
[86] G. Clément, M. Rebeller, and P. Trambouze, *Rev. Inst. Franç. Pétrole*, 1968, **23**, 702.

in other SCP production systems considerable effort is directed towards the maintenance of steady-state conditions for the growth process.

Probably the best example of a specifically designed reactor system for photo-autotrophic algae production is the inclined plane reactor system developed in Czechoslovakia for the cultivation of *Scenedesmus* spp.[85] A large pilot plant facility with a surface area of 900 m^2 and containing 54 m^3 of algal suspension was constructed at Třeboň in the late 1960's and subsequently a 200 hectare facility was constructed in Bulgaria. This latter facility has been claimed to have a production capacity of 6300 tonnes for a 6 month cultivation season.

In the pilot plant facility the inclination of the plane is 1:30 and it is oriented so as to face towards the south. During operation, the depth of culture on the inclined plane is 50 mm and turbulence is created by submerged glass baffles normal to the flow of culture down the plane. With *Scenedesmus obliques* and *Scenedesmus quadricauda* the maximum operating temperature is 34 °C and, in general, evaporative cooling is sufficient to maintain this. However, at times of high light intensities and temperature, supplementary cooling, using a spray tower, is required. The optimum carbon dioxide concentration in the culture has been reported to be 0.5%, and losses occur because the pH is maintained between 6.5 and 7.0 for contamination control reasons. Carbon dioxide is transferred to the culture by passing a portion of the culture through a spray absorption tower. During the night and during rain the culture is stored in tanks to prevent cooling in the first instance and dilution in the second. Mechanical centrifuges are used for the separation of the algae. The concentration of algae in suspension is maintained between 1.5 and 2.0 g l^{-1}. The daily average production of dry algae over a 150 day season is 10 g m^{-2}, although a production of 17.5 g m^{-2} is considered possible at the latitude of Třeboň (49 ° N).[87] In the case of the 900 m^2 facility, 10 g m^{-2} represents a productivity, in conventional terms, of only 0.00694 kg m^{-3} h^{-1} and 17.5 g m^{-2} one of only 0.0122 kg m^{-3} h^{-1}, neither of which are particularly impressive.

The cultivation of *Spirulina maxima* as a source of protein has attracted considerable attention for more than a decade. This alga is the predominant species in the phyto-plankton found in surface waters of Lake Chad, and has traditionally been collected for food.[86] Its filamentous nature permits easy separation from suspension, and it can be sun dried.

The development of technology for *Spirulina* production has been carried out, very largely, by the French Institute of Petroleum and co-operating organizations in France, Algeria, Mexico, Egypt, and elsewhere.[88] As in the case of Třeboň, productivities for *Spirulina* assessed in the conventional way for SCP in either pilot plant[86] or modified natural systems[89] are relatively unimpressive.

The 'energy crisis' in industrialized countries has resulted in renewed interest with respect to algal cultivation systems for energy capture and conservation.

[87] L. Enebo, *Chem. Eng. Progr. Symp. Ser.*, 1969, 65(93), 80.
[88] G. Clément, *Rev. Inst. Pasteur, Lyon*, 1971, 4, 103.
[89] G. Clément, in 'Single Cell Protein II', eds. S. R. Tannenbaum and D. I. C. Wang, p. 467, M.I.T. Press, Cambridge, Mass., 1975.

Goldman and Ryther[90] have discussed the energy balance in and the efficiency of such systems and Oswald[91] has examined algal systems for nutrient recovery from waste water recycle systems where the algae can be used either for animal feed or as a fertilizer.

In most algal biomass production systems solar energy utilization is usually considered to be 5 to 6% at the best. A recent claim by Pirt[92] suggests that this value could be 15 to 16%, and if this is subsequently verified, it could have major repercussions with respect to the potential of biomass production using algal systems.

Finally, one other venture that must be mentioned is the proposed use of the photosynthetic bacterium *Rhodopseudomonas gelatinosa* for SCP production from agricultural by-products,[93] again indicating the wide range of possible alternate routes for production that are available for consideration.

8 Bacterial Growth on Carbon Dioxide and Hydrogen

The concept of producing SCP by the cultivation of aerobic, facultative, chemolithotrophic, hydrogen-oxidizing bacteria or Knallgasbacteria,[94] the German name by which they are widely known, was developed much more as a bioregenerative system for space-craft[95] rather than as a presently economic means of producing SCP as an animal feed ingredient, although in the latter context it has been presented as a method for producing food from electricity.[96]

Knallgasbacteria were first isolated more than 70 years ago by Kaserer.[97] The representative genus of these bacteria is *Hydrogenomonas* and most of the studies concerned with the growth of these bacteria have used either *H. eutropha* or *Hydrogenomonas* H16, both of which are mesophilic bacteria with an optimum growth temperature within the range 30 to 35 °C. A thermophilic strain, *H. thermophilis*, with a temperature optimum for growth of 50 °C, has been isolated,[98] but this occurred after much of the work concerning the growth of *Hydrogenomonas* spp. had been terminated.

The formation of biomass from hydrogen, oxygen, and carbon dioxide is usually summarized by the equation:[99]

$$6H_2 + 2O_2 + CO_2 \rightarrow (CH_2O) + 5H_2O$$

[90] J. C. Goldman and J. H. Ryther, in 'Biological Solar Energy Conversion', eds. A. Mitsui, S. Miyachi, A. San Pietro, and S. Tamura, p. 367, Academic Press, New York, 1977.
[91] W. J. Oswald, in 'Proc. Regional Seminar on Microbial Conversion Systems for Food and Fodder Production and Waste Management', ed. T. G. Overmire, p. 213, KISR, Kuwait, 1977.
[92] S. J. Pirt, private communication, September 1978.
[93] R. H. Shipman, I. C. Kao, and L. T. Fan, *Biotechnol. Bioeng.*, 1975, **17**, 1561.
[94] H. G. Schlegel, *Adv. Comp. Physiol. Biochem.*, 1966, **2**, 185.
[95] L. H. Bongers, *Develop. Industr. Microbiol.*, 1964, **5**, 183.
[96] H. G. Schlegel, in 'Fermentation Advances', ed. D. Perlman, p. 807, Academic Press, N.Y., 1969.
[97] H. Kaserer, *Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg., Abt. II*, 1906, **16**, 681.
[98] J. M. McGee, L. R. Brown, and R. G. Fischer, *Nature*, 1967, **214**, 715.
[99] H. G. Schlegel and R. M. Lafferty, *Adv. Biochem. Eng.*, 1971, **1**, 143.

where (CH_2O) represents nitrogen and ash free biomass. More recently[100] the empirical equation:

$$38.7\ H_2 + 4.1\ CO_2 + 14.8\ O_2 + 0.78\ NH_4^+ \rightarrow C_{4.10}H_{6.99}O_{1.73}N_{0.78} + 35.9\ H_2O$$

has been obtained from material balance studies, suggesting rather different proportions of carbon, hydrogen, and oxygen in the cells and hence, an increased requirement for both hydrogen and oxygen relative to carbon dioxide. *Hydrogenomonas* spp. utilize urea as their nitrogen source.

The majority of growth studies concerning *Hydrogenomonas* spp. have been carried out in batch culture systems, although laboratory-scale turbidostatically controlled continuous culture systems have been described by Foster and Litchfield[101] and Bongers.[102] Using such systems Foster and Litchfield[103] failed to report any steady-state data, whilst Bongers,[102] although concentrating primarily on the mineral nutrition of *H. eutrophia*, did report a maximum growth rate in continuous culture of 0.42 h^{-1} and a maximum yield coefficient, based on hydrogen, of 5.6 at a growth rate of 0.31 h^{-1} and a dry weight of bacteria of 3.5 g l^{-1}. The value of the hydrogen based yield coefficient contrasts with a maximum value of 1.4 reported by Kodama *et al.*[100]

Schlegel and Lafferty[99] described both a continuous chemostat and a batch culture system with provision for internal electrolytic oxygen and hydrogen generation for the cultivation of *Hydrogenomonas* H16. In the former system, growth rates did not exceed 0.081 h^{-1} and maximum cell density reported was 3 g l^{-1} on a dry basis.

9 Yeast and Fungal Growth on Carbohydrates

Traditionally, micro-organisms have been grown in both the laboratory and in industry on carbohydrate substrates and most fermentation products are still manufactured from carbohydrate feedstocks even though the literature abounds with reports concerning potential production routes from non-carbohydrate feedstocks.

Fodder yeast was manufactured from carbohydrates, mostly in Germany, during both World Wars as a supplement for animal feeding. In addition, lipid rich food yeast was also produced during the same periods in an attempt to supplement a deficiency of fat in human diets. Much of the early work on fodder yeast was reviewed by Braude[104] and some of the later studies, including work in both the USA and Britain, were discussed by Rose.[105]

In general, fodder yeast production ceased with the end of hostilities, and it must be assumed that the processes developed could only maintain their attractive-

[100] T. Kodama, Y. Igarashi, and Y. Minoda, *Agr. Biol. Chem.*, 1975, **39**, 83.
[101] J. F. Foster and J. H. Litchfield, *Biotechnol. Bioeng.*, 1964, **6**, 441.
[102] L. H. Bongers, *Develop. Industr. Microbiol.*, 1970, **11**, 241.
[103] J. F. Foster and J. H. Litchfield, Proc. Conf. on Bioregenerative Systems, p. 67, NASA$_1$ Washington, D.C., 1968.
[104] R. Braude, *J. Inst. Brew*, 1942, **48**, 206.
[105] A. H. Rose, 'Industrial Microbiology', Butterworths, London, 1961.

ness when strategic arguments rather than a strict economic basis was used to judge them. A few isolated fodder yeast production plants continued to operate in both Europe and the USA, usually as a means for reducing water pollution from sulphite pulp manufacture, whilst in the USSR, new manufacturing capacity has been installed, presumably on the basis of either different economic evaluation procedures or strategic arguments. Fat production by the cultivation of the yeast, *Rhodotorula gracilis* (*Rhodosporidium toruloides*) was discussed by Lundin[106] and Törnqvist and Lundin.[107]

Most of the more recent investigations with respect to SCP production from carbohydrate feedstocks have been concerned with waste carbohydrate streams resulting from process industries based on either agriculture or forestry, although there are exceptions. For most SCP production processes based on waste stream, the most frequent restrictive factor with respect to the economic optimization of the process is that productivity is governed by the carbohydrate concentration in the waste stream, such that fermenter volume is no longer controlled by mass transfer capacity considerations.

One of the most interesting of the new generation of SCP processes based on carbohydrate feedstocks is the Symba Yeast process[108] which was developed in Sweden to handle starch from potato processing, although its application is not restricted to starch from this one source. The basis of the process is the application of a symbiotic mixed culture comprising *Candida utilis* and *Endomycopsis fibuliger*. The *E. fibuliger* produces α- and β-amylases which hydrolyse the starch such that dextrins, which cannot be used by the *C. utilis*, are converted into glucose and maltose which can be utilized. In batch systems, the concentration of dextrins evidently first increases and subsequently decreases, indicating that saccharifying activity is the limiting factor in the production process.[109] Symba yeast production is carried out in the continuous mode using a two-stage fermenter system.[110] Although the first commercial plant operates on waste potato starch with a yeast production of 6 tonnes per day, tropical starch products, such as tapioca, could be potential feedstocks for the process. Recently, a research paper[111] has suggested that instead of producing enzymes *in situ* it might be more appropriate to produce the enzymes under optimum conditions using micro-organisms other than *E. fibuliger* and then grow *C. utilis* on the enzymatically hydrolysed potato waste under conditions optimized for *C. utilis* production. The overall philosophy and projected economics of SCP production from starch have been discussed, relative to other SCP feedstocks, by

[106] H. Lundin, *J. Inst. Brew.*, 1950, **56**, 17.
[107] E. Törnqvist and H. Lundin, *Internat. Sugar J.*, p. 123, May 1951.
[108] M. Tveit in 'Biology and the Manufacturing Industries', ed. M. Brook, p. 3, Academic Press, London, 1967.
[109] K. Jarl, *Food Technol.*, 1969, **23**, 1009.
[110] K. Skogman, in 'Food from Waste', eds. G. G. Birch, K. J. Parker, and J. T. Worgan, p. 167, Applied Science, London, 1977.
[111] R. S. Moreton, *J. Appl. Bacteriol.*, 1978, **44**, 373.

MacLennan,[112] who emphasized the potential for producing agricultural crops specifically as industrial feedstocks.

Another approach to SCP production from carbohydrate feedstocks has been the promotion of an 'intermediate' technology process for developing countries rich in carbohydrate wastes. The essentials of this process are that a filamentous fungi, *Aspergillus niger*, is used as the production micro-organism and cultivation under batch culture conditions are proposed.[113] A major advantage in using filamentous micro-organisms is the ease with which product separation can be achieved. However, any such cost saving must be lost as a result of the intrinsically low overall productivity of batch culture systems. More recently,[114] a *Fusarium* sp. has gained favour as the process micro-organism.

In an alternative SCP production process,[115] based on carbohydrates, but directed towards human food rather than animal feed, the microfungi, *Fusarium graminearum* has been used. This latter process has been developed to use starch, sucrose, or lactose as the feedstock, rather than an ill-defined carbohydrate waste liquor and the production process is operated in the continuous mode. This process again benefits from the ease of product separation and the product produced is intended as an ingredient in textured food.

Another process development involving the use of a filamentous micro-fungi is the Pekilo process in which *Paecilomyces variotii* is used as the process micro-organism and the feedstock comprises the monosaccharide and acetic acid fractions of spent spruce sulphite liquor.[116] The scale of operation of this process is, of course, dependent on the scale of the pulping operation with which it is associated and productivity of the process is limited by the concentration of the feedstock. This process again benefits from the ease of separation of the product; a drum filter is in fact used. It has been evaluated on the pilot plant using a 15 m^3 fermenter and the fermenter size for the first commercial plant is 360 m^3.[117] Unlike some of the other carbohydrate based processes, this process also employs a relatively high operating temperature, 38 °C. The fermenter residence times used are 4.5 to 5.0 h and the biomass concentration obtained ranges from 13 to 16 g l^{-1}, giving a fermenter productivity range of 2.60 to 3.56 kg l^{-1} h^{-1}, which is obviously attractive even though it is feedstock concentration limited.

One final approach to SCP production from carbohydrate feedstocks is the

[112] D. G. MacLennan, in 'Continuous Culture 6: Applications and New Fields', ed. A. C. R. Dean, D. C. Ellwood, C. G. T. Evans, and J. Melling, p. 69, Ellis Horwood, Chichester, 1976.

[113] F. K. E. Imrie and A. J. Vlitos, in 'Single-Cell Protein II', ed. S. R. Tannenbaum and D. I. C. Wang, p. 223, M.I.T. Press, Cambridge, Mass., 1975.

[114] F. K. E. Imrie and R. C. Righelato, in 'Food from Waste', ed. G. G. Birch, K. J. Parker, and J. T. Worgan, p. 79, Applied Science, London, 1977.

[115] C. Anderson, J., Longton, C. Maddix, G. W. Scammell, and G. L. Solomons, in 'Single-Cell Protein II', ed. S. R. Tannenbaum and D. I. C. Wang, p. 314, M.I.T. Press, Cambridge, Mass., 1975.

[116] H. Romantschuk, in 'Single-Cell Protein II'. ed. S. R. Tannenbaum and D. I. C. Wang, p. 344, M.I.T. Press, Cambridge, Mass., 1975.

[117] H. Romantschuk, in 'Continuous Culture 6: Applications and New Fields', ed. A. C. R. Dean, D. C. Ellwood, C. G. T. Evans, and J. Melling, p. 116, Ellis Horwood, Chichester, 1976.

work that has been discussed by Rolz *et al.*[118] in which the cultivation of a *Verticillium* sp. on cane molasses is under investigation.

10 Fungal Growth on Cellulose

Cellulose is by far the most abundant renewable carbon feedstock from which SCP can be manufactured and since the major step change that occurred with respect to hydrocarbon prices in 1973, the interest in cellulose as a feedstock for SCP production has markedly increased. In addition, in many countries, an increased awareness of the pollution created by the production and processing of agricultural and forest products has created incentives for finding ways of economically utilizing the cellulose rich wastes that predominate in these industries. Of course, animal feed protein is only one possible product that can be produced from cellulose; in fact, just as with hydrocarbons, a broad range of conventional chemical products can be envisaged.[119] In the USA alone, cellulosic waste production has been estimated to be approximately 5×10^8 tonnes per annum.[120]

One major source of cellulose rich waste is the manure from intensive livestock production. To avoid public nuisance, it is essential to provide effective treatment and disposal techniques, and when the techniques allow either the utilization or recovery and recycle of valuable materials present in the manure they offer increased attractiveness. Bellamy[121] has considered the possible uses of large volume cellulosic wastes and concluded that conversion into animal feed protein offered both a higher added value and market where demand was increasing, and proposes a technology based on the use of thermophilic cellulolytic bacteria, actinomyces and sporocytophaga strains. A *Thermoactimomyces* sp. growing at 55 °C and derived from the above mentioned study has been further investigated by Humphrey *et al.*[122] The key problem in using cellulose as a feedstock for SCP is the achievement of optimized cell yield and productivity with acceptable levels of cellulose conversion. Experiments indicate maximum cell yields of 0.45 and growth rates of 0.45 h^{-1}.

Some other recent studies have investigated the conversion of all the components of wood, cellulose, hemicelluloses, and lignin, into protein using the white-rot fungus *Sporotrichum pulverulentum*[123] and the submerged cultivation of the edible white-rot fungi, *Phanerochaete chrysosporium*, *Polyporus anceps*, and *Pleurotus sapidus* on tree bark,[124] which is also a major waste material from

[118] C. Rolz, R. Espinosa, S. de Cabrera, O. Maldonado, and J. F. Menchú, in 'Continuous Culture 6: Applications and New Fields', ed. A. C. R. Dean, D. C. Ellwood, C. G. T. Evans, and J. Melling, p. 100, Ellis Horwood, Chichester, 1976.

[119] E. L. Gaden, in 'Proc. Internat. Symp. on SCP', Rome, ed. P. Davis, Academic Press, London, 1974.

[120] A. E. Humphrey, *Biotechnol. Bioeng. Symp. No. 5*, 1975, 49.

[121] W. D. Bellamy, in 'Single-Cell Protein II', ed. S. R. Tannenbaum and D. I. C. Wang, p. 263, M.I.T. Press, Cambridge, Mass., 1975.

[122] A. E. Humphrey, A. Moreira, W. Armiger, and D. Zabriskie, *Biotechnol. Bioeng. Symp. No. 7*, 1977, 45.

[123] K. E. Eriksson, in 'Proc. Regional Seminar on Microbial Conversion Systems for Food and Fodder Production and Waste Management', ed. T. G. Overmire, p. 167, KISR, Kuwait, 1977.

[124] A. J. Daugulis and D. H. Bone, *European J. Appl. Microbiol.*, 1977, **4**, 159.

forestry. A recent development with respect to the utilization of slurried poultry manure for SCP production has been discussed by Shuler *et al.*[125] The problem of repression of uric acid degradation was solved by either using a two-stage fermenter system or non-glucose repressed strains of bacteria and a single-stage fermenter system.

11 Yeast and Fungal Growth on Industrial Wastes

Numerous suggestions concerning the attractiveness of various waste streams as feedstocks for SCP production have been made during the past decade. In general, the vast majority of these have concentrated on wastes from agricultural or forest product processing, although from time to time attention is drawn to the potential of industrial wastes. Most industrial wastes are unsuitable for SCP production for a host of reasons. These include:

(i) Low carbon concentrations;

(ii) Mixed and of variable composition because of unsegregated drainage systems;

(iii) Contain potentially noxious compounds;

(iv) Total production would be insufficient to justify the required product testing programme.

An exception to this is waste water from cyclohexane oxidation[126] which contains a range of mono- and di-carboxylic acids. The process envisaged involves the use of *Candida lipolytica* and *Trichosporon cutaneum* as process micro-organisms. As far as published reports are concerned, this process seems to be an isolated successful example of using a strictly industrial waste, rather than an agro-industrial waste for SCP production.

12 Engineering Considerations

The process engineering problems concerned with the large-scale production of SCP are numerous. In most studies concerning such problems, too much emphasis has been placed on those problems that occur in the fermenter, and it is important to remember than an integrated production process will comprise some six or seven interacting unit operations.

Lainé[127] has reviewed some of the bio-engineering problems occurring in the production of SCP from hydrocarbons. Although this discussion was oriented towards SCP production from liquid n-alkanes, most of the problems discussed apply just as much to processes based on other feedstocks. Topiwala[128] has discussed the interactive nature of the various unit operations involved in SCP production from methane and methanol.

[125] M. L. Shuler, R. E. Austic, and H. W. Seeley, 'Proc. NSF Grantee-Users Conf. on Non-Conventional Proteins and Foods', p. 193, Madison, Wisconsin, 1977.
[126] J. W. Woldendorp, *Chemische Weekblad*, 1971, **67**(1), 13.
[127] B. Lainé, *Canad. J. Chem. Eng.*, 1972, **50**, 154.
[128] H. H. Topiwala, Proc. Internat. Symp. on Microbial Growth on C_1-Compounds, Tokyo, p. 199, 1974.

In order to achieve economy of scale in SCP production, it is essential that large-scale (> 30 000 tonnes per annum) continuous-flow production systems, operating at minimum productivities of between 3 and 6 kg m^{-3} h^{-1}, are employed. In addition, it is also essential that both a high yield coefficient, based on the carbon feedstock, and a high conversion of the carbon feedstock are achieved, and that the process water requirements and aqueous effluent discharge are minimized.

The item of process plant that will vary the most, but not entirely because of differences in the process routes, will be the fermenter. The operating volume of the fermenter or fermenters to be used for any SCP manufacturing venture will be controlled by both the total production required and the optimum productivity of the system, whilst total fermenter volume will depend on factors such as gas hold-up and gas disengagement requirements. The maximum productivity of any particular fermenter design is dependent on its mass and heat transfer capacity, and the maximum productivity will vary according to the carbon feedstock used in the fermenter. The type of reactor employed will depend on the level of gaseous substrate-conversion required and on the relative capital and operating costs of mechanically agitated and sparged/induced flow systems under particular economic environments. Using the methane route, conversion of the carbon feedstock will always be less than complete but, provided a gas-fired dryer is used, exhaust gases from the fermenter can be enriched with further natural gas to provide fuel for the drying operation.

In the design of fermenters for SCP production, mass transfer, heat transfer, and mixing are the most important factors, and a great deal has been written on these subjects. The problems of mass and heat transfer in hydrocarbon-based fermentations was first discussed by Darlington[129] and by Guenther[130] respectively, at a time when fermenter design was such that it seemed unlikely that substantial productivities could become a reality.

The operating costs of the fermenters, in all SCP production processes, represent a considerable proportion of the total operating costs of the process, such that any improvements in the efficiency of energy utilization in the fermenters will have a significant impact on the overall process profitability. The high operating costs for the fermentation step result from a combination of gas compression and broth agitation requirements.

Transmission of energy to the fermentation broth increases the gas-liquid interfacial area and, consequently, in a system where gas–liquid mass transfer is the rate-determining step, increases the reaction rate. The two standard energy sources in fermenters are compressed gas expansion and mechanical agitation. The efficiency of stirring generally exceeds that for compression, suggesting that the cost to transfer a unit mass of gas is less for mechanical agitation than for gas compression and subsequent expansion. However, in these latter systems, if axial dispersion of the gas phase can be minimized, higher driving forces for mass transfer than in mechanically agitated systems where the dispersed gas phase is

[129] W. E. Darlington, *Biotechnol. Bioeng.*, 1964, **6**, 241.
[130] K. R. Guenther, *Biotechnol. Bioeng.*, 1965, **7**, 445.

completely mixed can be achieved with identical power inputs and gas compositions. In systems designed to approach plug flow of the gas phase, there may be problems of nutrient concentration gradients in the liquid phase that will adversely affect the physiology, particularly the yield, of the culture.

It is important, when evaluating the potential performance of various fermenter designs, that they should not necessarily be compared on an equal productivity basis, but at the optimal productivity for each fermenter type. Fermenter conversion efficiency will affect downstream operations.

Fermenter cooling is of less importance than mass-transfer efficiency, but it is still of considerable significance. When any particular process plant is built at an established site, as opposed to greenfield site, it is an important aspect of the economics to be able to utilize existing services, *e.g.* cooling water from the integrated site system and the central facility for aqueous effluent treatment, provided, of course, that the new plant does not overload such services, and that the services are appropriate to the requirements of the new plant.

The use of mesophilic micro-organisms for high productivity industrial fermentations makes cooling, particularly with cooling water rather than refrigerant, a significant problem. The basis for calculating the cooling surface required for a particular design is the minimum temperature difference between the fermentation broth and the cooling water. As this difference decreases the heat exchange surface requirement, and therefore cost, increases. However, operating costs are directly concerned with the amount of cooling water needed, and are calculated on the basis of the average cooling water temperature. Cooling water temperature is dependent on a number of factors which include the origin of the cooling water, the climatic conditions and variations at the plant location, and the type of cooling water system employed, either once-through or recycled with a cooling tower. Use of saline cooling water will result in a need to use materials such as titanium for the heat exchanger surfaces.

Previous paragraphs have dealt with the process engineering problems concerning the fermenter and finally, in this section, some brief comments on medium sterilization, product separation, and drying are necessary. In general, there is a dearth of literature concerning these aspects of SCP production processes.

The question of sterilization of the liquid medium prior to fermentation is a disputed question. Without doubt, some measure of contaminant control or prevention is essential. This can take the form of heat, chemical, or filter sterilization, an essential if a food product is the ultimate objective, or varying levels of protective measures when the product is destined for either a feed or an industrial use. Such protective measures can involve use of an acidic medium, inhibitory intermediate addition with subsequent oxidation by the process micro-organisms in the fermenter, sterilization of medium components but no sterilization of the recycled medium, or the employment of defined structured mixed cultures in which all of the potential ecological niches have been filled by appropriate micro-organisms such that growth of stray contaminant micro-organisms in the reactor is effectively eliminated.

For any process employing a yeast as the process micro-organism, separation of product from the fermentation broth will be markedly easier and probably cheaper than separation of bacteria in those processes where they are used although, in the proposed routes employing yeasts, additional thermal lysis of the cells after separation and prior to drying may be necessary.

With bacterial-based routes, centrifuges are invariably proposed, but after first either flocculating or agglomerating the cells leaving the fermenter. This can be achieved by addition of either a flocculating agent or by adjusting the pH value of the fermentation broth. Care has to be exercised in the selection of flocculating agents for this use, because the agent could become associated with the final product and must, therefore, be free from any adverse toxicological properties. Provided process-water recycle is used, it is probably better to flocculate bacteria by addition of phosphoric acid, a reagent which is a necessary inorganic nutrient for the micro-organism. Centrifugal separators will be required to produce cell creams containing 20% cell dry weight as feed to the dryer. With yeast-based routes, flocculation is much less essential and a decanting operation followed by separation of cells on a drum filter is considered feasible.

With presently available equipment, asepsis during separation operations will be difficult to achieve and this will, of course, have important implications when process water recycle is employed to achieve process water economy. The primary dangers in operating process-water recycle are microbiological contamination of the fermentation, inhibition of the fermentation by recycling products of cell lysis, and inorganic nutrient build-up unless these are very carefully balanced.

For drying of SCP either spray or flash dryers seem to be preferred. The economy of this step in the process is largely dependent on the water content of the cream or slurry fed to the dryer. The higher the solids content the lower will be the fuel requirements for the drying operation. The most important aspect of the drying operation is that no significant heat damage of the product occurs, as this would undoubtedly adversely affect the nutritional value of the product.

Product storage will either be in the bags, which tend to be expensive, or in silos where both humidity and dust control will become important factors to which attention must be paid.

13 Concluding Remarks

In this review, an attempt has been made to be as comprehensive as possible. Obviously, some possible processes and variations have been omitted. The emphasis has been placed exclusively on the production process rather than on the very extensive studies that have been performed to assess the nutritional quality of SCP products.

The extensive research and development programmes that have been directed towards the production of SCP from various feedstocks have resulted in significant changes in fermentation technology, such that it will never be quite the same again even if SCP should fail as a commercially viable product. In the development of the necessary technology for SCP production, fermentation processes

have, virtually for the first time, been subjected to realistic process engineering investigations in addition to appropriate biological investigations. The success of some development programmes is such that it now seems probable that large quantities of SCP will be produced in certain regions of the world.