RESOURCES CONSERVATION BY NOVEL BIOLOGICAL PROCESSES*

I Grow Fats from Wastes

By C. Ratledge
DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF HULL, HULL HU6 7RX

1 Introduction

The world's annual production of oils and fats is about 48 million tons of which nearly 80 % is derived from plant sources (Figure 1). The U.K. imports over 1.1

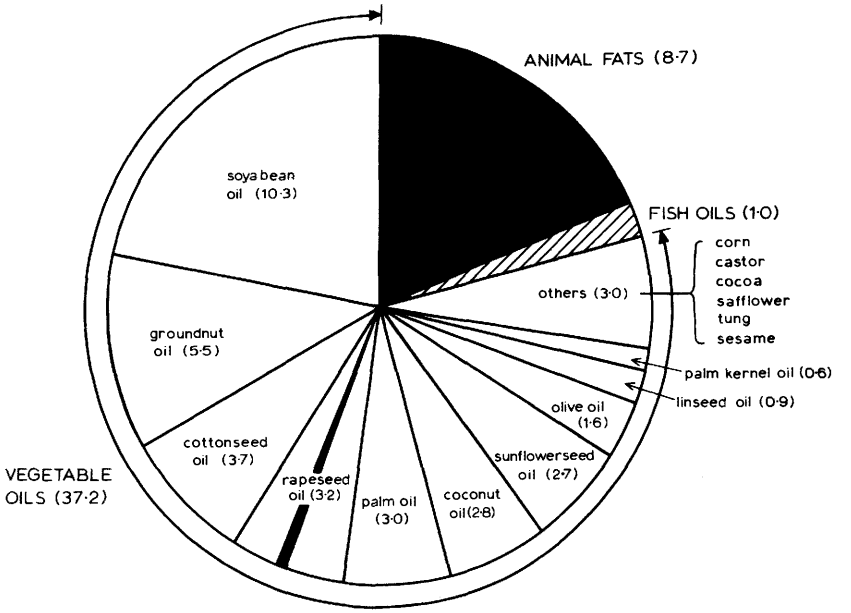

Figure 1 *Annual World production of oils and fats (Dark band in rape seed oil segment is the estimated total U.K. production of this commodity—about* 0.2 million tons)

*These papers were originally presented at a 'Resource Conservation by Novel Biological Processes' symposium, organized by the Chemical Society Industrial Division, Process Technology Group, and held at the Scientific Societies' Lecture Theatre, Savile Row, London W1, on 25th May 1978.

million tons of these commodities, either as such, or as oil seeds for processing here, at a cost in 1977 of about £380 million. This situation is not likely to change in the foreseeable future for, although we are now beginning to produce rape seed as an indigenous crop, the amount of oil which this is likely to yield (up to about 0.2 million ton) will probably be no more than is needed to satisfy the continuing increase in our demands.

Alternative sources of oils and fats should therefore be of considerable national importance though little interest in exploiting micro-organisms for this purpose is evident. This is somewhat surprising as a great deal of effort has gone into thinking about Single Cell Protein (SCP) and the volume of literature on this subject is now so massive that a newcomer to this field must be forgiven if he imagines that this is the sole bulk product that micro-organisms are capable of making.

It is the purpose of this article to try to redress the balance and to illustrate how we might usefully exploit micro-organisms for a bulk product besides protein. One of the major factors in favour of considering Single Cell Oil (SCO) is that a suitable microbial oil could command a much higher price than SCP which perforce must compete against soybean meal (selling at about £150/ton) or, if of a high protein content, against fish meal (at about £320/ton). The prices of oils and fats (see Table 1) vary from £300/ton for palm oil at the bottom of the

Table 1 *Prices of selected oils and fats* (*taken from* 'The Public Ledger')

(£ per metric ton)

	Linseed oil	*Groundnut oil*	*Soybean oil*	*Palm oil*
1974 Jan	465	355	325	215
1975 Jan	475	470	380	300
1976 Jan	375	360	205	185
1977 Jan	410	560	325	350
Dec	275	510	300	280
1978 April	360	570	330	300

April 1978 spot prices of other oils

Lard	£330—£360
Corn oil	£670
Castor oil	£600—£770
Groundnut oil	£750—£850
Olive oil	£1250
Tung oil	£1400

market up to £3000/ton for cocoa butter. Production of an SCO if aimed at the top end of the market, not necessarily cocoa butter which may be difficult to imitate but say at groundnut oil, should therefore be an attractive proposition.

2 The Nature of Microbial Oils

Just as vegetable oils contain a diversity of fatty acids to give the individual commodities their characteristic properties, so also do oils from micro-organisms. The main prerequisites for a micro-organism to be considered suitable for SCO production can be considered to be a combination of the types of fatty acids it contains with its total amount of oil. Table 2 shows twenty of the more prolific fat accumulating yeasts and moulds; other micro-organisms, bacteria, and algae, do not accumulate high levels of lipid and consequently an oleaginous micro-organism must be chosen from yeasts and moulds. Fat contents of up to 70 % of the total cell mass have been recorded in several species and contents over 30 % are quite commonplace.[6,10] The nature of the fatty acids is similar to many plant oils with high contents of oleic acid (18:1), linoleic acid (18:2), and palmitic acid (16:0) being the norm.

Polyunsaturated acids (such as α- or γ-linolenic acid; 18:3) occur in several species of fungi though short chain fatty acids (12:0 and 14:0) are not usually seen in either yeast or mould. The latter acids, which are abundant in palm kernel oil and coconut oil can, however, be found in species of *Entomorphthora*[11,12] though these are not recognized as fat-accumulating organisms. Hydroxy fatty acids, such as ricinoleic acid (12-hydroxyoleic acid) the principal component of castor oil, occur in species of *Claviceps* where both ricinoleic acid and 9,10-dihydroxystearic acid have been recognized.[13,14] Again it is not known whether these species can produce high concentrations of these entities in the laboratory.

Like all naturally-occurring fatty acids, ones of microbial origin are esterified usually as triacylglycerols (triglycerides). The content of triacylglycerols in the lipid of an oleaginous micro-organism may be from 70—90 % depending on the species[6] although, where the nature of the accumulated oil has been extensively examined, it is often found predominately to be composed of this molecular form. Uzuka *et al.*,[15] succeeded in isolating the oil globules, which are the form in which this fat is stored within the microbial cell (see Figure 2), from *Lipomyces starkeyi* and found that 86 % was composed of triacylglycerol with a further 6 % being diacylglycerol and 5 % being free fatty acid.

The distribution of fatty acyl moieties on the glycerol is also similar to that seen in plant oils. The central position (*sn*-2) is usually occupied by an unsaturated acid.[16,17,18] In *L. starkeyi*, however, although the major triacylglyceride was 16:0—18:1—18:1 (at 47 % of total) 16:0—16:0—18:1 was the next most abundant species (at 30 %) indicating a similarity to animal fats.[19] Although this

[10] J. B. Rattray, A. Schibeci, and D. K. Kidley, *Bacteriological Reviews*, 1975, **39**, 197.
[11] D. Tyrrell, *Canad. J. Microbiol.*, 1967, **13**, 755.
[12] D. Tyrrell and J. Weatherston, *Canad. J. Microbiol.*, 1976, **22**, 1058.
[13] P. G. Mantle, *Trans. Br. Mycol. Soc.*, 1972, **52**, 381.
[14] L. J. Morris, *Lipids*, 1968, **3**, 260.
[15] Y. Uzuka, T. Kanamori, T. Koga, K. Tanaka, and T. Naganuma, *J. Gen. Appl. Microbiol.*, 1975, **21**, 157.
[16] R. F. Thorpe and C. Ratledge, *J. Gen. Microbiol.*, 1972, **72**, 151.
[17] J. E. Haley and R. C. M. Jack, *Lipids*, 1974, **9**, 679.
[18] R. M. DeBell and R. C. Jack, *J. Bact.*, 1975, **124**, 220.
[19] T. Suzuki and K. Hasegawa, *Agric. Biol. Chem.*, 1974, **38**, 70.

Table 2 *Oleaginous micro-organisms: the 'top 20' fat-producers*

	Fat content (% w/w)	*Efficiency of fat* production*	*Relative percentage of fatty acid*									*Ref.*
			Saturated				*Unsaturated*				*Others*	
			14	16	18	20	16:1	18:1	18:2	18:3		
Yeasts												
Candida 107	37—44	22	1	36	14	4	1	36	8	tr		1
Cryptococcus terricolus	68	23	—	18	6	—	1	60	12	2	24:0 1%	2, 3
Endomyces vernalis (*Trichosporon pullulans*)	57	—	NOT DETERMINED									4
Lipomyces lipoferus 3—6B	49	—	—	28	—	6	47	7	—	—	17:0 + 17:1 2%	5
Lipomyces starkeyi	65	13	—	40	3	1	7	49	1	—		6
Lipomyces sp.	67	22	1	30	12	—	—	53	3	1		7
Rhodotorula gracilis	74	21	1	31	9	—	—	53	1	5		4, 6
Rhodotorula glutinis glutinis	58	—	2	31	5	—	2	48	11	1		8
Moulds												
Aspergillus nidulans	51	17	tr	18	12	1	4	43	21	tr	20:1 2%	6
Aspergillus ochraceus	48	13	—	38	tr	—	—	15	45	2		6
Aspergillus terreus	57	13	2	23	tr	—	tr	14	40	21		6
Chaetomium globosum	54	—	—	58	8	—	3	27	—	—	17:0 + 17:1 3%	6

Fusarium bulbigenum	50	15	NOT DETERMINED										4
Fusarium sp.	50—52	—	NOT DETERMINED										4
Mortierella vinacea	66	18	NOT DETERMINED										6
Mucor circinelloides	65	14	2	20	25	—	2	40	4	6†	16:2	1%	3, 4, 6
Mucor mucedo	51	5	1	17	11	—	1	31	32	6†			9
Penicillium lilacinum	56	17	tr	16	2	—	3	40	13	—			6
Penicillium spinulosum	64	16	tr	18	12	1	4	43	21	—	—		6
Phythium ultimum	48	—	8	23	7	5	9	22	15	2†	20:1	11%	6

tr = trace
*g fat produced per 100 g substrate
†γ-linolenic acid = 6,9,12 (all *cis*)-octadecatrienoic acid, all other micro-organisms produce 9,12,15 (all *cis*)-octadecatrienoic acid

[1] C. O. Gill, M. J. Hall, and C. Ratledge, *Appl. Environ. Microbiol.*, 1977, **33**, 231.
[2] T. A. Pedersen, *Acta Chem. Scand.*, 1961, **15**, 651.
[3] M. J. Hall and C. Ratledge, unpublished work.
[4] M. Woodbine, *Prog. Indust. Microbiol.*, 1959, **1**, 179.
[5] M. V. Zalashko, V. D. Andreyevskaya, and N. V. Obraztsova, *Priklad. Biokhim. Mikrobiol.*, 1972, **8**, 891.
[6] C. Ratledge, in *Economic Microbiology*, vol. 2, ed. A. H. Rose, Academic Press, London, 1978, p. 263.
[7] D. Watanabe, *Hakko Kyokai-shi*, 1974, **32**, 55; *Nippon Nogei Kagaku Kaishi*, 1975, **49**, 119.
[8] S.-O. Park, *Hanguk Nonghwa Hakhoe Chi*, 1974, **17**, 93.
[9] J. L. Sumner, E. D. Morgan, and H. C. Evans, *Canad. J. Microbiol.*, 1969, **15**, 515.

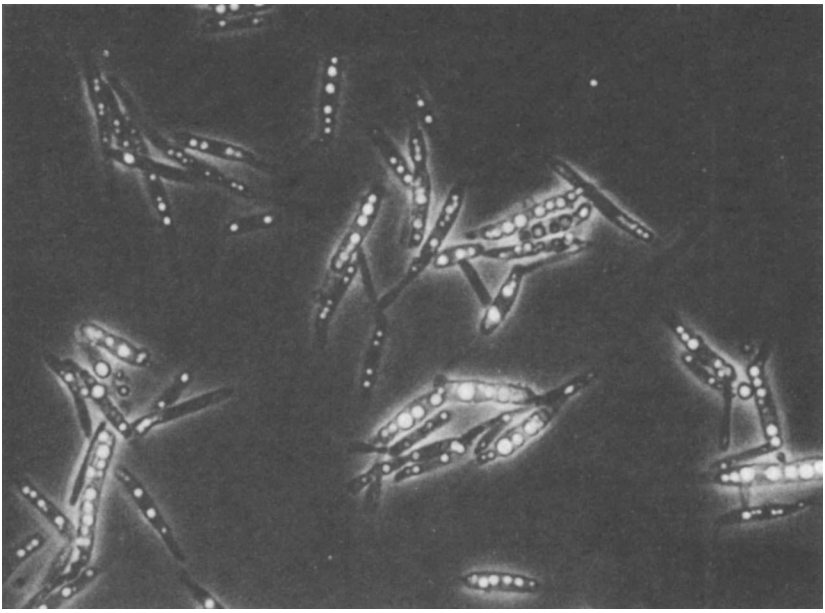

Figure 2 *Phase contrast micrograph of* Candida 107 (*magnification about* 1400 ×) *showing accumulated droplets of oil within each cell. Yeast grown in continuous culture to achieve about* 40% (w/w) *oil content; see text for further details*

seems to be an exception, in view of the paucity of data on this subject it is probably unwise to make too many generalizations until a good many other species have been examined.

3 The Course of Microbial Lipid Accumulation

The true reason for fat accumulation in micro-organisms is not yet known. It could be a production of a reserve storage compound which can then be utilized later to provide energy and carbon for cell maintenance in time of starvation or it could be a means whereby the cell ensured a continuation of metabolism so that intracellular metabolites, in particular NADPH, ATP, *etc.*, do not build up to the general detriment of the cell. Whatever the true biochemical reason, micro-organisms accumulate lipid when a nutrient, other than carbon, becomes exhausted from the growth medium and the excess carbon is then consumed by the cell and ultimately assimilated within the cell in the form of lipid. The process is essentially a two-stage system (see Figure 3A) and has been recognized in most oleaginous yeasts and moulds.

This process of fat accumulation is, though, a 'batch' process. That is, it has a time of starting and a finite time of finishing. It is perhaps thus not immediately apparent how such a two-stage process could be designed to be continuous other

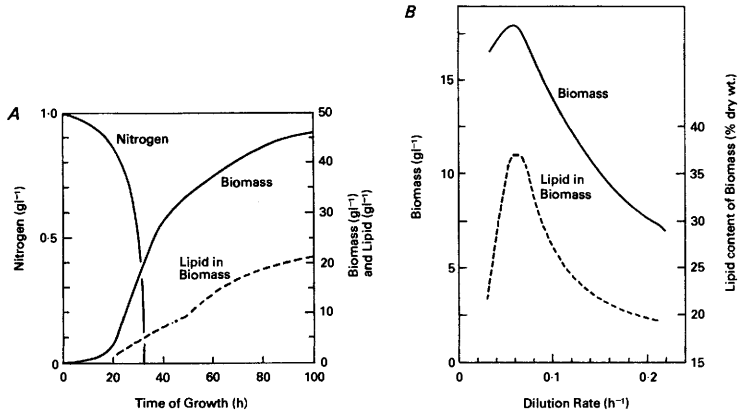

Figure 3 *Patterns of lipid accumulation in micro-organisms. A. in batch-culture; B. in continuous culture using nitrogen limited growth conditions. In the latter case, the dilution rate can be held indefinitely at any rate up to the maximum*

than as a two-stage continuous process as suggested by several groups of workers. This however would be extremely expensive for a potential SCO process.

Continuous culture of micro-organisms is considered to be the most efficacious manner of growing micro-organisms as not only can the environment of the organism be rigorously controlled but also the operating costs of such a process are minimized. It is therefore considered that any SCO process, to stand a chance of being economical, must be a single-stage continuous process.

We have recently shown that such a system can be attained. Using an oleaginous yeast, *Candida* 107, we found that by growing the yeast with nitrogen as the limiting nutrient and at a growth rate (which is controlled by the rate of flow of fresh medium into the chemostat vessel) of about a third of its maximum, lipid was accumulated to the same extent as previously achieved in batch culture (see Figure 3B).[1] A two-stage process, though feasible, offered no advantage over this system.[20] What happened was that we restricted the growth rate and therefore the synthesis of protein and nucleic acid by controlling the cultural conditions. These did not, though, alter the specific rate of lipid synthesis. Hence, as the growth rate of the yeast was lowered by decreasing the flow rate of fresh medium into the fermenter, lipid synthesis became more dominant and the cells, because there is no turnover of this lipid,[21] then accumulated a large amount of lipid in the form of discrete globules (*viz.* Figure 1).

A stable steady-state situation is created in a chemostat and consequently the product has a constant composition over many weeks. The relative proportions of fatty acids in the lipid of *Candida* 107 indeed have been found to be almost unvarying for periods of operation up to 6 months.[1] Under these conditions an

[20] M. J. Hall and C. Ratledge, *Appl. Environ. Microbiol.*, 1977, **33**, 577.
[21] P. A. Botham and C. Ratledge, *J. Gen. Microbiol.*, 1979, **114**, 361.

efficient utilization of the substrate takes place and conversions of glucose to lipid of up to 22 % (w/w) yield have been achieved with *Candida* 107.

Continuous culture, because of its very nature of ensuring a constant growth rate, is the ideal way to study influences of the growth conditions on an organism. Although many studies have been made on the influence of such parameters as temperature, oxygen levels, pH, substrate *etc.*, on the lipids of yeasts and moulds[10,22–24] there has been, as far as we are aware, only one study carried out with an oleaginous organism. This was with *Candida* 107 which was examined in the author's laboratory.[20] In brief, the fatty acid profile of the yeast proved remarkably resilient to changes in growth conditions; the only factor having much effect was the growth rate itself but, of course, this is the one parameter which cannot be changed without sacrifice of productivity.

The lack of effect of oxygen deprivation on the relative composition of fatty acids of *Candida* 107 (see Table 3) led us to look at oxygen demand of oleaginous

Table 3 *Effect of aeration on fatty acid composition of* Candida 107 *growing in continuous culture* (dilution rate = 0.1 h^{-1}) *on a nitrogen-limited medium* (taken from M. J. Hall and C. Ratledge, ref. 20)

Aeration rate (v/v min^{-1})	0.05	0.1	0.5	1.0
Biomass (g l^{-1})	5.3	9.4	10.3	10.6
Percentage lipid in biomass (w/w)	10.4	19.7	24.0	21.4
	Relative % of major fatty acids			
Fatty acid 16:0	27	26	25	24
18:0	12	9	7	9
18:1	39	43	41	42
18:2	19	19	23	21

yeasts. Lipid, being a chemically reduced compound, does not in fact require oxygen for its biosynthesis (save to generate one mole of ATP needed in the conversion of acetyl-CoA to malonyl-CoA). Consequently an organism accumulating high concentrations of lipid should not require as much oxygen as one accumulating, say, protein and, furthermore, this demand for oxygen would be further decreased because of the slower growth rate needed to promote lipid accumulation.

This lower demand for oxygen was found to be the case with both *Candida* 107 and *Rhodotorula gracilis*.[25] In particular, see Figure 4, the specific oxygen

[22] C. M. Brown and B. Johnson, *Ant. van Leeuwenhoek*, 1971, **37**, 477.
[23] K. Hunter and A. H. Rose, in 'The Yeasts', vol. 2, ed. A. H. Rose and J. S. Harrison, Academic Press, London, p. 211.
[24] C. Ratledge, in 'Food from Waste', ed. G. G. Birch, K. J. Parker, and J. T. Worgan, Applied Science Publishers, London, 1971, p. 98.
[25] C. Ratledge and M. J. Hall, *Appl. Environ. Microbiol.*, 1977, **34**, 230.

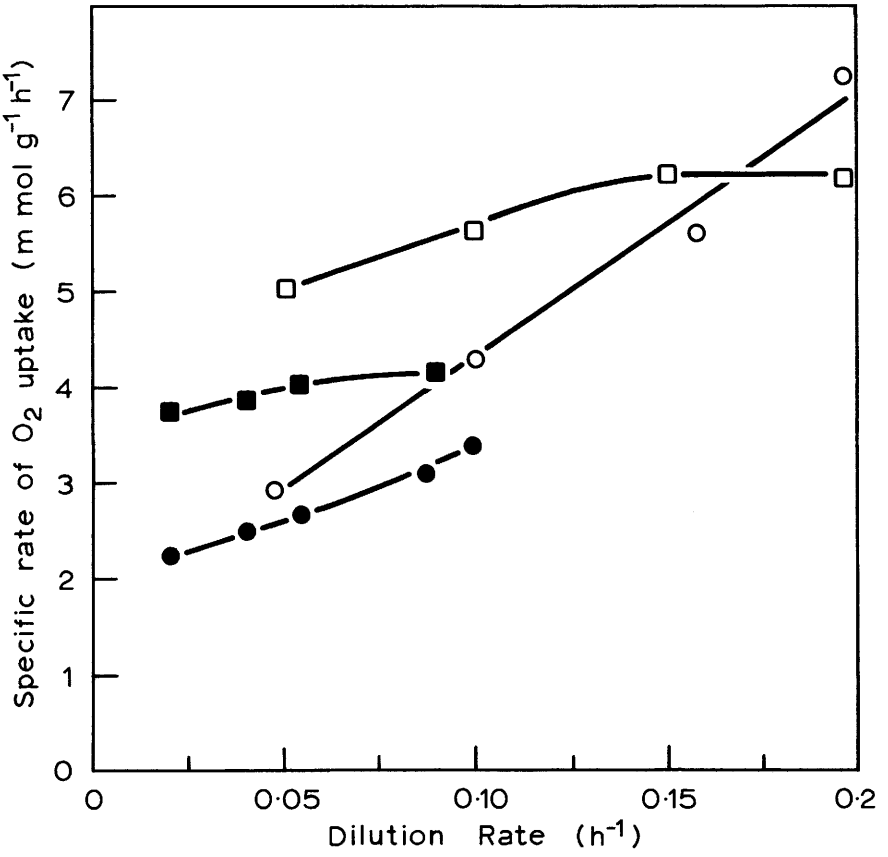

Figure 4 *Oxygen uptake rates in two oleaginous yeasts* (Candida 107, *open symbols, and* Rhodotorula gracilis, *closed symbols) growing in continuous culture under carbon limitation (squares) or nitrogen limitation (circles). Lipid accumulation occurs only under the latter condition (see Figure* 3B). (From C. Ratledge and J. M. Hall, ref. 25)

demand of *Candida* 107 decreased 2½-fold in going from a high growth rate to a slower growth rate coupled with lipid accumulation. Consequently, as oxygen transfer rate is the usual limiting constraint upon the greatest biomass density which can be maintained in a fermenter,[26] an SCO process should be able to maintain a cell population 2½ times as dense as that in an SCP process. Loss of productivity from the fermenter because of the lower dilution rate which an SCO process has to employ is therefore obviated.

4 Choice of Substrate

Even in the best circumstances the yield of oil per unit amount of a substrate is

[26] B. J. Abbot and A. Clamen, *Biotech. Bioeng.*, 1973, **15**, 117.

not high. If conversions of 22 % can be consistently achieved, this implies that 4½ tons of substrate would be required to produce one ton of oil. This immediately puts pressure on choosing a cheap and abundant substrate on which an SCO process could be based. There is, however, one important point to be made. Oil is not made in isolation. After the oil is extracted from a micro-organism, a protein-rich residue (see below) remains which is then saleable as SCP. Thus a micro-organism grown for oil is producing two eminently saleable products.

Almost every substrate which can be used to produce SCP could be used to produce SCO. Exceptions are methane and methanol which, for reasons as yet unknown, do not appear capable of promoting lipid accumulation. Methane is only utilized by some bacteria and would therefore be an unsuitable starting material but methanol is utilized by many yeasts but none of these produce more than a few percent lipid.

Substrates which can and have been used for growth of fat-accumulating organisms include molasses (sugar beet and cane), whey, waste sulphite liquors, starch, ethanol, acetic acid, hydrolysed organic materials such as peat and rice hulls, wastes such as date extracts and even blanching liquors from vegetable processing units. In short, any material currently being considered for 'upgrading' into SCP could be considered for fat production providing it had a high carbon to nitrogen ratio. An appropriate screening programme would always be needed to select the most suitable organism for a particular substrate. Some workers have shown that different fatty acid profiles arise by using different substrates and whilst this may be so there seems to be no overall trends which could be safely offered as guidelines. Our own work with *Candida* 107 grown at constant growth rates in continuous culture on sucrose, ethanol, or lactose revealed no major changes in yeast fatty acids (Table 4) and similar results have also been reported for the fatty acids of *Rh. gracilis* grown on ethanol or glucose.[27]

Table 4 *Fatty acids of* Candida 107 *grown in continuous culture using nitrogen-deficient medium with different carbon substrates*

Substrate	*Lipid* (% dry wt)	16:0	18:0	18:1	18:2	18:3	22:1
Sucrose	32	26	9	35	20	2	2
Ethanol	32	25	11	30	27	3	–
Lactose	21	25	9	31	31	2	7

The only substrates which do have a marked effect on fatty acids are n-alkanes which have featured prominently in many SCP processes. Their main advantage for an SCO process is that they can be used to 'tailor-make' particular ranges of fatty acids as the alkane is oxidized directly to the fatty acid which, if of the correct chain length, will be incorporated into the lipids.[28,29] However, because of

[27] V. Krumphanzl, V. Gregr, J. Pelechova, and J. Uher, in Advances in Microbial Engineering (Biotech. Bioeng. Symp. No. 4), ed. B. Sikyta, A. Prokop, and M. Novak, Part I, John Wiley & Sons, New York, 1973, p. 245.

[28] C. Ratledge, *Chem. and Ind.*, 1970, p. 843.

[29] C. Ratledge, in 'Developments in Biodegredation of Hydrocarbons', vol. 1, ed. R. J. Watkinson, Applied Science Publishers, London, 1978, p. 1.

their cost, they must be used as fractions or 'cuts' of alkanes which are still relatively expensive. Thus a range of fatty acids is produced which may be unacceptable as both odd- and even-numbered chain lengths will be present. Another disadvantage is that alkanes are recovered unchanged in the extracted lipid, thus making it highly unlikely that such a product would be acceptable as a human foodstuff even if it were to be refined with the total exclusion of residual alkanes. Oils intended for technical purposes would not be so restricted and thus alkanes should only be contemplated as a substrate if the product is not intended for inclusion in food.

Of the bulk, cheap substrates which are available, cellulose is the most abundant. Hydrolysis of the cellulose, chemical or enzymatic, would be needed as a pre-treatment as oleaginous micro-organisms are not yet known which can grow on native cellulose. Many schemes for the utilization of cellulose as a fermentation substrate have been devised[30] and some have been costed.[31–33] Table 5 shows the

Table 5 *Cost estimates for enzymatic utilization of waste cellulose for an* 833 ton/day *plant* (adapted from A. E. Humphrey, 'Symposium on Enzymatic Hydrolysis of Cellulose', ed. M. Bailey, T. M. Enari, and M. Linko, Finnish National Fund for Research and Development, Helsinki, Finland, 1975, p. 413)

	Sugar cost (£/ton)
A. 69 % conversion of cellulose to glucose	
I. No cost for waste paper (assumes 90 % process efficiency, and 50 % enzyme recovery)	28
II. As I but without enzyme recovery	34
III. As II but with waste paper collection charge $25/ton	70
B. 50 % conversion of cellulose to glucose	
IV. As I	42
V. As II	70
VI. As V but with waste paper collection charge $50/ton	135
C. Using wood pulp at $300/ton with a 90 % process efficiency, an 86 % conversion and 50 % enzyme recovery	196

various costs which are likely to be entailed in the enzymatic utilization of waste cellulose. A realistic price for the finished product would seem not to be below £70/ton. Whether chemical hydrolysis of cellulose would yield cheaper a glucose solution is uncertain. At this price, hydrolysed cellulose looks unpromising but

[30] C. Ratledge, *Ann. Rep. Ferment. Proc.*, 1977, **1**, 49.
[31] G. W. Gove and I. Gellman, *J. Water Pollut. Control Fed.*, 1976, **48**, 1234.
[32] D. Brandt, in 'Cellulose as a Chemical and Energy Resource', Biotech. Bioeng. Symp. no. 5, ed. C. R. Wilke, John Wiley and Sons, New York, 1975, p. 275.
[33] G. R. Cysewki and C. R. Wilke, *Biotech. Bioeng.*, 1976, **18**, 1297, 1315.

current research in Finland, New Zealand, and Canada[34–37] may succeed in developing more economical processes and if one should come about then many opportunities for fermentation processes, not only SCO, would become available.

5 Possible Costings

The choice of substrate for an SCO process is crucial as it is this which will decide whether it is a feasible proposition or not. It is possible to calculate the outline economics of an SCO process in two ways. The first and simplest is to compare a hypothetical SCO process with an SCP process. This is particularly relevant where a company may be considering the up-grading or 'waste disposal' of organic materials which have little intrinsic value. Here the 'cost' of the substrate can be taken as nil or even of negative value.

If we consider that one ton of fermentable carbohydrate is available, which product – protein or oil – will show the greatest profit? In Table 6 it has been

Table 6 *Biomass yields of SCP and SCO processes*

Biomass yield	*SCP process*	*SCO process**
	0.55 ton	*0.55 ton*
Oil	0.06 ton†	0.22 ton
Protein	0.30 ton	0.13 ton
Carbohydrate	0.09 ton	0.12 ton
RNA/DNA	0.06 ton	0.04 ton
Ash/residue	0.04 ton	0.04 ton

*Based on composition of *Candida* 107 grown in continuous culture
†Not extractable

assumed that the equipment being used would be the same in both cases and that the productivity (tons/year) would also be equivalent.

In the SCP process, the biomass has a 55 % protein content and should be saleable as an animal feedstuff, say, at £150/ton thus realizing £82.5/ton substrate utilized.

In the SCO process, the oil is extracted and, let us assume, sells only at the bottom end of the market, *e.g.* £300/ton. Thus £66 comes from sale of the oil. The biomass residue (0.33 ton) has a 40 % protein content and should, like the SCP, be saleable as an animal feedstuff though at only £110/ton (on a *pro rata* protein basis) thus realizing £36. The total sales therefore are £102. To the SCO costs must, however, be added the cost of extraction.

Extraction of fat from micro-organisms is not a difficult process and can be

[34] Symposium on Enzymatic Hydrolysis of Cellulose, ed. M. Bailey, T. M. Enari, and M. Linko, Finnish National Fund for Research and Development, Helsinki, Finland, 1975.
[35] D. A. Whitworth, *New Zealand Energy J.*, 1976, **49** (11), 173.
[36] D. A. Whitworth, *New Zealand Energy J.*, 1977, **50** (2), 14.
[37] 'Feasibility study of production of chemical feedstock for wood waste'; Pulp and Paper Research Institute of Canada, 1976.

accomplished using the existing technology which has already been developed for handling plant oilseeds. Solvent extraction in a rotary vacuum-filter system should cost no more than about £10—15 per ton of dry biomass to process if the throughput is at the 10—100 ton/day level. Thus, in the above calculations, the cost of extracting 0.55 ton biomass would be at most £10. Therefore, the SCO process is more profitable than the SCP process by a margin of at least 10 %.

The profitability will increase even further if the microbial oil sells, not at the bottom of the market, as we have assumed here, but at the middle or top-end of the price scale. An oil selling at £500/ton as a substitute groundnut oil might be achievable without undue difficulty.

The second method of trying to assess the economics of an SCO process is to calculate the absolute costs involved. This is an extremely complex process for which only approximate figures can be given.[38]

Let us assume that two tons of substrate, at a cost of £S/ton, will generate one ton of biomass selling at £B. Thus if F = cost of fermentation plus extractions (£/ton biomass product) then, for the process to break even,

$$2S + F = B$$

If the biomass consists of 40 % oil selling at £L/ton and 60 % protein residue selling at £P/ton then

$$B = 0.4L + 0.6P$$

$$\text{If } L = £300 \text{ and } P = £110$$

$$\text{then } B = £186$$

This immediately indicates that a small scale fermentation process, say up to 500 tons/year, is unlikely to be profitable as the total fermentation costs (capital repayment plus running costs) are likely to exceed £200/ton (see Forage in article III p. 309). At, say, 5000 ton/year or higher the fermentation costs will probably decrease to between £125—£150/ton but this still indicates an insufficient margin to buy the substrate *and* make a profit. Consequently production of a cheap microbial oil is not economically possible unless the substrate is truly of negative value.

If, however, in the above equations, the selling price of the oil is raised to £500/ton, and assuming fermentation costs are £150/ton, then one is left with £112 to buy two tons of substrate. If the substrate costs £50/ton, a profit of £12 is made (on an outlay of £250). Should the substrate cost only £30/ton, then a handsome profit of £56 will arise from an outlay of £210.

It is clear that in calculating the profitability of a process, the interplay of the scale of the operation coupled with the cost of the substrate has to be evaluated against the quantity *and* quality of oil being produced.

6 Conclusions

If an oil or oil-derived product costs £1000 or more /ton, then, if this can be

[38] C. Ratledge, *Chem. and Ind.*, 1975, p. 918.

obtained in good yield from a microbial source, there should be little difficulty in devising a profitable fermentation process based on the utilization of an existing substrate.

If an oil is desired which will sell for much less than £1000/ton then the cost of substrate becomes crucial and probably only substrates which are currently regarded as waste with little intrinsic value can make the process economical.

A microbial oil-producing process, though, will always be more profitable than a microbial protein producing process, and if waste processing, re-cycling *etc.* is being contemplated then serious consideration ought to be given to microbial oils and fats as a product. It is apparent, however, that there is a great deal of work still to be done on this subject which, for various reasons, has been a neglected area of investigation for over 20 years. I am confident that given only a fraction of the effort which has been put into developments for SCP processes that a Single Cell Oil could become a reality within the next 10 years.