4 Microbial Lipids

COLIN RATLEDGE
Hull, United Kingdom

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1 Introduction

Since the publication of the 1st Edition of “Biotechnology” and the earlier chapter on the biotechnology of lipids in 1986, a considerable number of developments have taken place in this field. Some microbial lipid products have now been produced commercially and prospects for other developments appear to be not too far away. In some cases, as e.g., with the bacterial lipid poly-β-hydroxybutyrate, no counterpart exists from plant or animal sources and consequently the economics of producing this product lie outside the normal oils and fats domain. With most other microbial lipids, these are the equivalent in composition to plant-derived oils and consequently must compete against these in any potential market place. Only the highest valued oils have any chance of being produced by biotechnological means as it is impossible for microorganisms to produce oils and fats as cheaply as the main commodity oils are produced from plant and animal sources. However, there is always the possibility of producing a microbial oil as an adjunct to some waste treatment process in a way similar to that often used to produce microbial proteins (SCP – single cell protein) for animal feed from some unwanted substrate. Microbial oils – which could then be referred to as single cell oils (SCO) – would have the double advantage over SCP in that they could probably sell for a higher price than SCP and, moreover, could be used for a technical purpose should the nature of the substrate prevent the product being returned into the food chain.

The major commercial plant oils continue to be dominated by soybean oil (current 1993 production is about 18·10⁶ t); palm oil, though, continues to be the fastest growing market with 14·10⁶ t now being produced compared to 6·10⁶ t in 1983. If the present rate of expansion in palm oil production continues in Malaysia and Indonesia (BASIRON and IBRAHIM, 1994; LEONARD, 1994), then palm oil will overtake soybean oil production by the end of this decade. Rapeseed oil (now 9·10⁶ t in 1993) is also expanding mainly due to increased cultivation in Europe and Canada. The variety now under cultivation is the low- (or zero-) erucic acid (20:1) oil which is then a permitted oil for food manufacture.

Overall production of plant and animal oils is increasing at about 3% per annum; production in 1992/93 was about 85·10⁶ t and is expected to reach 105·10⁶ t by the year 2000 (MIELKE, 1992). Pricing of these materials remains highly competitive as most products using oils can switch between the various types according to the day. The average price index for the major commodity oils is about US$ 500–550 per t though groundnut oil, e.g., is always significantly higher than the average at $ 800–850 per t. The highest priced commodity oil, excluding the speciality materials, is always olive oil at $ 1,500–2,000 per t. Its price depends on its quality which includes minor, but very important, flavour components. Animal fats (tallow and lard) have steadily declined in consumption over the past decade and are likely to fall even further to about 20% of the total market by 2001 (SHUKLA, 1994). Their prices are therefore usually at or below the average index level.

The trends in world oil and fats supplies are under constant surveillance and are frequently reviewed in various publications: the extensive reviews by SHUKLA (1994) and MIELKE (1992) can be recommended though for current information journals such as Lipid Technology (P. T. Barnes & Associates), Oils and Fats International (Chase Webb, St Ives PLC), INFORM (American Oil Chemists’ Society, Illinois) provide invaluable and continuously up-dated information in most areas. There are, in addition, a number of specialized trade reviews that provide weekly prices of the traded oils.

The fatty acid composition of the major commercial oils is given in Tab. 1. The nomenclature of lipids is given in Sect. 1.1. It will be appreciated that, unlike say animal feed protein, the composition of the fats varies considerably from species to species. In all cases, however, the oil or fat is composed almost entirely (>98%) of triacylglycerols (formerly known as triglycerides) – Sect. 1.1. For edible purposes, the oil or fat is retained in this form though the individual fatty acyl groups on the glycerol can be modified – usually by chemical means – without affecting the triacylglycerol structure per se. Some en-
**Tab. 1. Fatty Acid Composition of Fats and Oils of Animal and Plant Origin**

<table>
<thead>
<tr>
<th>Fats/Oils</th>
<th>Relative Proportion of Fatty Acyl Groups [% (w/w)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4:0–10:0  12:0  14:0  16:0  16:1  18:0  18:1  18:2  18:3  20:0  20:1  Others</td>
</tr>
<tr>
<td><strong>Animal Fats</strong></td>
<td></td>
</tr>
<tr>
<td>Butterfat</td>
<td>10  3  11  27  2  12  29  2  –  –  –  15:0 + 17:0, 3%</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>–  –  3  24  4  19  43  3  1  –  –  15:0 + 17:0, 2%; 14:1 + 17:1, 2%</td>
</tr>
<tr>
<td>Lard</td>
<td>–  –  2  26  3  14  44  10  –  –  –</td>
</tr>
<tr>
<td><strong>Plant Oils</strong></td>
<td></td>
</tr>
<tr>
<td>Coconut oil</td>
<td>15  47  18  9  –  3  6  2  –  –  –</td>
</tr>
<tr>
<td>Palm kernel oil</td>
<td>8  48  16  8  –  3  15  2  –  –  –</td>
</tr>
<tr>
<td>Cocoa butter</td>
<td>–  –  –  26  –  35  35  3  –  1  –  –</td>
</tr>
<tr>
<td>Olive oil</td>
<td>–  –  –  13  1  3  71  10  1  1  –  –</td>
</tr>
<tr>
<td>Rapeseed oil</td>
<td>–  –  –  4  –  2  62  22  10  –  –  –</td>
</tr>
<tr>
<td>Groundnut oil*</td>
<td>–  –  –  11  –  2  48  32  –  1  2  22:0 + 24:0, 5%</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>–  –  –  7  –  5  19  68  1  –  –  –</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>–  –  –  11  –  4  24  54  7  –  –  –</td>
</tr>
<tr>
<td>Corn oil</td>
<td>–  –  –  11  –  2  28  58  1  –  –  –</td>
</tr>
<tr>
<td>Cotton seed oil</td>
<td>–  –  –  1  22  1  3  19  54  1  –  –  –</td>
</tr>
<tr>
<td><strong>“Exotic” Plant Oils</strong></td>
<td></td>
</tr>
<tr>
<td>Borage seed oil</td>
<td>–  –  –  11  –  4  16  39  22b  –  4.5  22:1, 2.5% 24:0, 1.5%</td>
</tr>
<tr>
<td>Evening primrose seed oil</td>
<td>–  –  –  8  –  2  9  70  9b  –  –</td>
</tr>
<tr>
<td>Blackcurrant seed oil</td>
<td>–  –  –  6  –  1  10  48  17b  –  –  α-18:3, 13%</td>
</tr>
</tbody>
</table>

*a* Also known as peanut oil.

*b* γ-Linolenic acid, 18:3 (6, 9, 12).
zymatic reformulation of triacylglycerols occurs on an industrial scale using stereospecific lipases to transesterify palm oil fractions into the much more expensive cocoa butter-like triacylglycerols (OWUSU-ANSAH, 1993).

With some technical applications of oils, it is the fatty acid that is required; consequently saponification (hydrolysis) of the triacylglycerol is carried out and the fatty acid used either as such, e.g., with soap manufacture, or is modified to an appropriate derivative which is then used in a multitude of products: from detergents to adhesives.

The aim of all biotechnological processes is to produce products that are either cheaper than can be obtained from other sources, including possible chemical synthesis, or are not available by any other means. Within the field of lipids, the opportunities to produce triacylglycerol lipids are limited to the highest valued materials. The highest priced bulk (commodity) oil is cocoa butter whose price has varied between $8,000 to $3,000 per t over the past decade. At the higher price level, the prospects of producing a cocoa butter equivalent oil by yeast technology have looked favorable. This topic is specifically reviewed later (see Sect. 3.2.1).

Other very high valued oils are those in the health care market and which have had various claims made on their behalf for the amelioration of various diseases and conditions. Of current interest are oils containing the polyunsaturated fatty acids: γ-linolenic acid, 18:3 (ω-6); arachidonic acid, 20:4 (ω-6); eicosapentaenoic acid, 20:5 (ω-3); and docosahexaenoic acid, 22:6 (ω-3). Oils containing such fatty acids are found in a number of microorganisms and are reviewed in Sects. 3.3 and 3.4.

The very highest priced lipids though are probably the prostanoid compounds encompassing the prostaglandins, leukotrienes, and thromboxanes. These are mainly used for treatment of uncommon disorders or for experimental purposes. Consequently, the amounts required per annum are probably at the kilogram stage rather than the ton (or kiloton) stage with other lipid products. Prospects for producing such materials are briefly mentioned in Sect. 6.4.

Thus, if we view microorganisms as a potential source of the widest types of lipids then it is possible to identify a number of potentially attractive products. For the purposes of this article, I have therefore used the broad definition of a lipid as any material that is derived from a (micro)organism, is directly soluble in organic solvents, and is essentially a water-insoluble material. However, as there is still considerable interest in the manner in which microorganisms synthesize large quantities of lipids, much of the review will be taken up with the more conventional types of oils and fats that they produce. The entire subject of microbial lipids, encompassing all aspects and not just biotechnology, has been the above subject of a two-volume monograph by RATLEDGE and WILKINSON (1988a, 1989). The industrial applications of microbial lipids have also been the subject of a monograph edited by KYLE and RATLEDGE (1992). Details concerning the degradation of fats, oils, and fatty acids, including the action of lipases and phospholipases, which are not covered here have been recently reviewed elsewhere by the author (RATLEDGE, 1993).

It will be appreciated, of course, that although microorganisms remain a potential source of oils and fats, there is considerable effort being put into the production of oils and fats from conventional plant sources. Such efforts include the modification of peanut oils (groundnut oil) to produce changes in the fatty acid composition so that the more desirable oils can be produced more cheaply. The application of genetic engineering is now gathering pace as a means of producing “tailor-made” oils and fats in plants and is likely to supersede the traditional plant breeding approach as a means of creating what is wanted more quickly and with greater certainty. This review, however, will not include any detailed review of the current developments in plant genetic engineering as applied to the commodity oils and fats. Readers should though be aware that such advances are now likely to be a major influence in the availability of “improved” oils for everyday use and will undoubtedly ensure that these materials remain highly competitively priced for many years to come. The recent reviews by HARWOOD (1994a, b), MURPHY (1994a) and RATTRAY (1994) and the monographs
edited by Rattray (1991) and by Murphy (1994b) will be found particularly useful in this respect. Tab. 2 summarizes some of the current developments that are now taking place in this area.

The opportunities for microorganisms to produce oils and fats of commercial value for the bulk markets remain doubtful but where the product cannot be obtained from elsewhere then this provides a much better opportunity for a microbial oil than attempting to replicate what is already available from plant sources. Some opportunities nevertheless do exist but they have to be identified with some care. Hopefully, some of the following material may indicate to the astute reader where such opportunities may lie.

1.1 Lipid Nomenclature and Major Lipid Types

Fatty acids are long chain aliphatic acids (alkanoic acids) varying in chain length from, normally, C12 to C24 though both longer and shorter chain-length acids are known. In most cells (microbial, plant, and animal), the predominant chain lengths are 16 and 18. Fatty acids may be saturated or unsaturated with one or more double bonds which are usually in the cis (or Z) form. The structure of a fatty acid is represented by a simple notation system $-X:Y$, where $X$ is the total number of C atoms and $Y$ is the number of double bonds. Thus, 18:0 is octadecanoic acid, that is stearic acid; 16:1 is hexadecenoic acid, that is palmitoleic acid, with one double bond, and 18:3 would represent octadecatrienoic acid, a C18 acid with three double bonds. The position of the double bond(s) is indicated by designating the number of the C atom, starting from the COOH terminus, from which the double bond starts: oleic acid is thus 18:1 (9) signifying the bond is from the 9th (to the 10th) C atom. If it is necessary to specify the isomer, this is added as “$c$” (for “cis” = Z) or “$t$” for “trans” = E). In this review, the cis/trans system is used. Thus, cis, cis-linoleic acid is 18:2 ($c\ 9, c\ 12$). Most naturally-occurring unsaturated fatty acids are in the cis configuration and, unless it is stated otherwise, this configuration may be assumed.

With polyunsaturated fatty acids (PUFA), the double bonds are normally methylene-interrupted: $-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-$. Thus, once the position of one bond is specified all the others are also indicated. In num-

<table>
<thead>
<tr>
<th>Plant Target</th>
<th>Fatty Acid</th>
<th>Objective</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean and</td>
<td>16:0</td>
<td>increase</td>
<td>margarine</td>
</tr>
<tr>
<td>rapeseed</td>
<td>16:0</td>
<td>decrease</td>
<td>edible oil</td>
</tr>
<tr>
<td></td>
<td>18:0</td>
<td>increase</td>
<td>margarine; cocoa butter</td>
</tr>
<tr>
<td></td>
<td>18:1</td>
<td>increase</td>
<td>improved edible oil</td>
</tr>
<tr>
<td></td>
<td>$\alpha$-18:3</td>
<td>decrease</td>
<td>improved stability and odor</td>
</tr>
<tr>
<td>Rapeseed</td>
<td>22:1</td>
<td>increase</td>
<td>erucic acid for oleochemicals</td>
</tr>
<tr>
<td>Sunflower</td>
<td>18:1</td>
<td>increase</td>
<td>olive oil</td>
</tr>
<tr>
<td></td>
<td>18:2 + 18:3</td>
<td>decrease</td>
<td>substitute</td>
</tr>
<tr>
<td>Linseed</td>
<td>18:3</td>
<td>increase</td>
<td>oleochemicals</td>
</tr>
<tr>
<td>Groundnut</td>
<td>18:1</td>
<td>increase</td>
<td>improved edible oil</td>
</tr>
</tbody>
</table>
When fatty acids are esterified to glycerol, they give a series of esters: mono-, di-, and triacylglycerols (I, II, III). This is the preferred nomenclature to the older mono-, di-, and triglycerides.

\[
\begin{align*}
\text{I} & : \text{CH}_2\text{OH} \quad \text{CH}_2\text{OH} \quad \text{CH}_2\text{OH} \\
\text{II} & : \text{CH}\text{OH} \quad \text{CH}\text{OH} \quad \text{CH}\text{OH} \\
\text{III} & : \text{CH}_2\text{OH} \\
\end{align*}
\]

where R is a long alkyl chain and RCO− is, therefore, the fatty acyl group.

As various isomeric forms are possible, the position of attached acyl group must be specified in most cases. For this, the stereospecific numbering (sn-) system is used so that the two prochiral positions of glycerol (IV) can be distinguished as sn-1 and sn-3.

\[
\begin{align*}
\text{I} & : \text{CH}_2\text{OH} \\
\text{II} & : \text{CHOH} \\
\text{III} & : \text{CH}_2\text{OH} \\
\end{align*}
\]

With respect to the monoacylglycerols, there are obviously three possible isomers and similarly for the diacylglycerols.

Where different acyl groups are attached to the glycerol moiety, these can then be individually given. For example, 1-stearoyl-2-oleoyl-3-palmitoyl-sn-glycerol is the major triacylglycerol of cocoa butter with stearic, oleic, and palmitic acids on the three OH positions.

Phospholipids possess two fatty acyl groups at the sn-1 and sn-2 positions of glycerol with a phospho group at sn-3 which is also linked to a polar head group: choline, serine, ethanolamine, and inositol are the common ones. For the nomenclature and naming of phospholipids and other microbial lipids, the multiauthored treatise *Microbial Lipids*, edited by RATLEDGE and WILKINSON (1988a, 1989), may be helpful though there are numerous textbooks on lipids that provide similar information.

## 2 Accumulation of Lipid

### 2.1 Patterns of Accumulation

Not all microorganisms can be considered as abundant sources of oils and fats, though, like all living cells, microorganisms always contain lipids for the essential functioning of...
membranes and membranous structures. Those microorganisms that do produce a high content of lipid may be termed “oleaginous” in parallel with the designation given to oil-bearing plant seeds. Of the some 600 different yeast species, only 25 or so are able to accumulate more than 20% lipid; of the 60,000 fungal species fewer than 50 accumulate more than 25% lipid (RATLEDGE, 1989a).

The lipid which accumulates in oleaginous microorganisms is mainly triacylglycerol (see Sect. 1.1). If lipids other than this type are required then considerations other than those expressed here might have to be taken into account to optimize their production. With few exceptions, oleaginous microorganisms are eukaryotes and thus representative species include algae, yeasts, and molds. Bacteria do not usually accumulate significant amounts of triacylglycerol but many do accumulate waxes and polyesters (see Sects. 5.1 and 5.2) which are now of commercial interest.

The process of lipid accumulation in yeasts and molds growing in batch culture was elucidated in the 1930s and 1940s (see WOODBINE, 1959, for a review of the early literature, and RATLEDGE, 1982, for an updated review of these aspects). A typical growth pattern is shown in Fig. 1. This pattern is also found with the accumulation of polyester material in bacteria (see Sect. 5).

The key to lipid accumulation lies in allowing the amount of nitrogen supplied to the culture to become exhausted within about 24–48 h. Exhaustion of nutrients other than nitrogen can also lead to the onset of lipid accumulation (see GRANGER et al., 1993, for a recent reference) but, in practice, cell proliferation is most easily effected by using a limiting amount of N (usually NH_4^+ or urea) in the medium. The excess carbon which is available to the culture after N exhaustion continues to be assimilated by the cells and, by virtue of the oleaginous organism possessing the requisite enzymes (see below), is converted directly into lipid.

The essential mechanism which operates is that the organism is unable to synthesize essential cell materials – protein, nucleic acids, etc. – because of nutrient deprivation and thus cannot continue to produce new cells. Because of the continued uptake of carbon and its conversion to lipid, the cells can then be seen to become engorged with lipid droplets (Fig. 2). It is important to appreciate, however, that the specific rate of lipid biosynthesis does not increase; the cells fatten because other processes slow down or cease altogether and, as lipid biosynthesis is not

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**Fig. 1.** Typical lipid accumulation pattern for a yeast (*Rhodotorula glutinis* = *R. gracilis*) growing on a high C:N ratio medium in batch culture. Biomass □, % lipid content □, NH_4^+ in medium ○ (from YOON et al., 1982).

**Fig. 2.** Electron micrograph of *Cryptococcus curvatus* (=*Candida curvata* = *Apiotrichum curvatum*) strain D grown for 2 days on nitrogen-limiting medium (viz. Fig. 1) showing presence of multiple lipid droplets. Total lipid content approx. 40%, marker bar: 1 μm (from HOLDSWORTH et al., 1988).
linked to growth, this may continue unabated. The process of lipid accumulation (Fig. 1) can be seen as a two-phase batch system: the first phase consists of balanced growth with all nutrients being available; the subsequent “fattening” or “lipogenic” stage occurs after the exhaustion of a key nutrient other than carbon and, of course O₂. The role of O₂ during lipid formation was discussed briefly in the 1st Edition of “Biotechnology” (RATLEDGE, 1986).

Accumulation of lipid has also been achieved in single stage continuous culture (RATLEDGE et al., 1984) and a typical accumulation profile dependent upon the dilution rate (growth rate) is shown in Fig. 3. As with batch cultivation, the medium has to be formulated with a high carbon-to-nitrogen ratio, usually about 50:1. The culture must be grown at a rate which is about 25–30% of the maximum. Under this condition, the concentration of nitrogen in the medium is virtually nil and the organism then has sufficient residence time within the chemostat to assimilate the excess carbon and convert it into lipid. The rate of lipid production (i.e., g L⁻¹ h⁻¹) is usually faster in continuous cultures than in batch ones (EVANS and RATLEDGE, 1983; FLOETENMEYER et al., 1985).

The exact ratio of C to N chosen for the medium was originally considered to be of little consequence provided N was the limiting nutrient and sufficient carbon remained to ensure good lipid accumulation. However, YKEMA et al. (1986) showed that a range of lipid yields in an oleaginous yeast, Apiotrichum curvatum (originally Candida curvata but now Cryptococcus curvatus; see BARNETT et al., 1990) were traversed in continuous culture by varying the C:N ratio of the growth medium. There was a hyperbolic relationship between the C:N ratio and the maximum growth (dilution) rate that the organism could attain: the lowest growth rate was at the highest C:N ratio of 50:1 and this, in turn, controlled the amount of lipid produced and the efficiency of yield (g lipid per g glucose used) with which it was produced. Although the highest lipid contents of the cell (50% w/w) were obtained with a C:N ratio of 50:1 or over, the optimum ratio for maximum productivity (g L⁻¹ h⁻¹ lipid) was at a ratio of 25:1 with glucose (YKEMA et al., 1986) and at 30–35:1 when whey permeates were used with same yeast (YKEMA et al., 1988). Similar results for describing the optimum C:N ratio for lipid accumulation have been developed by GRANGER et al. (1993) using Rhodotorula glutinis.

Interestingly, YKEMA et al. (1986) commented that Apiotrichum curvatum simultaneously accumulated about 20% carbohydrate in the cells along with the 50% lipid. Such a phenomenon of carbohydrate formation had been conjectured by BOULTON and RATLEDGE (1983a) to be a likely event to account for an observed delay in lipid synthesis after glucose assimilation had been initiated. This carbohydrate was also recognized independently by HOLDSWORTH et al. (1988) in the same yeast and was considered to be glycogen. As YKEMA et al. (1986) pointed out, if the biosynthesis of the polysaccharide which, like lipid, is a reserve storage material, could be prevented then this would enhance the total amount of lipid producible with a cell.

Although most studies on microbial lipid accumulation have been conducted using batch cultivation and, for accuracy, in continuous culture, other growth systems have also been explored. In particular, fed-batch cul-

![Fig. 3. Typical lipid accumulation pattern for a yeast (Rhodotorula glutinis) growing on nitrogen limiting medium in continuous culture. Biomass ■, % lipid content O (from YOON and RHEE, 1983).](image-url)
ture has proved effective in increasing both the cell density and lipid contents of oleaginous yeasts: Yamauchi et al. (1983) used ethanol as substrate with *Lipomyces starkeyi* and achieved a biomass density of 150 g L\(^{-1}\) with a lipid content of 54%. Similarly, Pan and Rhee (1986) achieved 185 g (dry wt.) of *Rhodotorula glutinis* per liter with a lipid content of 43% using glucose as the fed-batch substrate. In this latter case, O\(_2\)-enriched air (40% O\(_2\) + 60% air) had to be used to sustain the cells. At the density recorded, the packed cell volume was 75% of the total volume of the fermentation medium. Without using additional O\(_2\), it seems likely that cell densities of up to 100 g L\(^{-1}\) could be achieved with most oleaginous yeasts (see, e.g., Ykema et al., 1988) though filamentous molds may pose other problems. Economic considerations, however, would probably be against the use of O\(_2\)-enriched air for any commercial process. Interestingly, it is suggested that higher rates of lipid formation may occur with fed-batch techniques than with batch- or continuous-culture approaches (Ykema et al., 1988).

At the end of the lipid accumulation phase (see Fig. 1), it is essential that the cells are promptly harvested and processed. If glucose, or other substrate, has become exhausted on the end of the fermentation, then the organism will begin to utilize the lipid as the role of the accumulated material is to act as a reserve store of carbon, energy, and possibly even water. Holdsworth and Ratledge (1988) showed with a number of oleaginous yeasts that after carbon exhaustion following lipid accumulation, the lipid began to be utilized within 1.5 h thus indicating the dynamic state of storage lipids in these organisms.

### 2.2 Efficiency of Accumulation

The efficacy of conversion of substrate to lipid has been examined in some detail in both batch and continuous culture. In general, the latter technique offers the better means of attaining maximum conversions as the cells are operating under steady state conditions and carbon is not used with different efficiencies at each stage of the growth cycle. Conversions of glucose and other carbohydrates including lactose and starch, to lipid up to 22% (w/w) have been recorded with a variety of yeasts (Ratledge, 1982; Ykema et al., 1988; Davies and Holdsworth, 1992; Hassan et al., 1993) which compares favorably with the theoretical maximum of about 31–33% (Ratledge, 1988). Somewhat lower yields appear to pertain with molds (Woodbine, 1959; Weete, 1980). The reason for this difference is not obvious though it may be due to a somewhat slower growth rate of molds than yeasts. It should be said, however, that there has not been the same amount of detailed work carried out with molds as with yeasts. Claims that microorganisms have achieved higher conversions of glucose or other sugars to lipid should be treated with caution: either there will be found to be additional carbon within the medium and not taken into the mass balance or, as may occasionally happen, the “lipid” has been improperly extracted and may contain non-lipid material. However, if experimental data are calculated so that the yield of lipid or fatty acids can be based on the fraction of glucose being used solely for lipid biosynthesis, then values close to the theoretical value have been attained in practice (Granger et al., 1993).

When ethanol is used as substrate, the theoretical yield of lipid is 54% (w/w) (Ratledge, 1988). Though only a 21% conversion of ethanol to lipid was recorded by Yamauchi et al. (1983) in the fed-batch culture of *Lipomyces starkeyi*, higher conversions were recorded by Eroshin and Krylova (1983) also using a fed-batch system for the cultivation of yeasts on ethanol: conversions of 26%, 27%, and 31% were obtained using, respectively, *Zygolipomyces lactosus* (*Lipomyces trassporus*) and two strains of *Cryptococcus albidus var. aerius*. The reason for these very high values lies in the efficiency by which ethanol can be converted to acetyl-CoA, the starting substrate for lipid biosynthesis (see below). With glucose, the maximum yield of acetyl-CoA can only be 2 mol per mol utilized whereas with ethanol the yield is 1 mol per mol. On a weight-to-weight basis, therefore, ethanol (MW 46) is almost twice as efficient as glucose (MW 180) in providing C\(_2\) units. Granger et al. (1993) have recorded direct