OCL 2013, 20(6) D602

DOI: 10.1051/ocl/2013029

OCL Oilseeds & fats Crops and Lipids

Available online at:
www.ocl-journal.org

Research Article – Dossier Open Access

Micro-organismes producteurs de lipides

Dossier

Microbial oils: an introductory overview of current status and future prospects

Colin Ratledge[a]

Department of Biological Sciences, University of Hull, HU6 7RX, United Kingdom

Received 28 juin 2013 – Accepted 18 juillet 2013

Abstract – A brief overview is provided of the microbial oils (single cell oils - SCOs) that have been, or are still being, produced commercially. Oils rich in gamma-linolenic acid (18:3 n-6), arachidonic acid (20:4 n-6), eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3) are covered. The prospects of using other SCOs for biofuels are discussed with the conclusion that it is highly unlikely that algae will provide oils that are competitive with alternative sources of fatty acids.

Keywords: Microbial oils / single cell oils / SCOs / biofuels

Résumé – Huiles microbiennes : une revue introductive de la situation actuelle et des perspectives d'avenir. Cet article propose une brève revue des huiles microbiennes (huiles d'organismes unicellulaires, SCOs) qui ont été, ou sont toujours, commercialisées. Les huiles riches en acide gamma-linolénique (1:3 n-6), acide arachidonique (20:4 n-6), acide eicosapentaénoïque (20:5 n-3) et acide docosahexaénoïque (22:6 n-3) sont traitées. Les perspectives d'avenir de l'usage des autres SCOs en tant que biocarburants sont également discutées, avec la conclusion qu'il s'avère très improbable que les algues fournissent des huiles compététitives avec les sources alternatives d'acides gras.

Mots clés : Huiles microbiennes / huiles d'organismes unicellulaires / SCOs / biocarburants

1 Introduction

Although the prospects of obtaining useful, and possibly even cheap, oils from microoganisms has been considered for nearly a century, it is only in the past two or three decades that they have begun to be produced commercially. All the oils in current production are high in their contents of polyunsaturated fatty acids (PUFA) and are destined mainly for human consumption as nutraceuticals though some are used for animal feeding, including feed for farmed fish. The oils are produced using various fungi and marine microorganisms that are grown heterotrophically, that is with a fixed form of carbon, usually glucose. These oils are likely to remain in commercial production for some years to come as alternative sources, principally genetically-modified plants, are still some way off being realized in spite of many claims of various research groups having achieved "major break-throughs".

More recently, however, microbial oils have begun to be considered as possible sources of biofuels. Both heterotrophic organisms (yeasts and fungi) as well as photosynthetic algae are being actively pursued as possible production organisms. The basic concept behind this work is the prospects of being able to produce fatty acids cheaply enough for them to be converted into fatty acid esters that would then be used as biodiesel much in the same way that cheap plant oils, such as those from sunflower, rapeseed (Canola) and palm oil, are currently being used for large-scale production of biodiesel.

There is, thus, a rapidly-growing interest in microbial oils and how best they might be produced and tailor-made to meet the demands of two very different branches of the oil industries: oils for human consumption and oils for biofuels. Both these aspects will be briefly reviewed in this introductory chapter but further information and details will be provided in the remaining contributions to this special issue of Oilseeds & Fats Crops and Lipids.

2 Background to microbial oils

The term, single cell oils (SCO), was created (Ratledge, 1974) as means of easily identifying those lipids of single-celled entities – the microorganisms – that would be suitable for human consumption as alternatives to plant and animal oils and fats that have total dominance of the edible oil market. Much for the same reason why producers of microbial proteins in the 1960s and 1970s considered that the term of single cell protein (SCP) would be an appropriate description for these materials that would avoid mention of the sources of them – bacteria, yeasts and fungi – so it was with SCOs; to avoid direct disclosure of the oils coming from sources that the general public might find difficult to appreciate. Thus, SCO

[a] Correspondence: c.ratledge@hull.ac.uk

Table 1. Fatty acid profiles of commercially-produced SCOs.

Organism	Major fatty acyl groups (relative % w/w of total fatty acids)													
	14:0	16:0	16:1	18:0	18:1	18:2	18:3 (*n*-3)	18:3 (*n*-6)	20:3 (*n*-6)	20:4 (*n*-6)	20:5 (*n*-3)	22:5 (*n*-6)	22:6 (*n*-3)	22:0 +24:0
Mucor circinelloides[a]	1	23	1	6	39	10	0.2	18	-	–	–	–	–	1
Mortierella alpina (DSM)[b]	–	8	–	11	14	7	–	4	4	49	–	–	–	–
Mortierella alpina (Car.)[c]	–	7.5	–	6	9	6	–	2.5	4	43	–	–	–	12.5
Crythecodinium cohnii[d]	20	18	2	<0.5	15	–	–	–	–	–	–	–	40	–
Schizochytrium sp.[e]	8	22	<0.5	0.5	1	–	–	–	–	–	–	17	41	–
Ulkenia sp.[f]	3	30	<0.5	1	–	–	–	–	–	–	–	11	44	–
Yarrowia lipolytica[g]	–	25	1	1	6	18	2.5	–	2	<1	56	–	–	–

[a] Oil of Javanicus, produced by J. & E. Sturge (UK): GLA-rich oil.
[b] ARA-SCO, produced by DSM (formerly Gist-brocades, The Netherlands).
[c] CABIO oil produced by Cargill Alking Bioengineering Co. Ltd (Hubei, China).
[d] DHASCO™, produced by Martek/DSM.
[e] DHASCO-S, produced by Martek/DSM.
[f] DHA CL, produced by Lonza (Switzerland); also sold as DHA*Aid*™.
[g] EPA-SCO, produced by E.I. du Pont, USA (from Xue *et al*., 2009).

has thus proved to be a useful euphemism to coin and continue using. In fact, single cell oils now gets many more "hits" than single cell proteins when Googled!

SCO was originally intended to denote the edible oils of microorganisms, that is the triacylglycerol fraction of the total cell lipid, and would thus be equivalent commercial plant and animal oils. However, the term is now expanded to include all fatty acid-containing lipids within a single cell; this then includes algal lipids in which the triacylglycercols may not be the predominant fraction: algal lipids contain a complex array of other lipid types including many glycosylated and sulphur-containing lipids involved with the photosynthetic apparatus of these organisms.

The process of oil accumulation in microbial cells is now well established. Although one or two bacteria, notably *Rhodococcus opacus*, can produce oils rich in triacylglycerols (see Bröker *et al.*, 2010), commercial production of oils is currently confined to fungi (including both yeasts and filamentous fungi) and algae, the latter mainly being cultivated heterotrophically rather than photosynthetically. The processes are similar: all rely on the organism of choice being grown in a medium in which the carbon source, usually glucose, is in excess but with nitrogen (usually as NH_4^+ salts or urea) being a limiting nutrient. Thus, after an initial phase where growth is balanced and all nutrients are available to the cells, cells become depleted of N and no longer are able to multiply (as clearly a supply of N is essential for new protein and nucleic acid biosynthesis). However, the oleaginous microorganisms continue to assimilate the available glucose (or whatever carbon source is available) and this is then preferentially channeled into lipid biosynthesis.

The biochemistry of lipid accumulation has been investigated mainly in yeasts and filamentous fungi (see Ratledge, 2004; Ratledge and Wynn, 2002). Little substantial work has yet been done with algae – either photosynthetically-grown species or in obligate heterotrophic algae. This is then a major research area that is in urgent need of addressing. Although the continued synthesis of acetyl-CoA, as the essential precursor of fatty acid biosynthesis, after the exhaustion of nitrogen from the growth medium is crucial for lipid accumulation, the key enzyme involved, ATP:citrate lyase, does not appear to be the rate-limiting step. This role has been proposed as being fulfilled by malic enzyme that then provides the essential reducing power, in the form of NADPH, to fatty acid synthase for the reduction of the CH_3CO- group of acetate into -CH_2-CH_2- as part of the growing fatty acid chain. However, this may not be the case in all oleaginous organisms as some yeasts, notably *Yarrowia lipolytica*, appear only to have an NAD^+-dependent malic enzyme (Beopoulos *et al.*, 2011; Zhang *et al.*, 2013). The NADP-dependent enzyme, though, does occur in other yeasts and in filamentous fungi. There is, therefore, still much that needs to be done to unravel the exact mechanism of lipid accumulation in many oleaginous microorganisms.

3 Commercially-produced microbial oils

3.1 Gamma-linolenic acid

The first microbial oil that was produced was from a filamenous fungus, *Mucor circinelloides*. This oil was rich in the essential fatty acid, gamma-linolenic acid (GLA; 18:3 n-6) and its production lasted from 1985 to 1990. It was produced by J & E Sturge (Selby, North Yorkshire, UK) using fermenters of 220 m^3 that were normally used for the production of citric acid using *Aspergillus niger*. This fatty acid had previously been exclusively produced using the seed oil of *Oenothera biennis* (the evening primrose) and was, until the arrival of the GLA-SCO, very expensive. The oil was considered useful for the treatment of multiple sclerosis, though this is a claim that has now been discounted. However, the oil from evening primrose was sold, and continues to be sold (mainly in the UK), as a dietary supplement useful for the alleviation of premenstrual tension in women. The fungal oil (see Tab. 1) was produced to provide a cheaper alternative to the evening primrose oil having almost twice the content of GLA as the plant seed oil. However, the launching of the oil, with the name of Oil of Javanicus, resulted in a price war between the two rival products with the result that the anticipated profits from the biotechnological process were insufficient to sustain production. Only low volumes of the oil were produced: about 5 to 10 tonnes

per year. Thus, in 1990, with the lower price of evening primrose oil, coupled with the arrival of borage oil from *Borago officinalis* with an even higher content of GLA at about 22% of the total fatty acids, it was considered that Oil of Javanicus was no longer economic. A full account of the development of the process and of the oil itself has been provided by Ratledge (2006).

Nevertheless, the launching of this first microbial oil for human consumption indicated that other microbial oils could probably be considered as being of sufficient safety to be offered for sale. Extensive trials of the GLA-SCO in various animals had been carried out prior to its launch into the specialty oils market and had indicated that the oil had an excellent safety record. This, coupled with its consumption by many humans buying the oil as an over-the-counter nutraceutical during the time it was being produced, showed that the oil had no deleterious properties that would adversely affect its place in the market. The time was now right for microbial oils to become part of the speciality oils market if the right oils could be found that would justify the high cost of the biotechnological process. This was subsequently realized by the production of various polyunsaturated fatty acids.

3.2 Polyunsaturated fatty acids (PUFAs)

3.2.1 DHA and ARA

In the late 1980s and early 1990s, a new company, Martek Inc, in the USA began to explore the possibility of producing long-chain polyunsaturated fatty acids using microorganisms. The key targets were initially eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) (Boswell *et al.*, 1992). Both these PUFAs were known to be components of fish oils but neither of them could be produced as individual fatty acids in any plant or animal oil. Whilst it was uncertain at this time whether there was a sufficiently large enough market for an EPA-only oil, it seemed to David Kyle, the founder and chief scientist of Martek, that there was a market for a DHA-only oil for the infant nutrition market (Kyle *et al.*, 1992). There was already considerable evidence that DHA was important for the development of visual acuity in infants and also in brain and neural development as the content of DHA both in retinal and brain tissues was extremely high. The company then began to exploit a known producer of an oil rich in DHA – namely, *Crythecodinium cohnii*. This is a marine dinoflagellate that is an obligate heterotroph having lost its photosynthetic ability. Nevertheless, it could be grown in large fermenters to a high yield and with a high oil content. The fatty acid profile of the oil is given in Table 1. Development of the process then occurred during the mid-1990s (see Kyle, 1996) with most, if not all, of the initial oils being provided to clinical research groups for investigations into the benefits of the oil for the improved nutrition of premature babies and neonatal children. The results were outstandingly good. Production of the DHA-SCO was then assured but there was a problem.

DHA, when administered as a single PUFA in an oil, either to young children or even to adults, can be retro-converted into EPA by simple loss of a C_2 unit. EPA was, however, contra-indicated as being of beneficial effect in babies and so the DHA oil was not as effective as originally hoped. It was then discovered that if DHA was given to infants along with another PUFA, arachidonic acid (ARA; 20:4 n-6), that the ARA would prevent this retro-conversion of DHA to EPA by the simple expedient of blocking the degradative pathway. A combination of DHA plus ARA was then the optimum choice for infant dietary supplementation (Sinclair and Jayooriya, 2010). Fortuitously, a process for ARA production, also using a microorganism, had been identified by Japanese workers in the 1980s (Totani *et al.*, 1987). The production organism was a filamentous fungus, related to *Mucor circinelloides* that had been used to produce GLA oil – see above; this was *Mortierella alpina*. The possibility of being able to produce ARA by fermentation was then developed by the Dutch company, Gist-brocades. An agreement was subsequently reached between Martek Inc and Gist-brocades for the latter company to produce the ARA-rich oil for exclusive sale to Martek. The fatty acid profile of this oil is given in Table 1. Martek, in the meanwhile, had developed large-scale technology for the growth of *C. cohnii* and for the recovery and purification of the oil (Kyle, 1996).

A combination of two volumes of ARA oil and one volume of DHA oil was found to be the most effective ratio to provide an oil appropriate for providing the key PUFAs to newly-born infants, including premature babies. The success of this mixed oil was quickly established and has now resulted in sales of the oil into over 70 countries with sales to 23 individual companies. Extensive safety trials, both in adults and in infants, have shown the oil to have an unimpeachable safety record (see Ryan *et al.*, 2010). The FDA of the USA gave approval and GRAS status to the oil in 2002.

Sales of DHA and ARA oils have steadily increased since they were launched in the late 1990s. Martek itself was taken over by DSM (Dutch State Mines), who had previously acquired Gist-brocades, the producer of the ARA-rich oil, in 2011. The selling price of the company was US $ 1.1 billion. For the last year of trading as an individual company, Martek BioSciences recorded a revenue of US $ 317 million for sales of the oil for infant nutrition. Although the selling price of the oil is commercially sensitive, it is considered that at least 2000 tonnes of the DHA-SCO are sold annually. This figure may now have reached 3000 tonnes meaning that 6000 tonnes of the ARA-SCO must also be produced.

The ARA-rich oil is also produced by Suntory in Japan, being sold under the trade name of SUNTGA40S, and by Cargill in conjunction with Wuhan Alking Bioengineering Co. Ltd in China where it is sold for incorporation into infant formulae. In 2012, the EU gave approval to allow for sales of the oil for infant nutrition in Europe. The Cargill product has the provisional name of CABIO oil (see Tab. 1).

Besides *Crypthecodinium cohnii* being used to produce a DHA-SCO, DHA is also produced commercially by various strains and species of thrautochyrids. These are marine microorganisms belonging to the heterokont group of algae; they comprise numerous species all of which appear to produce oils with varying amounts of DHA. The main organisms used for DHA production are *Schizochytrium, Aurantiochytrium* and *Ulkenia* (also known as *Labyranathula*). The original company that developed the process was OmegaTech in the USA led and pioneered by Bill Barclay. This company was acquired

by Martek in 2002. The oils produced by these organisms (see Tab. 1) all contain a second PUFA besides DHA: docosapentaenoic acid (DPA: 22:5 n-6). Initially, this fatty acid gave some cause for concern as it was not an n-3 fatty acid but was of the n-6 group of PUFAs. Nevertheless, when it was realized that DPA was a component fatty acid of human brain tissue objections to its presence in the oil were nullified and the oil went on sale for consumption by animals (poultry, fish and other animals) as well as humans; but not to infants. Martek, now DSM, continue to produce this oil. The organism being used, *Schizochytrium* sp., has the distinct feature of being the fastest growing oleaginous organism: it can reach cell densities of > 200 g/L in less than 72 h (Barclay *et al.*, 2010) with oil contents in excess of 40% of the biomass.

The oil is, however, also produced by a number of other companies using different strains or species of these thraustochyrids. These companies include Lonza Group in Switzerland and several other smaller companies in the USA and UK. The oil is also sold by mail order and is usually described as "an algal oil rich in DHA". No mention is made of the presence of DPA but if analyses are carried out on the oils, and if DPA is detected, then production organism is certainly a thraustochytrid. Some production also occurs in China (for example by Jiangsu Tiankai Biotechnology Co. Ltd) where, unlike in the USA and Europe, the oil is mixed with an ARA oil (presumably from Cargill) and sold for infant nutrition. No ill effects of the oil have been reported and so it may be presumed that DPA is not detrimental to young babies. Sales of these oils may therefore be expected to increase particularly in China and elsewhere in South East Asia.

3.2.2 EPA (20:5 n-3)

The nutritional benefits of EPA are less clear that those for DHA. Combinations of EPA and DHA, that are considered beneficial for the prevention of various cardiac problems, are easily satisfied by consumption of fish oils particularly those from the so-called oily fish. But not everyone, and of course, vegans and some minor religious groups, are willing to have fish products in their diet and so microbial oils containing these PUFAs will find a small but ready market. Algal oils contain a mixture of PUFAs: ARA, EPA and DHA in varying proportions. Some of these algae are grown phototrophically and may prove to be useful sources of this PUFA. Others, such as *Nitzschi laevis, Navicula pelliculosa* and *Cylindrotheca fusiformis*, have been identified (Tan and Johns, 1996; Wen and Chen, 2003, 2010) that can be grown heterotrophically using a fixed carbon source – usually glucose. *Nannochloropsis* spp. have also been suggested as possible producers (Zittelli *et al.*, 1999; Rodolfi *et al.*, 2009). Companies known to be developing technologies for the production of EPA using micro-algae include Algisys LLC (Cleveland, OH, USA), Qualitas Health Ltd (Israel), and Photonz (New Zealand). In these cases, the lipid being produced is not a triacylglycerol that is found in the oleaginous yeasts and moulds, but is a mixture of lipid types: galactolipids, phospholipids and some sulfolipids. Only a small proportion of the total EPA will be in a triacylglycerol fraction. The oil produced by Qualitas Health is produced by a photosynthetically-grown alga and is probably a mixture of EPA with other PUFAs; it is sold under the trade name of EicoOil.

EPA has been advocated as a highly desirable PUFA that can exert beneficial effects on blood pressure, platelet aggregation and various inflammatory responses. It has also been used for the alleviation of some neuropsychiatric disorders, including manic depression (bipolar disorder), depression, schizophrenia and also attention deficit hyperactivity disorder in children (see Ratledge, 2013 for further information). EPA, derived from fish oil fractionation (that is an extremely expensive process) and is used as its ethyl ester, is already used in the clinical management of hypertriglyceridemia – a high and potentially dangerous level of triacylglycerols in the blood. This preparation is produced by Amarin Corp plc. However, because of the high price of this oil and now a possible market for it, alternative sources of EPA from microbial sources now seem attractive. Hence, the companies mentioned above are actively pursuing various algae as possible sources of EPA.

An alternative, however, to using microalgae for EPA production has been developed by DuPont (Wilmington, DE, USA) using a genetically-modified yeast, *Yarrowia lipolytica*. To achieve synthesis of EPA in this yeast, that normally produces fatty acids only up to linoleic acid (18:3 n-3), between 15 and 20 genes had to be introduced into the cells. A profile of the fatty acids is given in Table 1. An account of the molecular procedures that have been used has been given by Xue *et al.* (2013). By using a naturally-occurring, oleaginous yeast, the DuPont team did not need to introduce genes into the organism to promote lipid accumulation as the final oil content of the cells remained at about 35–40% (Damude *et al.*, 2011). The EPA-rich oil has now received GRAS status from the FDA and is marketed and sold through a wholly-owned subsidiary company of DuPont, New Harvest. The oil appears to be primarily intended as an over-the-counter nutraceutical rather than being sold as a "medical food", as will probably be the case with EPA ethyl esters derived from algal lipids. This, however, may change.

4 Oils as biofuels

Over the past 10 years, considerable work has been carried out by numerous start-up companies, mainly in the USA, to produce oils using photosynthetic algae. Over 200 such companies are thought to have been registered for this purpose (Torrey, 2010). Regrettably, the science behind these intentions has been poorly appreciated and the economics look very doubtful (Ratledge and Cohen, 2008). Most of the preliminary investigations into the possibilites of using photosynthetic algae have used various photobioreactors that require illumination and agitation; even so, light penetration into cultures with biomass densities of no more than 4 g/L becomes limiting and further growth is then very slow. In addition, CO_2 must be fed into the bioreactors to keep the C level high: oleaginous algae are just like other oleaginous microorganisms and require an excess of carbon coupled with a deficiency in another nutrient (usually this will be nitrogen) in order to engender lipid accumulation. In this way, lipid contents of algal cells of up to 60% have been achieved: see for example, Toledo-Cervantes *et al.* (2013) who grew *Scendesmus obtusiusculus* in an air stream with 10% CO_2 to achieve lipid contents of 56% over

16–24 days. Similarly, Yoo *et al.* (2013) grew a species of *Etlia* with CO_2 between 5–10% of the air supply and achieved an oil content of 42% of the biomass over 16 days culture. But the biomass never exceeded 4 g/L. It is these types of results and similar earlier ones that have encouraged the concept of being able to extend the cultivation of algae into open ponds or lagoons in the expectation that similar productivities of lipid will be achieved. Unfortunately, these expectations have not been realized.

The main problems with large-scale algal cultivation may be seen as follows:

1. The cost of even the simplest photobioreactor, which is probably arrays of polythene tubing located in some appropriate warm and sunny clime, is still too expensive for the production of a relatively cheap oil needed for conversion into biodiesel. Such systems are used commercially but only for the production of high-value materials such as astaxanthin using *Haematococcus pluvialis* where the price of the biomass can be calculated to be about US $ 40/kg (Ratledge and Cohen, 2008). Thus, it is obligatory that open ponds, not raceways as these still are expensive to construct and run, or lagoons are used.
2. Supply of CO_2. It is considered that about 14 tonnes of CO_2 are needed to produce 1 tonne of extractable lipid from an algal (this is based under semi-optimal conditions involving closed bioreactors). How much is needed in cultures that are open to the atmosphere is unknown but clearly sophisticated mechanisms will be needed to distribute CO_2 evenly throughout large cultivation areas and attempt to hold it in solution other than being a transient gas requiring continuous addition. This then begs the question as to where this CO_2 is to be derived. Usually CO_2 discharges from coal-fired power stations are advocated as possible sources but power stations are not usually located in areas that will be used for algal cultivation. Piping CO_2 over large distances is clearly not an economic proposition. Neither is condensing and bottling the CO_2 in large containers an economic proposition.
   In addition to the sourcing of CO_2 and its delivery to algal cultures, Larkum (2010) has pointed out a serious flaw in the argument that algae can outcompete land-based plants for productivities: land plants have evolved to use CO_2 from the atmosphere whereas algae must fix it from water. The former process is some 10 000 times faster than the latter and thus all algae must either be limited by natural mixing and stirring, and therefore will grow slowly, or these cultures must be mechanically stirred which will inevitably increase production costs.
3. Water is another critical factor. With open algal ponds having to be located in warm (desert-like) regions of the world that have minimal rainfall (as tropical downpours would tend to wipe out algal cultures very quickly), evaporation of water becomes a major concern. The water has to be replaced daily otherwise the ponds will become hypersaline and, importantly, the replacement water must be relatively pure as, again, if seawater were used then continuous evaporation would lead to unacceptable salinity levels. The only acceptable solution would appear to grow algae in marine lagoons or in areas of brackish water that would then be automatically replenished with tidal seawater twice daily. This, however, poses additional problems of the surface of the algae rising and falling and, from this, there are major difficulties in achieving high cell densities and high lipid contents of the cells due to the poor mixing in such systems not to mention the problems of supplying a surfeit of CO_2 to the cells.
4. Night-time and winter time. What happens to algae during the periods of darkness seems to be largely ignored when calculations of lipid productivities have been announced. Lipids, being produced as storage materials, are used during the night as the cells switch from photosynthesis to respiration. In the dark, cells no longer have a source of energy and must therefore derive metabolic energy (for cell maintenance) from accumulated materials within the cells. Thus the content of lipid, that may have increased during daylight hours, now decreases as it is consumed to provide metabolic energy to the cell. Winter time also is something that has usually been ignored when calculations have been made to extrapolate laboratory data into possible yields and productivities obtainable outdoors. Few suitable places for algal culture do not have winters and almost all will have some rainfall during this time. Thus it may only be possible to run open algal ponds optimally for, at the very most, no more than 10 months of the year. These factors then seriously diminish the rather ambitious calculations for lipid productivities that were advanced when algal technology for biofuel production was first being proposed.
5. Finally, contamination of open ponds by adventitious protozoa or nuisance algal weeds that would result in large-scale disruption of the algal cultures cannot be ruled out. Also, open systems are not ‘good manufacturing practice’ as contamination from all manner of wild life is a serious and worrying possibility. Some microbial pathogens may also be able to grow in such conditions.

For these reasons, many of the original start-up companies have begun to look at producing higher value algal products than just oils for biodiesel. There has been a gradual realization that the hopes of being able to produce a biofuel, literally from the CO_2 in the air, were rather fanciful. By definition, oils for biodiesel must compete with plant oils, such as palm oil and sunflower oil, that are already in use. Also, the fatty acids of algal oils are far from the ideal needed for biodiesel that preferentially requires C16 and C18 saturated or mono-unsaturated fatty acids. Polyunsaturated fatty acids are not desirable as they would need hydrogenation; neither are fatty acids longer than C18 but, unfortunately, most algal lipids have C20 and sometimes C22 fatty acids in them. Where companies, such as Solazyme, have declared a continuing interest in producing algal oils for biodiesel, the alga in question are no longer being grown photosynthetically. Solazyme, probably the leading company in this field, is using a *Chlorella* sp. that is being grown on glucose in large stainless steel tanks. The process is then no different from other SCO processes except that, because a photosynthetic alga is being used, albeit not being grown photosynthetically, the process can then attract substantial tax relief from the US authorities! But, in principal, this process will then compete with other similar processes being developed by such companies as BP

using more orthodox approaches to produce appropriate SCOs as sources of biofuels. However, Solazyme are not just producing an oil for biodiesel but are diversifying the range of products to include food materials, possible lubricants as well as anti-wrinkle creams based on myristate and laurate fatty acids

Even the most up-beat summaries of the current prospects for the microalgal industry (for example see Stephens *et al.*, 2013) do not foresee much chance of algal oils fulfilling any significant role in the foreseeable future. The most realistic estimates still put algal biofuels some 10 years into the future (King, 2013). Instead, higher value products will therefore be the new focus of many of the enterprises.

5 The future

The production of key PUFAs, such as DHA and ARA, are likely to continue to be produced by microbial technology for many years to come. The existing oils produced by *C. cohnii*, *Mort. alpina* and the thraustochyrids will continue to be predominant. It is likely that EPA-rich oils or EPA esters will be soon be produced using either GM yeast (the DuPont project) or from various algae grown either photosynthetically or heterotrophically. The prospects of obtaining oils rich in these long-chain PUFAs from GM plants remains as distant to-day as it was 10 years ago in spite of many claims of near-success. However, undoubtedly plant geneticists will continue to strive to produce such plants and, maybe, one day we will all be surprised and enthusiasts for microbial oils, such as the present author, will then be confounded.

The prospects, however, of producing SCOs for biofuels remain uncertain, though it is highly likely that these will not be realized through photosynthetic algae. Plant-based oils will continue to be much cheaper than algal oils for the foreseeable future. The same might not apply to heterotrophically-grown microorganisms where both yeasts and algae, such as *Chlorella* used by Solazyme, might be profitable if a cheap enough carbon source, such as sucrose from sugar cane, can be provided in sufficient quantities to make very large-scale operations economically viable. But, all the speculations about microorganisms being a cheap source of energy, may be thwarted by the arrival of shale oil and fracking (hydraulic fracturing) as the newest and cheapest source of gas around the world. Such technology would clearly revolutionize our current thinking on energy provision and would, in all probability, render SCOs irrelevant in this respect. But for the provision of key long-chain polyunsaturated fatty acids, the foreseeable future must remain with microorganisms.

References

Barclay W, Weaver C, Metz J Hansen J. 2010. Development of a docosahexaenoic acid production technology using Schizochytrium: historical perspective and update, In: Cohen Z, Ratledge C, eds. Single Cell Oils. 2nd ed. Champaign, IL: AOCS Press, 75–96.

Beopoulos A, Mrozova Z, Thevenieau F, Le Dall MT, Hapala I, Papanikolaou S, Chardot T, Nicaud JM. 2008. Control of lipid accumulation in the yeast *Yarrowia lipolytica*. *Appl. Environ. Microbiol.* 74: 7779–7789.

Boswell KDB, Gladue R, Prima B, Kyle DJ. 1992. SCO production by fermentative microalgae. In Kyle DJ, Ratledge C, eds. Industrial applications of single cell oils. Champaign, IL: AOCS Press, 274–286.

Bröker D, Elbahloul Y, Steinbuchel A. 2010. Production of lipids for biofuels using bacteria. In: Cohen Z, Ratledge C, eds. Single Cell Oils, 2nd ed. Champaign, IL: AOCS Press, 291–314.

Damude HG, Gillies PJ, Macool DJ, Picatoaggio SK, Pollak DMW, Ragghianti JJ, Xue Z, Yadav NS, Zhang H, Zhu QQ. 2011. High eicosapaentaenoic acid producing strains of *Yarrowia lipolytica*, *US Patent*, 7,932,077 B2 (Apr. 26, 2011).

King A. 2013. Lean green microbe machines. *Chem World* 10: 44–47.

Kyle DJ. 1996. Production and use of a single cell oil which is highly enriched in docosahexaenoic acid. *Lipid Technol.* 8: 107–110.

Kyle DJ, Sicotte VJ, Singer JJ, Reeb SE. 1992. Bioproduction of docosahexaenoic acid (DHA) by microalgae, In: Kyle DJ, Ratledge C, eds. Industrial applications of single cell oils. Champaign, IL: AOCS Press, 287–300.

Larkum AWD. 2010. Limitations and prospects of natural photosynthesis for bioenergy production. *Curr. Opin. Biotechnol.* 21: 271–276.

Ratledge C. 1974. Microbial production of oils and fats. In: Birch GG, Parker KJ, Worgan JT, eds. Food from Waste. UK: Applied Science Publishers, 98–113.

Ratledge C. 2004. Fatty acid biosynthesis in microorganisms being used for single cell oil production. *Biochimie* 86: 807–815.

Ratledge C. 2006. Microbial production of gamma-linolenic acid, In: Akoh AA, ed. Handbook of Functional Lipids. Baco Raton: Taylor & Francis, 19–45.

Ratledge C. 2013. Microbial production of polyunsaturated fatty acids as nutraceuticals. In: McNeil B, Archer D, Giavasis I, Harvey L, eds. Microbial production of food ingredients, enzymes and nutraceuticals. UK: Woodhead Publishing Co., 531–558.

Ratledge C, Cohen Z. 2008. Microbial and algal oils: do they have a future for biodiesel or as commodity oils? *Lipid Technol.* 20: 155–160.

Ratledge C, Wynn JP. 2002. The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. *Adv. Appl. Microbiol.* 51: 1–51.

Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, Bonini G Tredici MR. 2009. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivatin in a low-cost photobioreactor. *Biotech. Bioeng.* 102: 100–112.

Ryan A, Zeller S Nelson EB. 2010. Safety evaluation of single cell oils and the regulatory requirements for use as food ingredients, In: Cohen Z, Ratledge C, eds. Single Cell Oils. 2nd ed. Champaign, IL: AOCS Press, 317–350.

Sinclair AJ, Jayasooriya A. 2010. Nutritional aspects of single cell oils: applications of arachidonic acid and docosahexaenoic acid oils, In: Cohen Z, Ratledge C, eds. Single Cell Oils. Champaign, IL: AOCS Press, 351–368.

Stephens E, Ross IL, Hankamer B. 2013. Expanding the microalgal industry – continuing controversy or compelling case? *Curr. Opin. Chem. Biol.* 17: 1–9.

Tan CK, Johns MR. 1996. Screening of diatoms for heterotrophic eicosapentaenoic acid production. *J. Appl. Phycol.* 8: 59–64.

Toledo-Cervantes A, Morales M, Novelo E, Revah S. 2013. Carbon dioxide fixation and lipid storage by *Scenedesmus obtusiusculus*. *Biores. Technol.* 130: 652–658.

Torrey M. (2010). Survey of commercial developments of microalgae as biodiesel feedstock. In: Cohen Z, Ratledge C, eds. Single Cell Oils. 2nd ed. Champaign, IL: AOCS Press, 243–270.

Totani N, Watanabe A, Oba K. 1987. An improved method of arachidonic acid production by Mortierella sp. S-17. *J. Jpn Oil Chem. Soc.* 36: 328–331.

Wen ZY, Chen F. 2003. Heterotrophic production of eicosapentaenoic acid by microalgae. *Biotechnol Adv* 21: 273–294.

Wen ZY, Chen F. 2010. Production of eicosapentaenoic acid using heterotrophically grown microalgae, In: Cohen Z, Ratledge C, eds. Single Cell Oils, 2nd ed. Champaign, IL: AOCS Press, 151–177.

Xue ZX, Sharpe PL, Hong SP, Yadav NS, Xie D, Short DR, *et al.* 2013. Sustainable source of omega-3 eicopentaenoic acid by metabolic engineering of *Yarrowia lipolytica*. *Nature Biotech.* 31: 734–740.

Yoo C, Choi GG, Kim SC, Oh HM. 2013. Ettalia sp. YC001 showing high growth rate and lipid content under high CO_2. *Biores. Technol.* 127: 482–488.

Zhang H, Zhang L, Chen H, Chen YQ, Ratledge C, Song Y, Chen W. 2013. Regulatory properties of malic enzyme in the oleaginous yeast, *Yarrowia lipolytica*, and its non-involvement in lipid accumulation. *Biotech. Lett.*, in press.

Zittelli GC, Lavista F, Bastianini A, Rodolfi L, Vincenzini M, Tredici MR. 1999. Production of eicosapentaenoic acid by Nannochloropsis spp. cultures in outdoor tubular photobioreactors. *Prog. Indust. Microbiol.* 35: 299–312.

Cite this article as: Colin Ratledge. Microbial oils: an introductory overview of current status and future prospects. OCL 2013, 20(6) D602.