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Catalytic Hydroprocessing of Single-Cell Oils to Hydrocarbon Fuels

Converting microbial lipids to fuels is a promising approach to replace fossil fuels

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Microbial lipids hold great promise as biofuel precursors, and research efforts to convert such lipids to renewable diesel fuels have been increasing in recent years. In contrast to the numerous literature reviews on growing, characterising and extracting lipids from oleaginous microbes, and on converting vegetable oils to hydrocarbon fuels, this review aims to provide insight into aspects that are specific to hydroprocessing microbial lipids. While standard hydrotreating catalysts generally perform well with terrestrial oils, differences in lipid speciation and the presence of co-extracted compounds, such as chlorophyll and sterols, introduce additional complexities into the process for microbial lipids. Lipid cleanup steps can be introduced to produce suitable feedstocks for catalytic upgrading.

Introduction

Oils derived from oleaginous microbes, such as algae, yeast and bacteria (so-called single-cell oils (SCOs)) have long held promise as a biofuel precursor due to the high lipid content (>20%), high growth rate and the ability of these microbes to be cultivated on feedstocks or in areas that do not compete for terrestrial food production (1). Historically, the availability of microbial lipids has been quite limited, but technology developments in food and feed applications may begin to benefit lipid production for biofuels as well. Corbion produces an algae oil high in oleic acid for culinary use, and an algae-based omega-3 fatty-acid-enriched product for fish feed. Qualitas Health, DSM, Evonik and Veramaris also produce omega-3 enriched oils for food and feed applications, though to our knowledge only Qualitas Health is producing the algae photoautotrophically. While the target omega-3 fraction is not ideal for fuel production due to long carbon chains and numerous double bonds that would increase hydrogen consumption during hydroprocessing, the process to enrich omega-3 oils likely generates a byproduct of lighter and more saturated fatty acids that may be more suitable for fuel production than the omega-3 fraction. Additionally, although many oil majors and startup companies have pivoted away from algal biofuels, a partnership between Synthetic

Genomics and ExxonMobil to improve algal biology for fuel production is still active. The increasing availability of cellulosic sugars may also pave the way for larger scale cultivation of heterotrophic oleaginous microbes.

While both the growth and characterisation of oleaginous microbes (1–13) and the conversion of vegetable oils to fuels (13–27) have been extensively studied, there has been considerably less research on converting SCOs to fuels, and especially on converting SCOs to so-called 'drop-in' hydrocarbon fuels that are compatible with existing fuel infrastructure (28–30). However, production of hydrocarbon fuels from SCOs is a growing area of research and hydroprocessing of these oils requires some considerations that are unique to the differing composition of SCOs. Enough recent studies have been published that a summary of findings and perspective on research directions is warranted, especially with respect to the differences between terrestrial oils and SCOs. In particular, lipid recovery, lipid cleanup and catalyst selection all appear to play a key role in hydroprocessing performance, and there are clear needs for deeper research in each of these areas.

Lipid Recovery

In most cases, SCOs are produced intracellularly, and thus necessitate separation from the other cell components prior to upgrading. Additionally, the microbial biomass is typically recovered with a very high water content (≥80%) relative to terrestrial biomass. Thus, while vegetable oils can be effectively extracted by pressing, solvent extraction or a combination thereof, these operations are usually not effective when applied directly to microbial biomass. Furthermore, it is energy intensive to dehydrate microbial biomass and therefore strategies that can extract oil in high yields from wet biomass are required. The first step is commonly to rupture the microbial cell wall which allows access to the lipids.

Various cell wall lysis methods have been applied for this application, such as high-pressure homogenisation (HPH), bead milling, ultrasound, pulsed electric field (PEF), osmotic shock, microwave, subcritical water hydrolysis, enzymatic hydrolysis, autolysis and chemical hydrolysis (31). From the perspective of energy consumption, pretreatment methods such as HPH, subcritical water hydrolysis, enzymatic and chemical hydrolysis are more attractive due to the relatively lower energy requirements. Moreover, all these methods can be scaled up to industrial application (32). Researchers at the National Renewable Energy Laboratory (NREL) have shown that dilute acid pretreatment is an effective, low cost, energy efficient and scalable means to recover lipids from wet microbial biomass (31–34).

It is also necessary to consider the compatibility of the cell lysis method with lipid extraction. For example, hexane is a commonly employed extraction solvent, but does not always maximise extraction yields. In many cases, modification of solvent polarity (for example employing a mix of hexane with ethanol or isopropanol) is necessary for high lipid recovery (35–39). However, incorporation of a polar solvent also often leads to more prevalent co-extraction of other polar materials, such as polar lipids, pigments, proteins and sterols, that require more severe cleanup of the lipid phase. In particular, extraction methods that incorporate chlorinated solvents, such as the well-known Folch and Bligh-Dyer extraction protocols, tend to extract significant amounts of polar lipids and other polar materials (36, 38). Combinations of hexane with alcohols, such as ethanol and isopropanol, have the potential to more selectively extract hydrophobic lipids while maintaining high extraction yields of these components (31, 39, 40). The optimal balance is thus a function of lipid speciation, content and speciation of other potential co-extractives, the lipid cleanup strategy and the hydroprocessing approach.

Lipid Composition

SCOs that are considered for biofuel production are mainly recovered from oleaginous algae, fungi (yeast and filamentous) and bacteria, with the number of known oleaginous species decreasing by family in the order listed. These microbes may be cultivated autotrophically (using only CO_2 as a carbon source), mixotrophically (using a mixture of CO_2 and organic carbon sources) or heterotrophically (using organic carbon sources, such as glucose and acetate). Several recent reviews have compiled oil content and broad composition of oleaginous microbes and their production of oils with an emphasis on wastes and lignocellulosic carbon sources (7–9, 11). A sampling of these compositions is reproduced in **Table I**, with comparison to common vegetable oils. It is important to note that analytical methods

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Table I Lipid Content and Speciation in Common Oleaginous Microbes and Terrestrial Plants

Species	Lipid content (% dry weight)	Fatty acids (% total lipid)											Reference
		C12:0	C14:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	
Microalgae and cyanobacteria													
Chlorella sp.	28–32	–	–	7–19	10–11	–	1–4	8–9	1–14	16–19	–	–	(41)
Chlorella zofingiensis	28–32	–	–	23	2	–	2	36	18	8	–	–	(41)
Crypthecodinium cohnii	23	–	13	23	–	–	3	8	–	–	–	–	(41)
Chatoceros muelleri	31–68	–	18–40	5–40	–	–	0.0–25.0	0–4	0–5	0–5	–	–	(41)
Schizochytrium linacinum	50–77	–	3–20	54–60	–	–	1–4	–	–	–	–	–	(41)
Various (10 species)	–	–	0.2–7.9	6.2–35.8	0.3–22.0	0.3–8.6	1.3–19.0	12.6–75.2	1.3–14.6	1.5–33.0	0.0–3.6	0.0–3.9	(42)
Dunalliela salina	6–27	6.9	2.8	9.8	14.7	–	3.8	8.0	4.9	12.9	1.7	24.8	(43, 44)
Synechocystis sp. PCC6803	18	19.9	20.9	43.3	–	–	11.3	1.5	1.1	1.4	–	–	(45)
Yeast													
Cryptococcus albidus	60	–	–	12	–	–	1	3	73	12	–	–	(41)
Cryptococcus curvatus	55–60	–	–	18.0–30.0	–	–	10.0–12.0	35–48	10–28	–	–	–	(10)
Lipomyces starkeyi	63	–	–	34	–	–	6	5	51	3	–	–	(41)
Rhodosporidium toruloides	66	–	–	18	–	–	3	3	66	–	–	–	(41)
Rhodosporidium toruloides	55–60	–	–	23–25	–	–	10–12	48–53	10–15	–	–	–	(10)
Rhodotorula glutinis	72	–	–	37	–	–	1	3	47	8	–	–	(41)
Trichosporon guehoae	65–78	–	–	20–22	–	–	10–25	35–38	12–22	–	–	–	(10)
Saccharomyces cerevisiae	30–50	–	–	8–10	32–43	–	4–7	42–48	–	–	–	–	(46)
Yarrowia lipolytica	36	–	–	11	–	–	6	1	28	51	–	–	(41)
Bacteria													
Acinetobacter baylyi	12.4	–	5	1	30	–	40	–	–	–	–	–	(47)

(Continued)

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Table I Continued

Species	Lipid content (% dry weight)	Fatty acids (% total lipid)											Reference
		C12:0	C14:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	
Alcanivorax borkumensis	23.2–26.9	–	2.9–15.0	36.8–85.3	tr–19.7	–	2.4–8.3	tr–41.2	–	–	–	–	(48)
Rhodococcus opacus	27.5–68.1	–	2.7–5.1	23.0–31.0	9.9–11.7	17.2–18.3	5.6–7.1	18.9–24.4	–	–	–	–	(49)
Rhodococcus opacus	19.0–26.0	–	–	–	–	–	3.0–19.0	6.0–74.0	–	–	–	–	(41)
Streptomyces coelicolor	64.2–83.0	–	8–27	28–38	–	1–3	1–3	–	–	–	–	–	(50)
Filamentous fungi													
Aspergillus sp.	12.4–22.1	–	–	0.8	14.4–14.8	22.5–29.6	43.4–48.7	0.4–1.6	0.4	0.1	3.0–12.2	0.1–2.9	(51)
C. echinulata	6.5–46.6	–	–	–	–	–	–	–	–	11.0–23.8	–	–	(52)
C. echinulata	5.3–8.4	–	–	22.5–24.7	–	–	6.2–8.2	35.3–38.5	18.3–20.5	12.0–13.8	–	–	(53)
M. isabellina	8.3–25.3	–	–	18.6–25.3	tr–4.9	–	2.0–6.2	45.9–50.4	13.6–20.6	3.5–5.5	–	–	(54)
M. isabellina	40–8–41.8	–	–	21.1–25.3	2.9–3.7	–	11.9–12.3	51.6–52.6	6.4–7.8	–	–	–	(55)
M. isabellina	34.0–50.0	–	–	20.3–29.0	2.0–2.7	–	3.0–5.0	41.2–55.0	3.0–27.8	–	–	–	(41, 56)
M. hiemalis	16.8–20.5	–	–	20.0–23.4	–	–	6.4–8.4	23.2–25.6	16.0–18.8	23.3–23.5	–	–	(53)
Mucor sp.	25.0–50.8	–	–	22.0–28.7	–	–	1.2–5.0	38.0–45.6	7.6–10.0	7.8–10.4	–	–	(41, 53)
Mucor sp.	1–9.4	–	–	13.8–24	tr–11.5	–	1.3–6.9	32.0–41.5	11.0–27.3	8.0–18.6	–	–	(54)
R. stolonifer	9.3–16.3	–	–	27.4–29.6	–	–	5.3–8.1	27.3–29.5	20.0–23.2	13.7–15.9	–	–	(53)
Rhizopus arrhizus	57	–	–	18	–	–	–	6	22	10	12	–	(41)
T. elegans	1–3.3	–	–	12.6–18.7	tr–2.3	–	4.8–12.4	38.7–73.3	5.6–15.7	2.4–23.0	–	–	(54)

(Continued)

Table I Continued

Species	Lipid content (% dry weight)	Fatty acids (% total lipid)										Reference
		C12:0	C14:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	
Z. moelleri	18.1–22.0	–	–	27.3–29.5	–	–	14.4–16.6	23.5–25.9	12.8–14.2	13.3–15.5	–	–	(53)
Plants													
Brassica napus (rapeseed)	45	–	–	4	–	–	2	62	22	10	–	–	(41)
Elaeis guineensis (palm)	50	–	1	44	–	–	4	38	10	1	–	–	(41)
Glycine max (soybean)	8.1–27.9	–	–	10.0–11.0	–	–	4.0	18.0–24.0	54.0–55.0	7.0–13.0	–	–	(41, 57, 58)
Zea mays (corn)	3.0–11.0	–	0.0–0.3	8.6–16.5	0.0–0.05	–	0.0–3.3	20.0–42.2	34.0–65.6	0.0–2.0	–	–	(59)

vary greatly across the references cited in **Table I**. The lipid content data, especially, should be taken with a grain of salt.

Both SCOs and terrestrial plant oils typically contain high proportions of palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1) and linoleic (18:2) acids. On the surface, the similar composition of both oils appears to make SCOs an attractive feedstock for catalytic upgrading. However, SCOs can contain a broader distribution of lipid classes than terrestrial plant oils, including higher proportions of polar lipids, free fatty acids (FFA), sterols, terpenes, carotenoids and chlorophyll, as well as unidentified compounds, that can be co-extracted with the desired monoacylglycerides (MAG), diacylglycerides (DAG), triacylglycerides (TAG) and FFA. Examples of these other lipid classes are shown in **Figure 1**. The proportions of each vary greatly depending on the organism, growth mode, growth condition, growth stage and postharvest processing conditions, including biomass and extracted oil storage and solvent extraction conditions (36, 60). For example, glycolipids and phospholipids can account for 17–90% of the total lipids in autotrophically-grown algae (61), compared to ~2% in soybean oil (62), and lipase enzymes present in the cells can convert acylglycerides to FFA during lipid or biomass storage (60).

TAGs and FFAs are usually considered as the favoured precursors for biodiesel or hydrocarbon-based biofuels such as renewable diesel or renewable jet fuel. Other components are often detrimental in catalytic hydroprocessing as they either increase hydrogen consumption due to a high degree of unsaturation (for example terpenes, carotenoids and chlorophyll), contain heteroatoms that can poison metal or acid catalysts (for example polar lipids and chlorophyll) or contribute to poor cold flow properties (for example sterol-derived cycloparaffins). For example, polar lipids mainly consist of phospholipids, glycolipids, lipoproteins and sulfolipids. A phospholipid molecule consists of a polar phosphorus-containing moiety (such as phosphate, phosphatidylethanolamine or phosphatidylcholine), whereas a glycolipid contains a polar carbohydrate moiety in place of the phosphor group. The sidechains of polar lipids (sugars, proteins or phosphorous-containing molecules) may also include other nitrogen, sulfur or phosphorus-containing moieties (21).

On the other hand, hydrotreating of some of these contaminants can be beneficial in that the

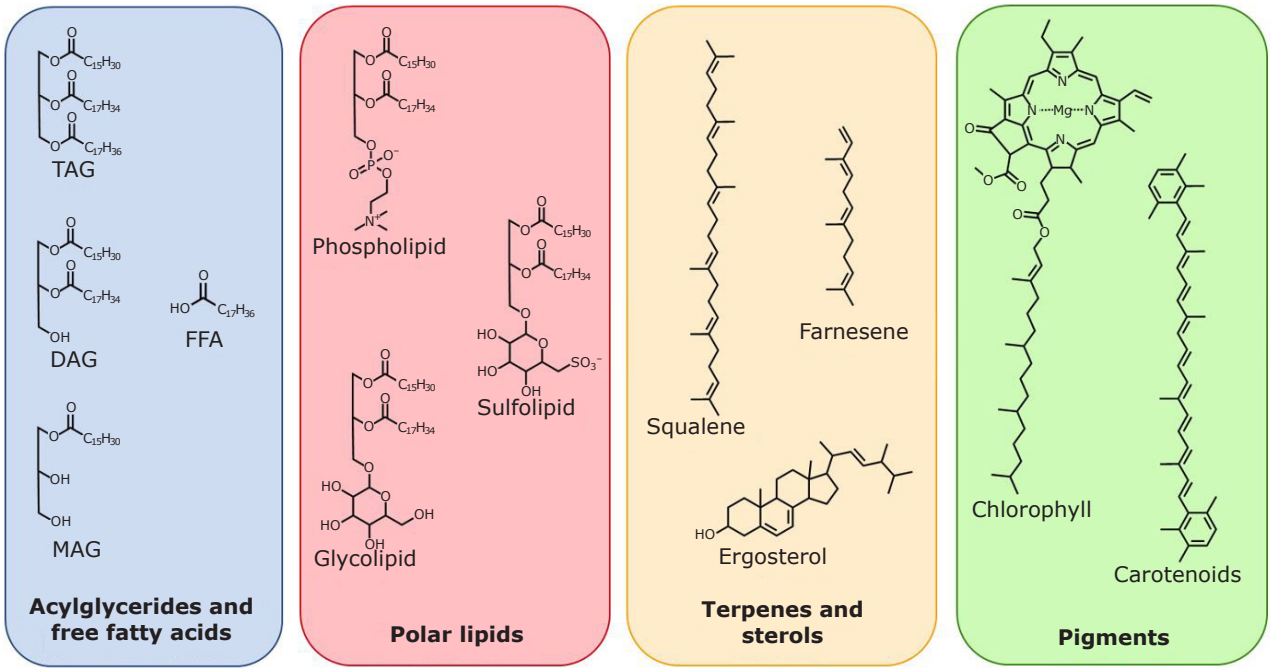

Fig. 1. Lipid classes present in single-cell oils

high degree of branching (for example in terpenes and the phytol chain of chlorophyll) can potentially improve cold flow properties and increase the value of the renewable diesel fuel. Additionally, when a large proportion of the lipid fraction comprises nominally undesirable molecules (such as polar lipids), removal of the entire molecule in a cleanup strategy would result in low fuel yields. Thus, while some cleanup of the oils is likely necessary, the most desirable cleanup strategy is not universal across SCOs, and tradeoffs between overall yields and catalyst longevity must be considered.

Lipid Cleanup

Cleanup of SCOs targets many of the same impurities that are removed in refining of vegetable oils for human consumption, and thus cleanup approaches can be broadly categorised into the same four operations: bleaching to remove pigments and polar compounds, degumming to precipitate phospholipids, deodorisation to remove FFAs and distillation to remove additional FFAs and other volatile matter. When refining SCOs for hydrotreating, some modifications may be necessary. For example, FFAs are one of the preferred feeds for hydrotreating so deodorisation and distillation will not always be needed. However, in some cases it may be advantageous to hydrolyse the entire lipid stream to FFAs, distil these FFAs away from impurities, and route the distillate to hydrotreating. In these cases, bleaching and degumming may not be needed. Researchers have explored to some extent each of these steps except deodorisation. These techniques and their advantages and disadvantages are summarised in **Table II**.

Bleaching

Crude extracted oils from autotrophically-grown microbes are often high in chlorophyll content. The removal of chlorophyll is important in both hydroprocessing and biodiesel production as chlorophyll contamination can deactivate catalysts and degrade fuel quality (60, 63, 64). For example, the porphyrin head of the chlorophyll molecule is highly unsaturated and contains the catalyst-poisoning heteroatoms nitrogen and magnesium. The two most common approaches to remove chlorophyll are adsorption on a solid material and dealkylation *via* acid treatment.

Bleaching earths, activated carbon and silica are common adsorbents for oil refining. Adsorption of chlorophyll is initiated by ion exchange of the Mg^{2+} centre of the porphyrin head with a proton. The metal-free porphyrin is then protonated and adsorbed (65). Adsorbents are commonly pretreated with mineral acids to increase the number of exchangeable protons (66). Ultrasound-assisted

Table II Strategies for Cleaning Up Single Cell Oils

Cleanup technology	Advantages	Disadvantages
Adsorbent bleaching	Relatively inexpensive Established technology Suitable for broad range of impurities	May not fully remove impurities May retain oil on solid, decreasing yields May retain polar lipids, decreasing yields significantly for oils high in these components
Acid bleaching	May be more effective than adsorbents for chlorophyll	Requires additional water to remove acid from oil Targets chlorophyll, may not be effective for other impurities
Degumming	Relatively inexpensive Established technology May be simple to integrate with cell lysis by dilute acid	Targets polar lipids, decreasing yields significantly for oils high in these components May not remove polar lipids that are non-hydratable May not remove impurities other than polar lipids
Hydrolysis and FFA distillation	Suitable for broad range of impurities and lipid compositions	Relatively expensive for cost of alkali Relatively energy intensive
(Trans)esterification and fatty acid methyl ester (FAME) distillation	Suitable for broad range of impurities and lipid compositions	Relatively expensive for cost of alkali, acid or methanol Relatively energy intensive

adsorption has also been demonstrated to increase the rate of chlorophyll removal from oils (67). It is worth noting that the loadings of polar adsorbents to fully remove chlorophyll from SCOs may be much higher than typically used for edible oil bleaching due to the higher levels of chlorophyll (and other adsorbing contaminants). For instance, Chen *et al*. employed bleaching earth to adsorb chlorophyll from microalgal biodiesel at a mass loading of 16 wt% (60), whereas a typical edible oil bleaching process uses a mass loading of 1.5 wt% (68). Increasing adsorbent loading, while effective in reducing chlorophyll content, decreases yields due to co-adsorption of lipids. This is particularly true of bleaching earths, which can retain up to a third of their mass in oil (69), though even higher retentions have been claimed (70). Similarly, Santillan-Jimenez *et al*. removed chlorophyll from crude extracted algae oil *via* adsorption on K10 montmorillonite clay and activated carbon, recovering only 58 wt% and 46 wt% of the original extract mass, respectively (71). Gas chromatography-mass spectrometry (GCMS) analysis of the recovered materials indicated a high content of fatty acids, hydrocarbons and phytol isomers, while ultraviolet-visible (UV-vis) spectroscopic and inductively coupled plasma (ICP) analyses revealed complete chlorophyll, phosphorus and magnesium removal. Notably, the relative abundance of hydrocarbons in the activated carbon-bleached material was lower than in the K10-bleached material, likely due to the greater hydrophobicity of the activated carbon retaining more of the hydrocarbons. This observation is also consistent with the lower total recovered yield from the carbon adsorbent. Thus, adsorbents with higher polarity may be more selective for removal of the target compounds.

Phosphoric acid-catalysed dealkylation was originally developed by Diosady to refine Canadian canola oil, which has anomalously high chlorophyll content for a terrestrial oil due to weather conditions in the region (70). The acid bleaching approach employs anhydrous acids, such as phosphoric acid or sulfuric acid to cleave the phytol side chain from the porphyrin structure, the latter of which is not soluble in oil. Thus, this precipitable form of pheophorbide can be easily separated from the oil by filtration while the hydrophobic phytol is preserved. Retaining the phytol in the oil confers the additional benefit of improved biofuel yield and performance due to a high degree of branching. Dong *et al*. employed a modified version of Diosady's method to remove 99.7% of chlorophyll from algae oil (63). In a direct comparison of adsorptive and dealkylative approaches, Kruger *et al*. found that phosphoric-acid-catalysed dealkylation was more effective than adsorption on silica for the removal of chlorophyll from algae oil (72). In the comparison, adsorption on silica gel removed 85% of the oil nitrogen content and still showed a detectable chlorophyll signal in UV-vis analysis, while acid bleaching removed 92% of the oil nitrogen and did not show a detectable chlorophyll UV-vis signal. Moreover, phosphoric acid bleaching is compatible with conventional vegetable oil refining practice,

since phosphoric acid is routinely added for oil degumming. However, for the same reason and also because the acid must be washed out of the oil by adding water, phosphoric acid bleaching may suffer from yield losses and poor bleaching performance in oils with high levels of phospholipids.

Apart from adsorption and acid treatment, other novel methods of chlorophyll removal have been reported. Sathish developed a multi-step process to remove chlorophyll from microalgal crude oil, involving sequential aqueous acid, base and acid treatments to lyse cells, saponify lipids and precipitate chlorophyll, respectively (73). Though the extraction process was not optimised, roughly 20% of the lipids remained with the biomass solids and another 20% with the precipitated chlorophyll. However, the purified lipids showed no chlorophyll signal in UV-vis analysis. The process is advantageous in that it does not require anhydrous conditions, but the sequential use of concentrated acids and bases is likely to be expensive and generate highly saline wastewater. On the other hand, Li *et al*. removed chlorophyll from intact microalgae by saponification with sodium hydroxide (74).

Degumming

Analogous to chlorophyll, SCOs can have higher phospholipid content than most terrestrial oils, and removal of these polar lipids is necessary to prevent catalyst fouling in downstream processes. Further complexity arises as a large portion of phospholipids in SCOs are non-hydratable, namely phosphatidic acid and phosphatidylethanolamine (75). These species complex with metal cations and cannot be removed by conventional water degumming processes that precipitate hydratable phospholipids. Other degumming methods have been developed to remove these non-hydratable phospholipids (76).

Acid degumming is the most common approach to removing non-hydratable phospholipids. An acid is added to decompose phospholipid salts to improve their hydratability. Phosphoric acid is routinely used in algae oil degumming given the co-benefit of chlorophyll reduction (32, 54, 60). A drawback of acid degumming, however, is that some decomposed phosphatic acid will remain in the oil phase (76). Subsequent addition of a small amount of diluted base – enough to neutralise phosphatic acid while avoiding saponification – has been shown to be effective in further reducing phosphorus content of acid-degummed oils (77). Other methods which target both hydratable and non-hydratable phospholipids, such as membrane or enzymatic degumming, are in nascent stages of development. While promising, the cost of these techniques are prohibitively expensive for SCO degumming in their current state (76).

Given the potentially high content of microbial polar lipids, a remarkable amount of microbial lipid may be lost in the degumming process (60). Nevertheless, in some SCOs degumming may be appropriate, and examples of both dilute acid and solvent degumming exist in the patent literature (78–80). For SCO streams containing large fractions of polar lipids, hydrolysis to a FFA stream is likely preferable for fuel production (34).

Hydrolysis and Distillation

As an alternative to removing impurities from the lipid stream, approaches have also been developed to remove only the desired components from the lipid stream and leave everything else. Within this realm, hydrolysis and methanolysis are the primary approaches applied, generating FFA and FAME, respectively, both of which are distillable.

Hydrolysis can employ acids, bases or high temperature water to cleave fatty acid and other esters. Alkaline hydrolysis (i.e. saponification) is advantageous in that it requires mild temperatures and short reaction times (typically 80°C and 1 h, respectively). However, the stoichiometric consumption of alkali likely makes this approach too expensive for a fuel production scenario. Acid hydrolysis is favourable in that the acid is catalytic and mild temperatures are also typically employed (100°C or less), but reaction times are typically 8–24 h. Acid hydrolysis was the basis of the industrial Twitchell process, which was subsequently superseded by steam splitting. Steam splitting *via* the Colgate-Emery process is the current industrial standard, employing temperatures of 250–330°C and reaction times of 2–3 h (81). Higher temperatures can significantly shorten the reaction time (for example to ~10 min), but also significantly increase the operating pressure (for example to ~2500 psig) (82). Steam splitting is effective with both acylglycerides and with phospholipids (82). Lawal *et al*. demonstrated the effectiveness of hydrolysing algal lipids to FFAs and distilling the hydrolysed FFAs away from impurities (83). An alternative approach involves engineering microbes to produce and excrete FFAs directly into the growth medium, which could simplify the cell lysis and lipid extraction operations. This approach

was recently demonstrated in the cyanobacteria *Synechocystis* sp. PCC6803 (45).

Alternatively, microbial lipids can be converted to methyl esters, which facilitates distillation without vacuum. The (trans)esterification to methyl esters is widely used on a commercial scale to produce biodiesel, and occurs under similarly mild conditions as saponification. Murzin and coworkers demonstrated the effectiveness of this cleanup approach for removing impurities in algae oil (84–86). However, this approach suffers from two significant drawbacks, namely that it requires a dry feedstock and that it consumes methanol stoichiometrically. Drying of microbial biomass is much more energy intensive than drying terrestrial crops, and may be economically untenable for fuel production. Process engineering may make it feasible to recycle the methanol (or valorise it, for example in a methanol-to-gasoline-type process) though this has not been explored to our knowledge.

Hydroprocessing of Single Cell Oils

Several types of SCOs have been upgraded to hydrocarbon fuels with and without prior cleanup. Among these, algae oils are by far the most common. Hydroprocessing approaches and cleanup approaches for use on algae oils have varied significantly, though many employed techniques to specifically remove chlorophyll. Hydroprocessing of yeast oils, which do not contain chlorophyll but may contain sterols, has also been reported by a few researchers. Among other microbes, to our knowledge only lipids from the methanotrophic bacterium *Methylomicrobium buryatense* have been upgraded to hydrocarbons. A summary of SCO hydroprocessing literature is provided in **Table III**.

Lawal and coworkers explored deoxygenation of algae oils from *Nannochloropsis salina* and *Chlorella vulgaris* over platinum, rhodium and nickel-molybdenum-based catalysts (83, 87, 88). The oils were obtained from Valicor, which employed a thermochemical pretreatment of the algal biomass to render the lipids extractable (83, 106). Notably, this pretreatment reduced the amount of phosphorus and iron in the *Chlorella* oil by nearly 100%, while sulfur was only reduced by 30%. For the *N. salina* oil, however, the sulfur and phosphorus content remained relatively high at 2033 ppm and 246 ppm, respectively. Additionally, the *N. salina* oil contained only 47% material that could be identified as acylglycerides or FFAs (83, 87). Analysis showed that the oil had up to 0.8% chlorophyll, 0.5% carotenoids, 5% sterols, 1–5% mannitol and a large fraction of unsaponifiable matter that was not identified (83). While most reaction conditions explored showed significant catalyst deactivation within 7 h time on stream (TOS), Zhou and Lawal were able to find a set of conditions for both a $NiMo/Al_2O_3$ and a Pt/Al_2O_3 catalyst that did not show significant deactivation within this timeframe, and obtained hydrocarbon yields above 60 wt% (87, 88). The optimal conditions for the platinum and nickel-molybdenum catalyst were significantly different, underscoring the need for reaction engineering studies for each combination of catalyst and oil.

Crocker and co-workers explored deoxygenation of algae oils from *Scenedesmus acutus* grown on power plant flue gas as a carbon source using nickel-based catalysts (71, 89–91). While the focus of these experiments was generally on model compounds, all of the catalysts employed with algae oils (Ni/Al_2O_3, Ni-Al layered double hydroxide and $Ni\text{-}Cu/Al_2O_3$) deactivated faster with the algae oils (within 4 h TOS) than with vegetable oils or model compounds such as triolein, despite the fact that the oil concentration was only 1.3 wt% in a dodecane solvent. The fast deactivation may have been in part due to the low temperature employed (260°C). In particular, the Ni-Al layered double hydroxide catalyst showed significantly less deactivation at 300°C. The faster deactivation with algae oils is also notable in light of the cleanup procedures employed: the lipids were purified by column chromatography using either K10 montmorillonite clay or activated carbon with silica gel, which was effective at removing chlorophyll and other pigments, magnesium and phosphorus. K10 montmorillonite appeared to be a more effective adsorbent than activated carbon, resulting in a greater fraction of products in the diesel range. The authors hypothesised that several unquantified components in the algae oil, including FFAs, polar lipids, sterols or fatty amides could be contributing to the deactivation (90). However, experiments with mixtures of model TAGs and FFAs suggested FFAs were not the culprit (90). A more detailed analysis of impurities also suggested that the oil cleanup quantitatively removed phosphorus and magnesium, though a small amount of nitrogen remained (71). Santillan-Jimenez *et al*. (71) also noted that the residual extraction solvent (namely chloroform) was likely the source of chloride detected on the catalyst in post-reaction analysis (71), underscoring the observation that

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Table III Summary of Single Cell Oil Hydroprocessing Reports

Reference	SCO type	Oil recovery	Oil cleanup	Deoxygenation			Hydroisomerisation		
				Conditions	Catalyst	Results	Conditions	Catalyst	Results
(87)	Algae, *Nannochloropsis salina*	Obtained from Valicor	1 mm filtration	1.3 wt% oil in n-$C_{12}H_{24}$, 280–360°C, 300–500 psig H_2, flow	NiMo/Al_2O_3	63 wt% hydrocarbon yield	N/A	N/A	N/A
(88)	Algae, *Nannochloropsis salina*	Obtained from Valicor	1 mm filtration	1.3 wt% oil in n-$C_{12}H_{24}$, 280–360°C, 300–500 psig H_2, flow	Pt/Al_2O_3, Rh/Al_2O_3, NiMo/Al_2O_3	76 wt% hydrocarbon yield (Pt/Al_2O_3), 56% hydrocarbon yield (Rh/Al_2O_3), 63 wt% hydrocarbon yield (NiMo/Al_2O_3)	N/A	N/A	N/A
(71)	Algae, *Scenedesmus acutus*	Modified Bligh-Dyer extraction	K10 montmorillonite clay or activated carbon in column chromatography	1.3 wt% oil in n-$C_{12}H_{24}$, 260–300°C 580 psig H_2, flow	Ni-Al layered double hydroxide	Hydrocarbon yields not quantified, but GCMS suggested nearly complete conversion to hydrocarbons at 1 h TOS, decreasing to 65–70% at 4 h TOS at 260°C, while conversion remained nearly complete through 4 h TOS at 300°C	N/A	N/A	N/A
(89)	Algae, *Scenedesmus acutus*	Modified Bligh-Dyer extraction	Activated carbon, silica gel in column chromatography	1.3 wt% oil in dodecane, 260°C, 580 psig H_2, flow	Ni/Al_2O_3, Ni-Cu/Al_2O_3	Hydrocarbon yields not quantified, but GCMS suggested incomplete conversion to hydrocarbon even at 1 h TOS. Catalyst deactivated faster with algae oil than with triolein	N/A	N/A	N/A
(90)	Algae, *Scenedesmus acutus*	Modified Bligh-Dyer extraction	K10 montmorillonite clay in column chromatography	1.3 wt% oil in dodecane, 300°C, 580 psig H_2, flow	Ni-Al layered double hydroxide	95% conversion, 73% selectivity to C10–C17	N/A	N/A	N/A
(91)	Algae, *Scenedesmus acutus*	Modified Bligh-Dyer extraction	K10 montmorillonite clay in column chromatography	1.3 wt% oil in dodecane, 260°C, 580 psig H_2, flow	Ni-Al layered double hydroxide	55–65% hydrocarbon in product mixture, lower hydrocarbon yield but increasing diesel-range selectivity with increasing TOS	N/A	N/A	N/A

(Continued)

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Table III (Continued)

Reference	SCO type	Oil recovery	Oil cleanup	Deoxygenation			Hydroisomerisation		
				Conditions	Catalyst	Results	Conditions	Catalyst	Results
(92)	Algae, not specified	Obtained from Verfahrenstechnik Schwedt GmbH	None	1.3 wt% oil in dodecane, 260°C, 580 psig H_2, flow	Ni/HBEA zeolite	100% conversion, 78% hydrocarbon yield, 60% *n*-C_{18} yield	N/A	N/A	Noted 3.9–11% isooctadecane yield in batch reactions
(93)	Algae, not specified	Obtained from Verfahrenstechnik Schwedt GmbH	None	1.3 wt% oil in dodecane, 270°C, 580 psig H_2, flow	Ni/ZrO_2	100% conversion, 75% hydrocarbon yield, 70% *n*-C_{17} yield	N/A	N/A	Noted modest isomerisation activity, but did not report isomer yields
(94)	Algae, not specified	Obtained from Verfahrenstechnik Schwedt GmbH	None	1 g oil in 100 ml dodecane, 260°C, 580 psig H_2, batch	Ni/HBEA zeolite	100% conversion, 79% hydrocarbon yield, 40–43% *n*-C_{18} yield, 25–27% *n*-C_{17} yield	N/A	N/A	2–3% isooctadecane yield
(95)	Algae, *Chlorella* sp.	Supercritical hexane	None	1 g oil in 100 ml dodecane, 300°C, 440 psig H_2, batch	Ni/HY zeolite, Ni/SiO_2, Mo_2N/MoO_2	32–49% FFA yield, 4–7% hydrocarbon yield	N/A	N/A	N/A
(96)	Algae, *Chlorella* sp.	Supercritical hexane	None	1 g oil in 100 ml dodecane, 300°C, 440 psig H_2, batch	Ni/SiO_2	15% conversion, 6% hydrocarbon yield, 42% FFA yield	N/A	N/A	N/A
(72)	Algae, *Scenedesmus acutus*	Acid pretreatment, hexane liquid liquid extraction	Silica gel in column chromatography, H_3PO_4 bleaching	25 wt% oil in hexane, 450°C, 1300 psig H_2, flow	Pd/C	73–74% liquid hydrocarbon yield, 15–17% C_4–C_{11}, 82–85% C_{12+}	Neat hydrodeoxygenation product, 350°C, 500 psig H_2	Pt/SAPO-11	77–81% liquid hydrocarbon yield, 30–50% isomer content, –12 ≤ CP ≤ –21°C
(97)	Algae, *Scenedesmus acutus*	Acid pretreatment, hexane liquid liquid extraction	Silica gel in column chromatography, H_3PO_4 bleaching	25 wt% oil in hexane, 450°C, 1300 psig H_2, flow	Pd/C	69% liquid hydrocarbon yield, 21% C_4–C_{11}, 79% C_{12+}	Neat hydrodeoxygenation product, 350°C, 500 psig H_2	Pt/SAPO-11	69% liquid hydrocarbon yield, 32% isoparaffin content, cloud point = –3.5°C
(98)	Algae, not specified	Obtained from Phycal	None	Neat oil, 350°C, 800 psig H_2, flow	Pd/C, Pt/Al_2O_3	85% of liquid product stream as *n*-alkanes (95% with second pass), polishing with Pt/Al_2O_3 converted residual oxygenates	Neat hydrodeoxygenation product, 350°C, 800 psig H_2	Pt/USY	50% isomerisation conversion, solvent dewaxing resulted in product that remained liquid at –20°C

(Continued)

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Table III (Continued)

Reference	SCO type	Oil recovery	Oil cleanup	Deoxygenation			Hydroisomerisation		
				Conditions	Catalyst	Results	Conditions	Catalyst	Results
(99)	Algae, not specified	Obtained from Xian Lyphar Biotech Co Ltd	None	Neat oil or 5 vol% oil in heptane, 260–300°C, 580 psig H_2, batch	Co/ natural clay	84–100% conversion of oil, 83–86% yield of alkanes	N/A	N/A	N/A
(100)	Algae, *Chlorella*	Extraction with CH_2Cl_2	None	Oil in H_2O (ratio unspecified), 330–370°C, batch	Pt/C	Yields not quantified, but increase in heating value and carbon content of oil phase, and highly isomerised products identified in liquid product	N/A	N/A	N/A
(101)	Algae, not specified	Obtained from Solix Biofuels	None	10 mg oil in 10 ml hexanes, 290°C, 440 psig H_2, batch	Fe/MSN	67% conversion, 16% alcohols, 33% unsaturated hydrocarbons, 18% saturated hydrocarbons, remaining FFAs all saturated	N/A	N/A	N/A
(102)	Algae, *Scenedesmus obliquus*	Extraction with $CHCl_2$:methanol	None	Neat oil, 290–350°C, 60 bar H_2, batch	NiMo/ Al_2O_3	84–87% liquid yields, increasing selectivity toward alkanes at higher temperature and catalyst loading	N/A	N/A	N/A
(103)	Algae, *Nannochloropsis* sp.	Incubation, high pressure homogenisation, hexane extraction	None	Neat oil, 300–375°C, 50–120 bar H_2, batch	CoMoP/ Al_2O_3	>99% conversion, up to 80% liquid yield, giving way to cracking and polyaromatisation at higher pressures	N/A	N/A	N/A
(104)	Algae, *Dunaliella* sp.	Electroflocculation and osmotic lysis, self-separation	Hot water hydrolysis	Oil in dodecane, 300°C, 19 bar 6% H_2, fed batch	Pd/C	98% conversion to *n*-alkanes	N/A	N/A	N/A
(105)	Cyanobacteria, *Synechocystis* PCC 6803	Excreted FFAs, recovered by solvent washing adsorbent	Recrystallisation, activated carbon, saponification, reverse phase liquid chromatography	1.15 g oil in 22.5 g *n*-$C_{12}H_{24}$, 300°C, 15 bar of 5% H_2, fed batch	Pd/C	32–88% alkane yield	25 g *n*-alkane, 300°C, 500 psig H_2	Pt/CaY zeolite	20–36% conversion, 18–31% yield to isoparaffins, 92–95% isomerisation selectivity

(Continued)

Table III (Continued)

Reference	SCO type	Oil recovery	Oil cleanup	Deoxygenation Conditions	Deoxygenation Catalyst	Deoxygenation Results	Hydroisomerisation Conditions	Hydroisomerisation Catalyst	Results
(10)	Yeast, *Rhodosporidium toruloides*	Acid pretreatment, hexane liquid liquid extraction	None	25 wt% oil in hexane, 450°C, 1300 psig H_2, flow	Pd/C	80% liquid phase yield, 60% C_{12}–C_{20} hydrocarbons, 12% C_7–C_{11} hydrocarbons, 25% C_{20+} hydrocarbons	Neat hydrodeoxygenation product, 350°C, 500 psig H_2	Pt/ SAPO-11	68% liquid hydrocarbon yield, 33% isoparaffin content, 70% C_{12}–C_{20} hydrocarbon, 20% C_7–C_{11} hydrocarbon, 7% C_{20+} hydrocarbon, cloud point –14.5°C (after cold filtration)
(34)	Bacteria, *Methylomicrobium buryatense*	Alkali-acid pretreatment, hexane extraction	Alkali-acid pretreatment, hexane extraction	0.5 ml oil in 25 ml decane, 360°C, 40 bar H_2, batch	Pd/SiO_2	100% conversion, ~100% yield to C_{13}–C_{17} paraffins	N/A	N/A	N/A

the extraction protocol can impact downstream processes through routes other than influencing the types of lipids extracted.

Lercher and coworkers explored nickel-based catalysts for algae oil deoxygenation under similar conditions to those above (though also in batch mode), using commercial algae oil of an unspecified species (92–94). In these experiments, support acidity (HBEA zeolite or zirconia) was shown in mechanistic studies with model compounds to play a key role in the reaction kinetics, and the nickel particle size was also key to catalyst activity. The oil was not analysed for impurities (only fatty acid composition), but did not deactivate the catalysts for at least 72 h (for Ni/ZrO_2) (93) or 120 h (for Ni/HBEA) (92) TOS. Notably, some isoalkanes, which improve the cold flow properties of the diesel blendstock, were observed in the products due to the support acidity.

Nguyen *et al*. explored both nickel and molybdenum-based catalysts in deoxygenation of *Chlorella* algae oil extracted by supercritical hexane (95, 96). In these studies, the catalysts were rapidly deactivated (within 1 h) and produced less than 10% yield to hydrocarbons. In contrast, FFAs were the major product, with acylglycerides featured prominently as well. The authors noted that the crude extracted lipids comprised only around 50% fatty acids and 3–4% sterols, with the remainder unidentified (95). While low conversion of the FFA and acylglycerides was observed, no conversion of the sterols or steryl esters was found (96). Nguyen *et al*. also noted that while molybdenum nitride catalysts had much lower initial activity than nickel-based catalysts, the molybdenum nitride catalysts did not show as strong a deactivation as the nickel catalysts did (95). The low activity of the molybdenum nitride catalysts represents a significant challenge nonetheless. However, recovery of the lipids as methyl esters and purification over a bleaching clay produced a feedstock that could be readily converted over a Ni-HY zeolite catalyst (84–86). Interestingly, a Pd/C catalyst did not perform as well as the Ni-HY catalyst in deoxygenation of the purified methyl esters, possibly due to the lower acidity of the carbon support.

Researchers at NREL demonstrated both deoxygenation and hydroisomerisation of *Scenedesmus* algae oil recovered after acid pretreatment and hexane extraction (72, 97). Deoxygenation employed a Pd/C catalyst, while hydroisomerisation employed a Pt/SAPO-11 catalyst. Kruger *et al*. found that while relatively

severe temperature and pressure (450°C and 1300 psig) and dilution of the algae oil in a hexane solvent were required for complete conversion and stable performance of the Pd/C deoxygenation catalyst, bleaching the oils with either silica gel or with phosphoric acid did not improve the hydroisomerisation performance, at least through 10 h TOS (72). The deoxygenation step also resulted in significant denitrogenation as well. The severe conditions required for deoxygenation led to significant cracking and lower yields to diesel range products, but hydroisomerisation produced renewable diesel blendstocks with cloud points below –10°C (72). The latter step is important to demonstrate given the non-zero levels of residual nitrogen in the deoxygenated feedstock that could poison acidic isomerisation catalysts.

Robota *et al*. also employed a Pd/C catalyst for deoxygenation of a neat algae oil, and then hydroisomerised the resulting alkanes with a Pt/USY zeolite catalyst (98). Despite the higher concentration of oil, less severe conditions were required for nearly complete conversion of the oil to alkanes than was observed by Kruger *et al*. (72), though a second pass and a polishing step to remove residual oxygenates were used to produce a clean alkane stream for hydroisomerisation. With the large-pore Pt/USY zeolite catalyst, isomerisation and cracking were competitive, resulting in a 40% or more mass loss to naphtha-range hydrocarbons. Though cloud points of the resulting products were not measured, solvent dewaxing yielded a product that remained liquid at –20°C.

Soni *et al*. (99) evaluated cobalt/natural clay catalysts for deoxygenation of a commercial algae oil, and achieved hydrocarbon yields around 85% after 8 h in a batch reactor at 580 psig hydrogen. This oil contained nearly 70% docosahexaenoic acid in its FFA profile, suggesting that the product alkanes would require some degree of cracking to serve as a diesel blendstock.

Fu *et al*. (100) demonstrated hydrogen-free decarboxylation of *Chlorella* oil at 330–370°C for 2 h in a batch reactor. The heating value and carbon content of the organic phase both increased and the organic product contained significantly isomerised compounds, though conversion, yield, oil characterisation and catalyst characterisation were not reported.

Kandel *et al*. (101) reported the conversion of an algae oil from Solix Biofuels into hydrocarbons using a nanoparticle iron catalyst supported on mesoporous silica. After 6 h at 290°C and 440 psig hydrogen, 67% of the algae oil had been converted to both saturated and unsaturated alcohols and hydrocarbons. The authors noted that the presence of unsaturated compounds suggests hydrogen may have been limiting.

Tang *et al*. (102) evaluated deoxygenation of a *Scenedesmus* algae oil in a batch reactor over a $NiMo/Al_2O_3$ catalyst. As the reaction temperature increased from 290°C to 350°C (and as catalyst loading increased from 0 wt% to 50 wt% of the oil), the product spectrum shifted from acids, esters and alcohols to paraffins, olefins, naphthenes and aromatics, with a small amount of isoparaffins also present. The authors used multiple solvent combinations to extract the lipids. Dichloromethane:methanol was the highest yielding solvent on a weight basis, but the primary components were sterols and tocopherols rather than acylglycerides and FFAs. Other solvents were lower yielding, but the extracts were also not analysed.

Poddar *et al*. deoxygenated *Nannochloropsis* lipids over a phosphorus-promoted $CoMo/Al_2O_3$ catalyst, achieving nearly complete conversion to alkanes and nearly 80% yield to liquid-phase products in 6 h at 375°C and 50 bar hydrogen (103). The authors conducted a detailed kinetic analysis but did not report detailed product analysis or post-reaction catalyst characterisation.

Wang *et al*. explored deoxygenation of a FFA stream derived from *Dunaliella* lipids (104). The lipids were hydrolysed in 250°C to release FFAs, which self-separated from the aqueous phase and were decarboxylated in fed-batch mode over a Pd/C catalyst. The catalyst maintained steady operation over 5 h of reaction, even with a hydrogen-poor headspace in the reactor.

Finally, Schulz *et al*. (105) deoxygenated *Synechocystis* (cyanobacteria) lipids over a Pd/C catalyst and isomerised the resulting alkanes over a Pt/CaY zeolite catalyst. The lipids were unique in that they were recovered in the form of excreted FFAs (mainly C12), adsorbed on and eluted from a resin. Once recovered, the lipids were also subjected to a number of cleanup protocols, including recrystallisation, activated carbon treatment, saponification and preparatory-scale liquid chromatography. These protocols had varying effectiveness in reducing sulfur and phosphorus contaminants in the oils (especially sulfur), which were reflected in varying performance of the Pd/C deoxygenation catalyst across FFA streams, giving 33–88% alkane yield depending on the stream. The two most promising streams gave relatively

low conversion, but high isomerisation selectivity over the Pt/CaY catalyst.

Aside from algae lipids, yeast lipids have also been evaluated for deoxygenation and hydroisomerisation. Sànchez i Nogué *et al*. (10) employed the same conditions as those used by Kruger *et al*. (72) and Knoshaug *et al*. (97) to deoxgygenate lipids from *Rhodosporidium toruloides* yeast. The yeast lipids produced a renewable diesel blendstock of similar quality as that from the algae oils. Process optimisation to determine if the yeast lipids could be satisfactorily converted under less severe conditions due to the lack of impurities such as chlorophyll was not conducted. The lipids were found to contain a small amount of steryl esters and a significant amount of polar lipids as well.

Chuck and coworkers have also evaluated conversion of yeast lipids to fuels, though not through conventional hydroprocessing approaches (107, 108). In these studies, the yeast lipids were either catalytically cracked (107) or metathesised with ethylene using a Hoveyda-Grubbs catalyst (108). Although the Pd/C cracking catalyst and reaction temperatures were similar to those used for deoxygenation by other researchers, the gas atmosphere was quite different. Interestingly, however, the catalytic cracking experiments were performed with a yeast lipid from *Metschkownia pulcherrima* that included 9 wt% sterols, and the authors inferred that the sterols can act as hydrogen donors as they undergo aromatisation at 350–400°C in an argon atmosphere, which improved the yield to linear alkanes from the triglyceride fraction.

Among other oleaginous microbes, the only report of hydroprocessing to our knowledge is that of Dong *et al*. (34), who converted polar lipids from *Methylomicrobium buryatense* to linear alkanes over a Pd/SiO_2 catalyst. The bacterial biomass was hydrolysed by a sequential alkaline-acid method that both ruptured the cell wall (rendering the lipids extractable) and cleaved the polar head groups off the predominantly polar lipids, producing a clean FFA stream for deoxygenation. The high degree of polar head group cleavage was confirmed by the presence of only 4 ppm phosphorus in the oil, though the oil contained sulfur, nitrogen, sodium and halogens in higher amounts. With a relatively high catalyst loading and deoxygenation in batch mode, full conversion to linear alkanes was achieved. The authors also noted that a preliminary catalyst screening with nickel- and copper-based catalysts confirmed the hypothesis that these catalysts would be rapidly deactivated in the presence of phosphorus-containing feeds, while noble metals performed better.

Summary and Outlook

Several interesting themes emerge in lipid composition, cleanup and hydroprocessing of microbially-derived oils. First, the studies reporting the longest and most stable catalytic performance have used refined algae oils, for example from Phycal or Solix. Although the details of the refining process are not readily available, these processes appear to sufficiently clean the oils of catalyst-deactivating impurities. In contrast, lipid streams extracted by researchers directly prior to catalytic processing have tended to deactivate catalysts more rapidly or perform more poorly, even when diluted by a factor of 100 into an inert solvent. Perhaps this is unsurprising given the differing descriptions of the oils' appearances, for example bright orange transparent liquid *vs*. dark brown semisolid. The situation is additionally confounded by the variety of techniques and solvents used to extract the lipids, which may co-extract different undesirable components. Hexane is the simplest solvent employed, but can result in low extraction yields and form emulsions with wet biomass. Alcohols, such as methanol and ethanol can be added to increase the extraction yields and minimise emulsions, but can also increase co-extraction of impurities. Halogenated solvents can serve the same purpose as alcohols, but further confound the process by introducing additional heteroatoms (typically chloride from chloroform or dichloromethane) that can remain in the extract in trace amounts even after solvent removal.

Second, studies using noble metal catalysts have tended to report better performance than those using base metal catalysts. This is frequently true in other catalysis applications as well, wherein base metals such as nickel, molybdenum and copper are more prone to coking, oxidation by feedstock oxygen and poisoning by feedstock heteroatom impurities. Indeed, in the examples where microbial oils have been thoroughly characterised for heteroatom impurities, it is tempting to infer that supported noble metals may even be able to tolerate some degree of phosphorus, nitrogen and sulfur in the feedstock. However, times on stream in the literature reviewed here, less than 200 h TOS, have typically been too short to make a meaningful assessment. Nevertheless, while noble metals are frequently avoided due to the high cost, there may

be environmental advantages (at least for metals such as ruthenium and platinum) (109), and some of the cost can be recouped by regeneration and recycling of the spent catalysts (110). It is worth noting that industrial processes producing green diesel fuels from vegetable oils, such as Honeywell-UOP's Ecofining, Neste's NExBTL, Axens' Vegan and Renewable Energy Group's Bio-Synfining processes employ nickel-molybdenum or cobalt-molybdenum sulfide catalysts similar to those used in petroleum refining (111). These catalysts tend to favour hydrodeoxygenation over decarbonylation or decarboxylation, which preserves carbon in the product, but also consumes more hydrogen in the process. At least one evaluation of the tradeoffs between carbon yield and hydrogen consumption concluded that decarboxylation would be economically favourable, provided that hydrogen consumption through subsequent methanation and reverse water-gas shift reactions is not significant (112). On the other hand, the deployment of large-scale wind and solar power has at times resulted in excess power production and low-cost electricity that can be used for water electrolysis to produce inexpensive hydrogen. Nickel-molybdenum catalysts are also frequently employed for hydrodesulfurisation and hydrodenitrogenation, which may indicate a higher level of robustness to heteroatoms than other types of catalysts. In particular, nickel-molybdenum catalysts typically require a co-fed source of sulfur, often hydrogen sulfide or dimethyl disulfide, to mitigate conversion of the active sulfide phase to a less-active oxide. Concomitantly, these catalysts tend to leach sulfur into the product (21), which may inhibit the ability of these catalysts to meet increasingly stringent sulfur limits in fuels. It is unknown whether the sulfur-containing species in microbial oils can satisfy this requirement of nickel-molybdenum catalysts.

Third, lipid stream cleanup by a number of approaches appears promising, but all of the techniques have some drawbacks and only a few can be reasonably expected to apply universally across microbial lipids. Saponification of the lipid stream to FFA followed by distillation is perhaps the most robust as it should be effective even with lipid feeds containing high levels of polar lipids, sterols, chlorophyll and metals. However, the distillation step is energy intensive compared to most of the other bleaching methods and the cost of the alkali is high. Methyl esterification and distillation should be similarly effective, but the methanol would need to be recovered for high material and economic efficiency, or else recovered as methane after hydrotreating. Degumming (precipitation of phospholipids) and phosphoric acid bleaching can work well for lipid streams that are low in polar lipids, but would suffer from significant yield losses otherwise, and the phosphoric acid must be thoroughly washed out of the cleaned lipid stream to avoid contamination from the bleaching agent. In lipid streams with high polar lipid content, hydrolysis to FFAs may prove more economical. Adsorption on a polar material such as silica, bleaching clay or activated carbon appear effective for some target impurities (for example chlorophyll and some metals), but similar to degumming, may result in unacceptable yield losses for lipid streams that contain large amounts of polar lipids. Additionally, post-adsorption oils have still tended to deactivate the catalysts. Interestingly, even when chlorophyll is removed to below detection limit, some nitrogen frequently remains in the oils, likely in the form of hydrophobic protein. There is thus a clear need for both fundamental science and process development on this topic.

Looking forward, converting microbial lipids to fuels is a promising approach to displace conventional fossil fuels, especially for diesel and jet fuels which share the same carbon number range as the microbial lipids. While previous research has focused primarily on algae lipids, the advent of third generation cellulosic sugars may spur further development of heterotrophic oleaginous microbes as well. Finally, to best allow for economically-viable biofuels production from SCOs, it is critical to design an integrated process, in which certain complementary units can be intensified to maximise the yield of high quality products for a given step. For example, the lipid hydrolysis step can be integrated with the biomass pretreatment or cell wall rupture step to simultaneously rupture the cell walls for bulk oil extraction and liberate FFA from polar lipids. In addition, the selectivity of the extraction solvent should be carefully considered to increase extraction of fatty acids while reducing the co-extraction of impurities. With these considerations, SCOs are poised to make a significant impact in sustainable fuel production.

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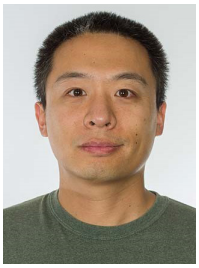

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