Current progress in lipid-based biofuels: Feedstocks and production technologies

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Abstract

The expanding use of fossil fuels has caused concern in terms of both energy security and environmental issues. Therefore, attempts have been made worldwide to promote the development of renewable energy sources, among which biofuel is especially attractive. Compared to other biofuels, lipid-derived biofuels have a higher energy density and better compatibility with existing infrastructure, and their performance can be readily improved by adjusting the chemical composition of lipid feedstocks. This review thus addresses the intrinsic interactions between lipid feedstocks and lipid-based biofuels, including biodiesel, and renewable equivalents to conventional gasoline, diesel, and jet fuel. Advancements in lipid-associated biofuel technology, as well as the properties and applicability of various lipid sources in terms of biofuel production, are also discussed. Furthermore, current progress in lipid production and profile optimization in the context of plant lipids, microbial lipids, and animal fats are presented to provide a wider context of lipid-based biofuel technology.

Keywords: Lipids; Biofuel; Biodiesel; Renewable hydrocarbon; Renewable fuels

1. Introduction

Greenhouse gases derived from the combustion of fossil fuels have caused serious environmental problems, which are only set to worsen in years to come (Correa et al., 2019). In order to ensure energy security and reduce both climate change and particulate emissions driven by fossil fuel consumption, many countries are interest in developing renewable energy sources. Common renewable energy sources include solar, wind, geothermal, hydropower and ocean energy, as well as biofuel, which comprises fuel produced via renewable and sustainable approaches. Biofuels have relatively high energy density and are compatible with existing infrastructure compared to other sources of clean energy, and therefore are especially useful (Liao et al., 2016). According to recent reports from the International Energy Agency, to keep up

with the Sustainable Development Scenario, the biofuel consumption for transportation use needs to be tripled by 2030 (https://www.iea.org/reports/transport-biofuels; accessed 3 March 2021).

The choice of feedstock greatly impacts the technological development of biofuel production, and every major theoretical upgrade of biofuel technology has been closely linked to the properties of its associated substrates. Depending on the choice of feedstock, the implementation of biofuel technology can also have different economic and social outcomes (Li et al., 2018). For instance, edible oils and sugars are not ideal choices for the production of inexpensive biofuels since the cost of the feedstock is high and their availability is unstable due to market competition. On the contrary, the use of non-edible lipids, such as waste animal fat, can lower the cost and increase the market value of biofuels (Othman et al., 2017). Although industrial by-products and waste have a lower initial price, their use in biofuel production requires pre-processing, which may also lead to higher expenditures. Progress in biofuel technology therefore depends fundamentally on minimizing the economic and social costs of obtaining the feedstock and improving the quality and functionality of the product. As such, development of alternative feedstocks and processing technologies has been gaining interest to further augment the economic feasibility of biofuel production (Perin & Jones, 2019).

Plant oils, microbial lipids, and animal fats are rich in storage lipids [mainly triacylglycerol (TAG)], which provide excellent feedstocks for biofuel production. In terms of the carbon chain length, saturation degree, and branching, the chemical properties of the fatty acid moiety in these lipids are generally similar to those of traditional fuels. Consequently, many types of biofuels derived from lipids, including biodiesel and renewable equivalents to conventional gasoline, diesel, and jet fuels (Fig. 1), have similar physiochemical properties with conventional fossil fuels, which allows for excellent compatibility with existing infrastructure. In terms of physical properties, the lubricity, viscosity, and cold flow properties of fuels all depend heavily

on the composition of the lipid feedstock (Table 1). Since lipids are a very diverse group of organic compound, oils and fats with distinct physicochemical properties, including the presence of unique functional groups, bring great diversity to the composition of available biofuel products. The blending or genetic modification of lipid-producing species allows for even further optimization of lipid feedstocks for biofuel production, thus improving fuel performance.

Although several recent papers have summarized the synthetic routes and economic value of biofuels, a comprehensive review is needed to address the influence of lipid feedstocks in biofuel production. This review summarizes the unique properties of various lipid-derived biofuels, their production methods, and the influence of lipid feedstock on biofuel properties. Furthermore, the applicability of plant lipids, microbial lipids, and animal fat to biofuel production, as well as progress in lipid biotechnology were also discussed, which could be used to tailor lipid sources to better fit the requirements of optimal feedstocks for biofuel production.

2. Lipid-derived biofuels

2.1 Production of biodiesel from lipid feedstocks

Biodiesel commonly refers to renewable fatty acid alkyl esters (FAAE) that comprise a combination of a fatty acid moiety with alcohol. FAAE is mainly produced through the transesterification of TAG with alcohol (methanol or ethanol), producing glycerol as a by-product. Many methods are currently available to produce biodiesel, including chemical transesterification, enzyme-catalyzed transesterification, and biosynthesis. Among these methods, conventional chemical transesterification is the most widely applied due to the low cost of the catalyst and high product yield (Ertugrul Karatay et al., 2019). At present, the industrial production of biodiesel mainly relies upon the use of alkaline catalysts to mediate transesterification between TAG and primary alcohol when the lipid feedstock has low free fatty acid and water contents (Thanh et al., 2012). The catalysts used in this process consist of alkaline

hydroxides (e.g., NaOH or KOH), alkoxides (e.g., sodium methoxide) or carbonates. Compared to the relatively cheap alkaline hydroxide catalysts, alkaline alkoxides can achieve significantly higher yields in a shorter reaction time. In contrast to alkaline catalysts, acid catalysts are used when the oil feedstock contains more than 1% free fatty acids and water as a means of preventing the formation of soap (Thanh et al., 2012). However, its catalytic activity is relatively low, which translates into the need for a higher temperature and longer reaction time (Worapun et al., 2012).

Biodiesel can also be produced using lipase as the catalyst, which is more eco-friendly and has a higher specificity (Canet et al., 2016). Lipase, which is a ubiquitous enzyme distributed across biological kingdoms, is commonly found in microorganisms such as bacteria and fungi. Lipase can catalyze the synthesis of biodiesel through the one-step alcoholysis of TAG or a two-step reaction consisting of TAG hydrolysis followed by esterification. The yield of lipase-dependent reactions can be optimized through the adjustment of several factors such as pH, enzyme activity and substrate concentration. In addition, lipase immobilization has become the focus of much research in recent years (Zhao et al., 2015). This method can improve the resistance of lipase to reaction conditions, and also facilitates recovery and reuse of the lipase following transesterification, which reduces costs.

Unlike chemical production, the microbial synthesis of biodiesel is an emerging biotechnology with unique advantages. Microorganisms such as bacteria and yeast have rapid growth rates and promising potential in large-scale fermentation, and the rapid development of advanced molecular tools has facilitated metabolic engineering approaches (Liao et al., 2016). However, the production of lipid-based biofuel in eukaryotic microbes can be hindered by their complex cellular structure and compartmentalization between cellular organelles. For example, the FAEE product titer in engineered *Yarrowia lipolytica* was 7.1 mg/L when a wax-ester synthase localized in the cytosol but increased to 136.5 mg/L and 110.9 mg/L when the same

enzyme was targeted to the endoplasmic reticulum and peroxisome, respectively (Xu et al., 2016), suggesting that targeting enzymes to specific subcellular organelles can improve biodiesel synthesis. Currently, the production of biodiesel in engineered microbial cell factories cannot compete with conventional chemical transesterification. However, since the microbial synthesis of biodiesel does not require harsh reaction conditions, it holds great promise in environmental protection and energy security, which are the main goals of biofuel development.

2.2 Production of gasoline-equivalent fuel from lipid feedstocks

Gasoline comprises hydrocarbons in the C_4 to C_{12} range, with C_3 to C_9 being the most common alkanes and C_5 species predominating (Mascal & Dutta, 2020). Biogasoline is a liquid fuel for spark-ignition engines that can be obtained from biomass. Through deoxygenation, the lipid-based feedstock can form hydrocarbons that are similar to those in conventional gasoline. To produce gasoline-like biofuel, it is necessary to have specific amounts of branched and aromatic components to match the fuel properties (Yeletsky et al., 2020). For example, the treatment of palm kernel oil over a beta zeolite catalyst can lead to the production of branched and aromatic products as a consequence of partial isomerization, which occurs simultaneously with deoxygenation and cracking of the lipid (Sousa et al., 2018). Hydro-processed lipids from a marine microalgae *Nannochloropsis* spp. treated over a sulfide cobalt molybdenum phosphorus/aluminum oxide catalyst under a hydrogen atmosphere can lead to the production of 35-50% of gasoline, with enhanced aromatics and cycloalkanes during the reaction (Poddar et al., 2018). In another study, various plant oils including those from soybean, sunflower, linseed, palm, and jatropha were converted to biogasoline through a catalytic hydrothermal process using molybdenum doped zeolite (Robin et al., 2017). Non-edible lipid feedstocks such as beef tallow, yellow grease, and brown grease can also be converted to biogasoline using a non-catalytic two-step hydrolysis-pyrolysis technology (Asomaning et al., 2014).

The emerging concept of co-processing has also received much attention in recent years, where lipids can be used with other refinery feedstocks and/or products to increase gasoline yield while decreasing carbon intensity as a desirable side-effect. Vacuum gas oil (VGO) is one of the outputs of vacuum distillation columns in a refinery, and it is usually applied in Fluid Catalytic Cracking (FCC) to increase the yield from crude oil to a gasoline or diesel product. Catalytic cracking of vegetable oils in FCC plants can produce fuels out of biomass and enhance gasoline yields (Abbasov et al., 2016). The aromatic content and octane number of end-products from co-processing with VGO are also affected by the unsaturation degree of the lipid feedstock (Bielansky et al., 2011), which again points to the influence of feedstock on biofuel production.

2.3 Production of diesel equivalent from lipid feedstocks

Compared to biodiesel, which is essentially a mixture of esters, traditional diesel fuel can be described as a mixture of hydrocarbon molecules in the C_{12} to C_{20} range and is comprised mostly of straight and branched alkanes (Mascal & Dutta, 2020). The production of renewable diesel from various edible or non-edible lipids has been successfully demonstrated, with the deoxygenation of lipids being essential to its production (Othman et al., 2017). In terms of plant oil, the deoxygenation of canola oil over gamma-alumina catalysts supported with NiMo/CoMo in a fixed-bed reactor has led to the production of hydrocarbons that consisted predominantly of diesel-like alkanes (Afshar Taromi & Kaliaguine, 2018). A similar catalyst has been used to hydrotreat jatropha oil to produce 80% hydrocarbons, which is 97% of the theoretical yield (del Río et al., 2018). A large C_{17} fraction in the resulting product was generated from C_{18} lipids, which indicates that the main reaction pathway was the decarboxylation or decarbonylation route. Hydrothermal liquefaction has also been used to process microalgae-derived lipids with CoMo and NiMo as catalysts without hydrogen, resulting in a yield of 50% green diesel (Biller et al., 2015). In addition to TAG, renewable diesel equivalents can also be produced from free fatty

acids. For instance, palmitic acid and oleic acid can both be deoxygenated to diesel equivalent fuel using nickel supported on zirconia with activated carbon and nickel phosphide as catalysts (de Oliveira Camargo et al., 2020; Hongloi et al., 2019). The deoxygenation of palm fatty acid distillate, the by-product of palm oil refining that mainly, led to a hydrocarbon product in which over 85% was within the range of diesel fuel (Kamaruzaman et al., 2020).

2.4 Production of jet fuel equivalent from lipid feedstocks

Aviation fuel is a type of hydrocarbon fuel specifically designed for aircraft engines. There are two main types of aviation fuel: aviation gasoline and aviation jet fuel. Jet fuel is expected to be free of physical impurities and moisture, and have a low freezing point, high energy density, adequate stability and volatility, and ignite under extreme conditions. All of these characteristics are closely related to its composition. Specifically, unsaturated hydrocarbons and sulfur should be kept low, as the former can form gum and negatively affects engine performance and the latter, particularly mercaptan, may lead to a substantial increase in the corrosion of engine parts (Jimenez-Diaz et al., 2017; Wang & Tao, 2016).

Methods for preparing aviation jet fuel from biomass mainly include the gas-to-jet, oil-to-jet, and alcohol-to-jet ones. With lipids as a feedstock source, the oil-to-jet method largely consists of hydro-processing, hydrothermal liquefaction, and hydro-treated depolymerized cellulosic jet technology (Jimenez-Diaz et al., 2017; Wang & Tao, 2016). One basic feedstock for hydro-processed renewable jet production is lipid from plants such as *Jatropha curcas* and *Camelina sativa*. Since oils from different plant sources tend to have different degrees of unsaturation, a catalytic hydrogenation process is required to reduce the number of double bonds through the addition of hydrogen, thus decreasing the degree of unsaturation (Pradhan et al., 2020). Hydrocracking and hydroisomerization processes, on the other hand, can convert a proportion of straight-chain alkanes derived from lipids into branched structures to lower the

freezing point of the fuel, which enhances cold flow properties (Wang & Tao, 2016).

3. Improving lipid sources for biofuel production

Since lipids are a major resource in biofuel production and their properties have essential influence on biofuel quality, there has been increased interest in the development of methods to improve the productivity and composition of suitable lipid feedstocks. Metabolic engineering approaches are being pursued to improve the quality and content of storage lipids, including the enhancement of precursor and cofactor supply for lipid biosynthesis, acceleration of TAG assembly, and downregulation of TAG degradation.

3.1 Plant storage lipids for biofuel production

3.1.1 Modifying the plant TAG biosynthetic pathway for improved oil production

Seed oil production can be improved by engineering genes related to the biosynthesis of storage lipids (Fig. 2). The overexpression of key enzymes involved in the biosynthesis of lipid precursors, such as glycerol-3-phosphate dehydrogenase, which provides a critical link between carbohydrate and lipid metabolism, can effectively enhance the oil yield (Chhikara et al., 2018). Acyltransferases responsible for TAG assembly, such as glycerol-3-phosphate acyltransferase and diacylglycerol acyltransferase (DGAT) that catalyzes the first and last steps of this process, have also been targeted to improve TAG biosynthesis (For reviews, see Xu et al., 2018; Jayawardhane et al., 2018). Moreover, regulating genes responsible for TAG synthesis can also assist in the enrichment of unusual fatty acids, which can benefit the physicochemical properties of fuel products. For instance, expressing *Ricinus communis LYSOPHOSPHATIDIC ACID ACYLTRANSFERASE 2* in *Lesquerella* led to increased hydroxy fatty acid levels at the *sn*-2 position of TAG (Chen et al., 2016), which could potentially improve the lubricity of the biofuel product. Likewise, the heterologous expression of *Neurospora crassa NcDGAT2* not only

increased maize kernel oil content but also altered its fatty acid composition through a reduction in linoleic acid and an increase in oleic acid (Oakes et al., 2011).

3.1.2 Modulating the activity of plant transcription factors for improved oil production

Modification of transcription factor activity is also an effective approach to increase oil content since it can effectively regulate a series of genes affecting carbon partitioning and lipid metabolism. Wrinkled1 (WRI1), which is a member of the AP2/ EREBP transcription factor family, has been extensively studied in this context (Kong et al., 2020). WRI1 positively regulates a number of genes controlling glycolysis and plastidial fatty acid biosynthesis, such as those encoding pyruvate kinase subunits, acetyl-CoA carboxylase subunits, acyl carrier protein, and 3-ketoacyl-ACP synthase. In line with this, the overexpression of *WRI1* up-regulates the expression of these genes, thus promoting lipid accumulation in plants and alleviating feedback inhibition on fatty acid biosynthesis (Kong et al., 2020).

Genes within the LAFL network, which is regulated by LEAFY COTYLEDON 2 (LEC2), ABSCISIC ACID INSENSITIVE3, FUSCA3, and LEC1 transcription factors, also control various aspects of seed development including embryogenesis and the accumulation of lipids. They moderate the partitioning of seed storage compounds such as lipids, starch, and protein. In line with this, the overexpression of *LEC1* in peanuts increased the transcription of genes associated with lipid biosynthesis, which led to higher oil content and seed weight without negative effects on agronomic traits (Tang et al., 2018). In addition, DNA BINDING WITH ONE FINGER proteins are another group of interesting transcription factors that are involved in the regulation of fatty acid biosynthesis (Zhang et al., 2014).

3.1.3 Tailoring the composition of plant lipid feedstocks for better biofuel production

The fatty acid composition of lipid feedstocks has a great influence on the performance of biofuel. As discussed above, reducing the amount of saturated fatty acids can improve the cold

flow performance of biofuel, while decreasing the content of polyunsaturated fatty acids (PUFA) can improve oxidation stability. In brief, the key steps determining chain length and degree of saturation of plant lipids include the Δ9 stearoyl-ACP desaturase (SAD)-catalyzed desaturation of saturated acyl-ACP to monounsaturated acyl-ACP, the fatty acid thioesterase (FAT)-catalyzed release of free fatty acids with high specificity for chain length and saturation, and further desaturation by fatty acid desaturase (FAD) to generate PUFA (Fig. 2).

Therefore, regulating the enzymatic activities of SAD, FAT, and FAD can effectively change the composition of fatty acid feedstocks. For instance, soybean oil comprises only approximately 25% monounsaturated fatty acids but over 55% PUFA, and therefore increasing fatty acid saturation degrees is important for soybean oil improvement. High oleic acid soybean lines (85% of seed oil) have already been achieved by inhibiting the expression or activity of *FAD2* seeds (Wagner et al., 2011). While the fatty acid chain lengths of common vegetable oils are generally within the range of C16-C18, medium-chain fatty acids (C8-C14) are more similar to gasoline, and there has also been a drive to tailor the composition of plant lipid feedstocks in this context. *Cuphea hookeriana* and *Cuphea pulcherrima* accumulate more than 70% of seed oil as medium-chain TAG (C8 and C10). Although *Cuphea sp.* has yet to be domesticated for large-scale agricultural production, the heterologous expression of its *FATB* in *Camelina* led to more than 11% medium-chain fatty acids as seed oil content (Kim et al., 2015).

Although the vast majority of unmodified plants possess vegetative tissues with substantially lower oil contents than their seeds, engineered vegetative tissues are promising potential resource for biofuel production and worth of further investigation (Vanhercke et al., 2019). Lipid content in leaves or stems of some plant species can increase significantly under stress conditions or during plant senescence, indicating the feasibility of oil accumulation in vegetative tissues. In certain cases, the overexpression of genes encoding key enzymes or

transcription factors involved in TAG synthesis can effectively shunt the metabolic flow of carbon from carbohydrate synthesis to lipid accumulation. For example, the co-expression of *DGAT1*, *WRI1* and *OLEOSIN* in *Nicotiana benthamiana* leaves increased oil content to over 30% of leaf dry weight, which is similar to some typical oilseeds (Vanhercke et al., 2017).

3.2 Microbial lipids for biofuel production

3.2.1 Oleaginous microalgae

Oleaginous microorganisms can accumulate up to 70% of their dry cell weight as lipids and thus are ideal for biofuel production (Table 2). Moreover, microbial lipids meet the requirement as feedstock for advanced biofuels. Some oleaginous microalgae have high photosynthetic capability, rapid growth rate and capacity to accumulate high amount of lipids under stress conditions, which makes them a great source for lipid-based biofuel production. The control of growth conditions and the acquisition of promising microalgal strains are two important factors in algal lipid production (Arutselvan et al., 2021). In terms of growth conditions, light is the most important component as it affects both the growth rate and lipid accumulation (Feng et al., 2020). The carbon source typically derives from CO_2 in the atmosphere or industrial exhaust gas, or soluble carbonate (Markou et al., 2014). During the high-density cultivation of microalgae, increasing the concentration of CO_2 may enhance lipid yields (Tanadul et al., 2014). In many cases, nitrogen and phosphorus serve as important control ingredients for creating nutritional stress for lipid accumulation. Nitrogen deprivation can accelerate the accumulation of lipids in microalgae; however, this effect is often associated with a reduced growth rate (Breuer et al., 2012). A two-stage cultivation method has been used to mitigate this issue, whereby microalgae were first cultured in a medium with sufficient nutrition to accumulate high biomass and lipid accumulation was induced in the second stage by nutrition starvation (Nayak et al., 2019). In addition, some microalgal species can adapt to mixtrophic and

heterotrophic conditions. While the cost of heterotrophic cultivation is relatively high, rapid and large-scale cultivation of microalgae can be achieved (Morales-Sánchez et al., 2013).

Molecular tools have been rapidly developed and applied in metabolic engineering of algal species to improve lipid production. For instance, phospholipid: diacylglycerol acyltransferase (PDAT) catalyzes the acyl-CoA-independent synthesis of TAG. The heterologous expression of *Saccharomyces cerevisiae PDAT* fused with a chloroplast transit peptide under the control of a chimeric Hsp70A-RbcS2 promoter led to a 32% increase in TAG content in *Chlamydomonas reinhardtii* (Zhu et al., 2018). In another study, the RNA interference-mediated down-regulation of *PYRUVATE DEHYDROGENASE KINASE* in *Nannochloropsis salina* leading to more rapid TAG accumulation without negative impact on cell growth (Ma et al., 2017). Moreover, CRISPR-Cas9 has also been applied on microalgae as a means of achieving marker-less genetic modifications (Chang et al., 2020; Shin et al., 2019). For example, the ADP-glucose pyrophosphorylase encoding gene in green microalga *Tetraselmis sp*. was targeted for mutation with a CRISPR-Cas9 ribonucleoprotein delivery system, which inhibited starch synthesis and significantly improved lipid content (Chang et al., 2020).

3.2.2 Oleaginous yeast and other fungi

An important advantage of using yeast and bacteria to produce lipids derives from their capacity to utilize a wide variety of carbon sources, including glucose and many agricultural and food waste, for biofuel production (Carmona-Cabello et al., 2021). For example, after enzymatic saccharification of lignocellulosic biomass, certain oleaginous yeasts such as *Rhodosporidium toruloides* can simultaneously convert both hexose and pentose sugars in the pretreated feedstock to >50% of dry cell weight as lipids and valuable bioproducts such as carotenoids (Wen et al., 2020). In addition, some studies have attempted to use yeast and bacteria as a platform for the

direct synthesis of biofuels, but the efficiency of *in vivo* synthesis remains low and further investigations are required to improve it for commercial production (Wang & Zhu, 2017).

Yeast storage lipids can be synthesized by *de novo* or *ex novo* lipid accumulation (Fig. 3A). In budding yeast, *de novo* fatty acid biosynthesis begins with the synthesis of fatty acyl-chain from acetyl-CoA, followed by elongation and desaturation in the endoplasmic reticulum (Athenstaedt, 2021; Chattopadhyay et al., 2021). The acyl-CoAs are then integrated into TAG by the typical Kennedy pathway including four steps catalyzed by glycerol-3-phosphate O-acyltransferase, lysophospholipid acyltransferase, phosphatidate phosphatase, and diacylglycerol O-acyltransferase, respectively (Athenstaedt, 2021; Chattopadhyay et al., 2021). TAG can also be synthesized from diacylglycerol and phospholipid catalyzed by phospholipid: diacylglycerol acyltransferase without the involvement of acyl-CoA (Athenstaedt, 2021; Chattopadhyay et al., 2021). *Ex novo* lipid accumulation, on the other hand, only occurs in a small number of non-conventional yeasts, such as *Y. lipolytica*, which has the ability to assimilate hydrophobic substrates (alkanes, TAG, etc.) and convert them into storage lipids (Huang et al., 2017).

Similar to microalgae, the lipid-accumulating ability of oleaginous yeast can also be enhanced by nutritional stress. Under nitrogen deprivation, carbon flux in yeast will be prioritized towards lipid accumulation for energy storage. Therefore, controlling the carbon/nitrogen ratio in the culture medium can be a very effective means for improving lipid yields in yeast systems. Oil contents of over 70% have been achieved in *R. toruloides* by using a medium with a high carbon/nitrogen ratio (González-García et al., 2017; Saini et al., 2021). Moreover, various genetic modifications, such as altering genes related to fatty acid biosynthesis, TAG assembly, transcription factors, and lipid degradation, have been successfully used to improve lipid accumulation in yeasts (Table 2; Xu et al., 2017). For instance, the overexpression of TAG assembly genes and the deletion of lipase responsible for TAG turnover turned oleaginous yeast

Y. lipolytica into an even more obese form with 30%-70% lipid content (Bhutada et al., 2017; Friedlander et al., 2016). Interestingly, although *S. cerevisiae* is not commonly recognized as an oleaginous species, it can accumulate 50%-65% lipids as dry cell weight after systematic metabolic engineering (Arhar et al., 2021; Knoshaug et al., 2018).

Some oleaginous moulds such as *Mortierella alpina*, *Mortierella isabellina*, and *Mucor circinelloides* have also attracted much attention due to their high yield of long-chain unsaturated fatty acids (Chang et al., 2022; Fazili et al., 2022). A generalized scheme of the lipid biosynthesis in mould is outlined in Fig. 3B. Compared with oleaginous yeast, mould has a more diverse range of fatty acid composition. For example, some moulds can accumulate large amounts of arachidonic acid and γ-linolenic acid. Similar to yeast, lipid accumulation of moulds may also be induced by the deprivation of certain nutrients. In terms of metabolic engineering, enhancing the supply of NADPH and precursors have been reported to be able to increase lipid synthesis in moulds. For example, the overexpression of malic enzyme, which involved in the formation of NADPH and the conversion of malate into pyruvate, resulted in an increase of lipid production in *M. circinelloides* by more than 2-fold (Rodríguez-Frómeta et al., 2013).

3.2.3 Oleaginous bacteria

Oleaginous bacteria are attractive for their easy cultivation, rapid growth rates and other advantages. The main neutral storage lipids accumulated in bacteria are TAG, wax esters and polyhydroxyalkanoates. Gram-negative bacteria generally accumulate wax esters or minor amounts of TAG, while some Gram-positive actinomycetes accumulate large amounts of TAG. In brief, the formation of TAG begins with *de novo* fatty acid synthesis (Fig. 3C). Subsequently, acyl-CoA:*sn*-glycerol-3-phosphate acyltransferase and acyl-CoA malonyl-CoA: lysophosphatidic acid acyltransferase (PlsB and PlsC in *Escherichia coli*) catalyze the sequential acylation of glycerol-3-phosphate to form phosphatidic acid using acyl-ACP and acyl-CoA as substrates.

Following the dephosphorylation of phosphatidic acid catalyzed by phosphatide phosphatase (PgpB in *E. coli*), diacylglycerol is produced. Unlike plants, microalgae and yeast, the final step of TAG synthesis in bacteria is mainly catalyzed by a bifunctional wax ester synthase and acyl-coenzyme A: diacylglycerol acyltransferase with low substrate specificity, which can use both diacylglycerol or fatty alcohol as the acyl receptor to form triglycerides or wax esters (For recent reviews, see Martin et al., 2021; Soong et al., 2021; Steinbüchel & Wältermann, 2020).

Similar to oleaginous yeast and microalgae, there is also a competitive relationship between lipid accumulation and cell growth under nutrient-limited conditions in bacteria. Under stress conditions, some aerobic bacteria can accumulate high amount of TAG. The well-studied fast growing model species *E. coli* has been engineered for the production of TAG and acyl esters with various gene combinations or for proof-of-concept studies (e.g., Wang et al., 2020a). Other bacteria species such as *Corynebacterium glutamicum*, *Cupriavidus necator* (also known as *Ralstonia eutropha*) and *Rhodococcus* spp. have also attracted attention for their remarkable ability to accumulate large amounts of TAG (up to 80%) (Alvarez et al., 2021; Chatterjee et al., 2020; Plassmeier et al., 2016). *Rhodococcus* strains appear to be very promising in terms of TAG production, and various genetic tools have been developed and applied in its metabolic engineering (Alvarez et al., 2021). *Rhodococcus opacus* PD630 can accumulate a large amount of TAG under nitrogen limitation (Alvarez et al., 2021; Kim et al., 2019; Liang & Yu, 2021). In fed-batch fermentation with nitrogen limitation, this strain could accumulate 110.7 g/L dry biomass with over 75% TAG content (>90% of the theoretical yield), and metabolic engineered strains could produce 21.3 g/L biodiesel and 5.2 g/L hydrocarbon (Kim et al., 2019).

In addition to various genetic engineering approaches, another promising potential of oleaginous bacteria is that many species can utilize a wide variety of carbon sources, including but not limited to sugars, organic acids, alcohols, and even lignocellulosic biomass (Chatterjee et

al., 2020). For instance, *R. opacus* could convert industrial by-products and waste, lignocellulosic substrate, alkanes and aromatic hydrocarbons into lipids (Herrero et al., 2018; Roell et al., 2019). When lignin phenolic compounds were supplied as co-substrate with glucose, *R. rhodochrous* accumulated more than 40% of its cell dry weight as lipids (Shields-Menard et al., 2017).

3.2.4 Co-fermentation of mixed microorganisms for the production of lipids

Although monoculture is the dominate fermentation process, co-fermentation of mixed microorganisms is a promising novel strategy for cell growth and the production of lipids and other valuable compounds (Kapoore et al., 2022; Kumsiri et al., 2021; Zhou et al., 2015). For example, the co-fermentation of oleaginous *yeast R. toruloides* with immobilized amylase-producing yeast *Saccharomycopsis fibuligera* could directly convert starch into approximately 65% single cell oil, where the hydrolysis of low-value starch and the synthesis of microbial lipids were assigned to different microorganisms (Gen et al., 2014). Likewise, the co-fermentation of wild-type and engineered *Rhodococcus* strains with either lignin degradation or lipid biosynthesis enabled the efficient conversion of glucose and lignin into lipids (Li et al., 2019). Such co-fermentation not only reduced the metabolic stress of a single cell but also increased productivity by promoting mutual growth through the transmission of matter and energy-related compounds.

The co-fermentation of microalgae with yeast or bacteria for lipid production also achieved good progress in recent years (Chen et al., 2019). Such a co-culture system has many advantages. Yeast and bacteria can convert complex carbon sources into simple sugars, which can be directly assimilated by microalgae, can provide CO2 for microalgae and consume the oxygen produced by microalgae, and may sometimes provide essential nutrients such as vitamin B12 and nitrogen for microalgae (Arora et al., 2019; Kazamia et al., 2012; Le Chevanton et al., 2013). In line with this, the co-culture of rhizobium and microalgae could increase the biomass and lipid accumulation of microalgae strains (Do Nascimento et al., 2013). Even when industrial

wastes were used as the feedstock, a mixed culture of oleaginous yeast and microalga led to faster growth rates and lipid productivities, which demonstrated the promising prospect of co-fermentation in terms of converting low-cost waste materials into lipid and lipid-based biofuel (Cheirsilp et al., 2011). Nevertheless, although the mixed culture system has been proven to be promising in lipid production, how to synchronize the growth of mixed microorganisms and maintain a stable equilibrium for the co-fermentation system still requires further investigation. It would be necessary to study the relationship among microorganisms in co-fermentation systems from physiological, metabolic and genetic perspectives.

3.2.5 Exploring low-cost and mixed carbon sources for the production of microbial lipids

Although microbial lipid production via fermentation is technically feasible, the costly production process has hindered its application in biofuel production. The utilization of cheap substrates such as lignocellulose, non-food carbohydrate, industrial by-products and industrial wastewater is a very promising approach to reduce the cost (Zhang et al., 2018). Common forms of lignocellulosic biomass, such as agricultural and forestry waste, mainly consist of cellulose, hemicellulose and lignin in a complex structure. For efficient fermentation, the lignocellulosic feedstock needs to be pre-treated to break down the complex structure, as well as remove microbial inhibitors (Nargotra et al., 2018). In general, effective pre-treatment should increase the accessible surface area and porosity of the lignocellulosic feedstock, decrease the crystallinity and polymerization degree of cellulose, and thus improve the efficiency of saccharification and fermentation (Nargotra et al., 2018; Sun et al., 2016). This process is essential for microorganisms to mobilize the lignocellulosic feedstock as the carbon source.

As one of the most abundant resources on earth, lignocellulosic biomass is renewable, inexpensive, and widely available, and thus is considered to be an ideal raw material for microbial oil production. However, lignocellulosic hydrolysate contains furfural, phenols and

other by-products, which may seriously affect the growth and metabolism of many microorganisms. Related research has made interesting progress in enhancing the tolerance of microorganisms to the toxic components, including the isolation of novel high-tolerance strains; the targeted metabolic engineering; and the microbial domestication in lignocellulosic hydrolysates (Agu et al., 2019; Wang et al., 2013). Moreover, fermentation with mixed carbon sources can also improve cell growth and lipid production in lignocellulosic fermentation. After the pre-treatment of lignocellulosic biomass, the resulting hydrolysates are rich in both pentose and hexose. When co-fermenting glucose and xylose under non-sterile conditions, the oleaginous yeast *Lipomyces starkeyi* could produces 63.8% microbial lipids (Liu et al., 2020). Likewise, a *Trichosporon cutaneum* strain could simultaneously assimilate glucose and xylose during fermentation of corn-stover hydrolysate and accumulate 39.2% lipids (Hu et al., 2011).

Nevertheless, not all microorganisms have the ability to use both pentose and hexose for fermentation, and even they can, the preferences for each sugar can be very different. For many microorganisms, the metabolism of pentose can only begin if glucose concentrations are significantly reduced (Ha et al., 2011). Therefore, overcoming natural preferences and promoting the rapid and simultaneous utilization of mixed sugars will be essential for ample product yield. Such an objective can be achieved through metabolic engineering (Liu et al., 2018). For instance, the engineered intracellular hydrolysis of cellobiose can reduce glucose repression when co-fermenting with xylose in *S. cerevisiae* (Ha et al., 2011). However, since molecular tools are still rather limited in many non-conventional yeasts, their metabolic engineering remains a challenge.

Other inexpensive carbohydrates, such as starch, can also be used as raw materials for microbial oil production. Since many plant starches are edible, it is considered uneconomical and somewhat controversial to convert them into lipid-based biofuels. In any case, the starch from Jerusalem artichoke (*Helianthus tuberosus*), which has relatively lower economic value, has been

examined for its potential in microbial lipid production. When cultivating the oleaginous yeast *R. toruloides* Y4 with Jerusalem artichoke extracts and hydrolysates, batch and fed-batch fermentations led to lipid contents of 40%-56% (w/w) (Zhao et al., 2010).

3.3 Waste animal fat for biofuel production

3.3.1 Tallow and lard

Meat processing and animal rendering industries are the primary sources of animal fat by-products, which include tallow, lard, and poultry fat, and is primarily made up of TAG. In addition, fats can also be generated as by-products from fish processing and leather industries (Adewale et al., 2015). The rendering industry is responsible for converting waste animal tissues into usable materials, including lipids, which can be used for energy applications such as the production of second-generation biofuels (Bedoić et al., 2020). The production of biofuel from tallow has been demonstrated by hydroprocessing esters and fatty acids to obtain diesel and jet fuel equivalent, as well as through the use of classic transesterification processes to achieve biodiesel (Seber et al., 2014). However, biodiesel produced from tallow feedstock tends to have a relatively low pour point because of the high saturation degree of the fat (Pereira et al., 2012). Lard, pig fat with a saturation degree higher than poultry fat but lower than beef tallow, can also being used to produce high-quality biodiesel through transesterification via traditional and catalytic routes (Ezekannagha et al., 2017). In addition, mixing soybean oil with lard as feedstock could improve the cold flow properties of the resulting biofuel (Sarantopoulos et al., 2014).

3.3.2 Poultry fat and fish oil

Compared to beef tallow and lard, the saturation degrees of poultry fat and fish oil are significantly lower, which has advantages in producing biodiesel with high cold flow requirements (Cardoso et al., 2019). The poultry industry generates a large amount of by-product consisting of the dry or wet rendered portion of the clean carcass, which is generally rich in fatty

acids that can be used either in the rendering industry or applied to biofuel production (Cardoso et al., 2019). Oleic acid is the main component of poultry fat, which accounts for nearly 40% of the total fatty acids and has been studied for biofuel production (Kirubakaran & Arul Mozhi Selvan, 2018). Hydrocracking of poultry fat over a nickel-tungsten catalyst could result in 80% renewable hydrocarbons comprising 40% gasoline-equivalent and 30% diesel-equivalent (Hanafi et al., 2016). Chicken carcasses, with a crude fat content of approximately 40%, have also been subjected to hydrothermal and microwave treatment to produce bio-oil, which demonstrates the potential of producing biofuels directly from poultry industry waste (Zhang et al., 2020). In addition, fish discard is a PUFA-rich by-product of fish processing, and waste oil extracted from it could also have great potential for biofuel production due to its balanced ratio of saturated and unsaturated fatty acids (Karkal & Kudre, 2020).

4. Research needs and prospects

To ensure energy security and cut down on the emissions of greenhouse gases and toxic compounds, many efforts have been undertaken to develop clean energy including biofuel in recent years. Feedstock choice has a substantial impact on the cost-effectiveness, environmental friendliness and performance of biofuel production, and lipids from plants, microorganisms and animal fats are among the most promising ones. The carbon-rich fatty acid moieties in lipid feedstocks provide the lipid-derived biodiesel and hydrocarbon equivalents with a high energy density, and the resulting hydrocarbon equivalents can be used as drop-in fuels.

Although conventional chemical transesterification and deoxygenation still predominate the production of biodiesel and hydrocarbons, technological innovations such as lipase-catalyzed reactions and direct production in microbes have made great progress recently (Zhao et al., 2015). To be applied on an industrial scale, the cost of lipase for biodiesel production must be substantially lowered by increasing enzyme productivity, optimizing enzyme immobilization for

reuse, or other novel approaches. Similarly, although the *in vivo* synthesis of fatty acid alkyl esters and hydrocarbons has been demonstrated in both eukaryotic and prokaryotic microbial cell factories, this technology is only just taking off and the yield of biofuel from metabolically engineered cells needs to be further increased to be cost-effective in an industrial setting.

It is clear that the composition of lipid feedstocks has a great impact on biofuel product quality in terms of both chemical and physical properties. Since there is a great diversity of lipid feedstocks, products with very different physicochemical properties can be obtained. In terms of production scale, various oil plants have overwhelming advantages over any other lipid source, and both biodiesel and hydrocarbons produced from plant storage lipids have been studied extensively. However, since many vegetable oils can also be used for food purposes, their use in biofuel production remains controversial. In contrast, non-edible plant biomass, microbial lipids, and animal waste oil may prove to be better alternatives.

Oleaginous microorganisms such as bacterial, fungi and microalgae have received much attention due to their high oil content and low arable land requirement, as well as their advantage in environmental protection such as lowering carbon footprint (e.g. CO2 fixation by microalgae) and the recycling of waste materials (e.g. agricultural wastes, forest industry wastes, and wastewater sludge) (Behl et al., 2020; Qin et al., 2017; Saini et al., 2021; You et al., 2020; Yang et al., 2020; You et al., 2020; Zhang et al., 2021). Moreover, metabolic engineering can greatly improve microbial strains, in terms of effective utilization of waster materials, rapid growth and lipid accumulation, and ideal fatty acid profile. In addition, co-fermentation and the utilization of low-cost and mixed carbon sources are emerging and promising approaches, and future research in those aspects would make important contribution in microbial biofuel production. The rapid development of metabolic engineering, biotechnology, fermentation engineering and their integration would lead to efficient biofuel production.

5. Conclusions

Taken together, lipids have been shown to be versatile, clean, and highly efficient as a source for biofuel production. With the continuous development of lipid biotechnology in plants, microorganisms and animal fat processing, the fatty acid profiles of various lipid feedstocks could be tailored by blending or genetic engineering, to provide optimal feedstocks for biofuel production. Improvements in the performance and cost-competitiveness of biofuels can be achieved without dramatically altering the design of existing infrastructure, which could facilitate a global transition to a future of clean energy.

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Figure Captions

Fig. 1. Concise chart for various lipid-based biofuels. Drop-in biofuels refer to the hydrocarbon ones identical to petroleum fuels and fully compatible with existing engines and infrastructures.

Fig. 2. Schematic storage lipid biosynthesis in seeds. Abbreviations: ACP, acyl carrier protein; CPT, CDP-choline:1,2- diacylglycerol cholinephosphotransferase; DGAT, acyl-CoA: diacylglycerol acyltransferase; FAD2, oleate desaturase; FAD3, linoleate desaturase; FATA/B, fatty acyl thioesterase A/B; GPAT, glycerol-3-phosphate acyltransferase; LACS, long chain acyl-CoA synthetase; LPAAT, lysophosphatidic acid acyltransferase; LPCAT: lysophosphatidylcholine acyltransferase; PAP, phosphatidate phosphatase; PC, phosphatidylcholine; PDAT, phospholipid: diacylglycerol acyltransferase; PDCT, phosphatidylcholine: diacylglycerol cholinephosphotransferase; PLA_2, phospholipase A_2; SAD, stearoyl-ACP desaturase. Enzymes are presented with the nomenclature of the plant community.

Fig. 3. Schematic storage lipid metabolism in fungi and bacteria. **(A)** Non-polar lipid metabolism in yeast. **(B)** Overview of the metabolic pathway for storage lipid assembly in long chain polyunsaturated fatty acid (PUFA)-producing oleaginous mould. **(C)** Storage lipid biosynthesis in bacteria. Other abbreviations: ACCase, acetyl-CoA carboxylase; ACP, acyl carrier protein; ALA, α-linolenic acid; ARA, arachidonic acid; ALE1, lysophospholipid acyltransferase; APP1, phosphatidate phosphatase; CoA, coenzyme A; DGA1, diacylglycerol O-acyltransferase 1; DGLA, dihomo-γ-linolenic acid; ELO, fatty acid elongase; EPA, eicosapentaenoic acid; ETA, eicosatetraenoic acid; FAA1, long chain fatty acyl-CoA synthetase; FAS, fatty acid synthase; GLA, γ-linolenic acid; GPT2, glycerol-3-phosphate O-acyltransferase 2; LA, linoleic acid; LOA1, lysophospholipid acyltransferase; LRO1, phospholipid: diacylglycerol acyltransferase; ME, malic enzyme; OA, oleic acid; OLE, acyl-CoA delta-9-desaturase; PA, palmitic acid; PgpB, phosphatide phosphatase; PlsB, acyl-CoA: glycerol-3-phosphate acyltransferase; PlsC, acyl-CoA

975 malonyl-CoA: lysophosphatidic acid acyltransferase; SA, stearic acid; SCT1, glycerol-3-
976 phosphate *O*-acyltransferase 1; SDA, stearidonic acid; SLC1, lysophospholipid acyltransferase;
977 WS/DGAT, wax ester synthase/ diacylglycerol acyltransferase. The enzymes are presented
978 according to the nomenclature of the related communities.

Table 1. Impact of lipid source on the biofuel properties

Property	Impact on fuel performance	Relationship with lipid feedstock	Other determinants	Reference
Lubricity	Lower friction between engine parts in relative motion	Increases somewhat with greater chain lengths and degrees of unsaturation	Determined by the presence of oxygenated functional groups	(Awang et al., 2021; Khan et al., 2022)
Viscosity	Affects fuel atomisation and combustion quality	Increases with greater chain lengths and saturation degree	The viscosity of fatty acid ethyl esters (FAEE) is slightly higher than fatty acid methyl esters (FAME)	(Hadhoum et al., 2021; Shanmugam et al., 2020)
Cloud point and pour point	Affects the operation ability of fuel under low temperature	Decreases with unsaturation degree and branching	Can be improved by the treatment with a cold flow improver	(Zahran et al., 2021)
Cetane number	Affects the ignition quality of the fuel product	Increases with chain length, decreases with branching and unsaturation degree	Decreases with the presence of aromatic compounds	(Mofijur et al., 2022)
Oxidative stability	Resistance of fuel to oxidation during processing and storage	Decreases with unsaturation degree	Exposure to oxygen or light, as well as the presence of heat or metal	(Sneha et al., 2021)

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Table 2. Lipid content of some representative microorganisms

Microorganism	Engineering strategies*	Cultivation conditions*	Major fatty acids	Lipid content/ titer	Reference
Microalgae					
Chlorella pyrenoidosa	Wild type	Synthetic saline wastewater influent; photobioreactor	C16:0 (up to 32%), C18:1 (up to 41%), C18:0, C18:2, C18:3	65.2%	(Yang et al., 2021)
Chlorella vulgaris	Surface display of carbonic anhydrase-dockerin complex	100 mL bold basal medium in 500 mL flasks	C18:1 and C18:2 (up to 70%) C16:1, C16:0, C18:0	23.3%	(You et al., 2020)
Monoraphidium sp.	Wild type	Strigolactone treatment and nitrogen deficiency	C16:0 (35%), C18:1 (24%), C18:2 (16%), C18:3 (17%)	53.7%	(Song et al., 2020)
Chlorella ellipsoidea	Overexpressing a soybean transcription factor *GmDof4*	Mixtrophic culture	C18:1 (31%), C18:2 (27%), C16:0 (21%), C18:3 (14%), C18:0	52.9%	(Zhang et al., 2014)
Parachlorella kessleri	Heavy-ion beam irradiation-induced mutagenesis	Photobioreactor	NA	66%	(Takeshita et al., 2018)
Chlorella sp.	Wild type	Two-stage cultivation; nitrogen starvation; photobioreactor	C18:1 (22.6-29.3%), C16:0 (20%), C18:2, C18:3	36.7%	(Nayak et al., 2019)
Chlamydomonas reinhardtii	Knockdown long-chain acyl-CoA synthetase (cracs2)	Tris–acetate–phosphate (TAP) medium	C18:3 (41.1%), C16:4 (25.3%), C16:0	14.%	(Jia et al., 2016)
Yeast					
Yarrowia lipolytica	Overexpression of heterologous DGA1 and DGA2, and deletion of the native TGL3	Glucose, batch fermentation	NA	77%	(Friedlander et al., 2016)
Yarrowia lipolytica	Engineering of reducing agent supply by overexpressing TKL1 & DGA1	Glycerol	C18:1, C18:0, C16:0, C18:2, C16:1	25%	(Dobrowolski & Mirończuk, 2020)

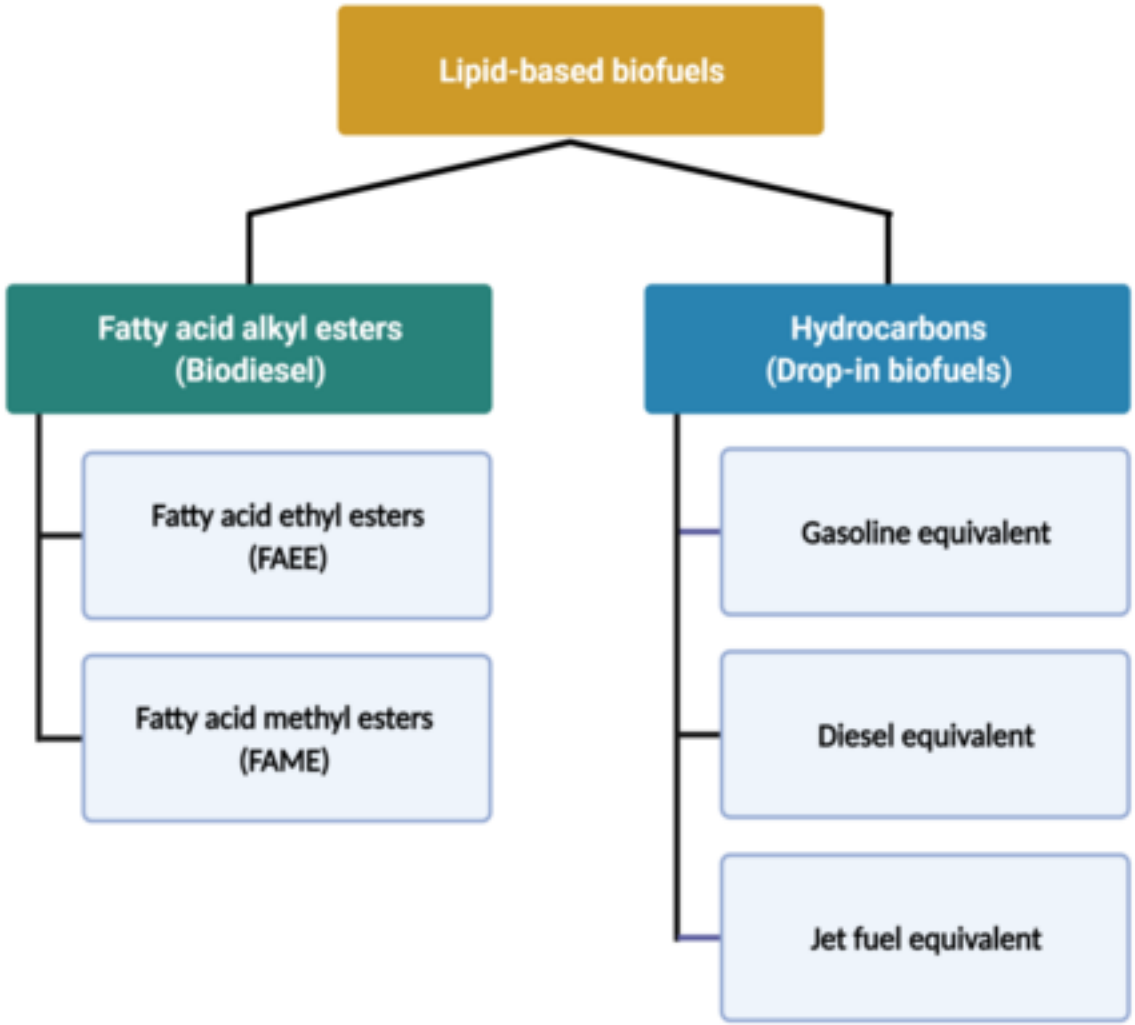
Yarrowia lipolytica	Deleting POX1–6 and TGL4, overexpressing DGA2, GPD1, HXK1 & SUC2	Molasses/glycerol	C18:1 (~50%), C16:0 (~20%), C16:1, C18:2	31%	(Rakicka et al., 2015)
Yarrowia lipolytica	Deletion of TGL4 and GSY1 with overexpression of DGA2 and GPD1	Glycerol	C18:1, C16:0, C18:2, C18:0, C16:1	52.4%	(Bhutada et al., 2017)
Rhodosporidium toruloides	Overexpression of ACC1, DGA1 and SCD1	Glucose	C18:1, C16:0, C18:0, C18:2	89.4 g/L	(Zhang et al., 2016)
Rhodosporidium toruloides	Expression of *Vitreoscilla* hemoglobin (VHb)	Glucose	C16 and C18 long-chain fatty acids	61%	(Wang et al., 2020)
Rhodosporidium toruloides	Adaptation and screening in tea waste hydrolysate	Nitrogen-limited tea waste hydrolysate medium	C16:0 (~28%), C18:0 (~15%), C18:1 (~30%), C18:2 (~20%), C18:3 (~4%)	42%	(Qi et al., 2020)
Rhodosporidium toruloides	Overexpression of OLE1	Glucose	C16:0 (10%), C18:1 (70%)	~4g/L	(Tsai et al., 2019)
Rhodosporidium toruloides	Wild type	Crude glycerol media with L-proline as the anti-stress agent	C16:0 (55%), C18:0 (13.5%), C18:1 (23.2%)	64%	(Kamal et al., 2020)
Rhodosporidium toruloides	Wild type	Glucose, nutrient limitation in two-step batch culture	NA	77.0%	(González-García et al., 2017)
Lipomyces starkeyi	Wild type	Glucose	C16:0 (36%), C16:1 (3%), C18:0 (14%), C18:1 (43%)	54.8%	(Calvey et al., 2016)
Candida tropicalis	Ectopic expression of repressor activator protein 1 (CtRAP1)	Glucose/galactose	NA	37%	(Chattopadhyay et al., 2020)
Saccharomyces cerevisiae	High lipid content segregant from mating AWRI1631 with CEN.PK. Mutated Acc1p, overexpression of DGA1, deletions of CKB1, TGL3, ARE2, GSY2	Glucose	C16:0 (~15%), C16:1 (~35%), C18:1 (~43%)	65%	(Arhar et al., 2021)
Saccharomyces cerevisiae	Knocking out SNF1 and overexpression of DGA1, XYL1, XYL2, and XKS in a oleaginous strain D5A	Xylose/Glucose	C18:1 (~45%), C16:1 (30%-45%) C16:0, C18:0	up to 50%	(Knoshaug et al., 2018)

Bacteria

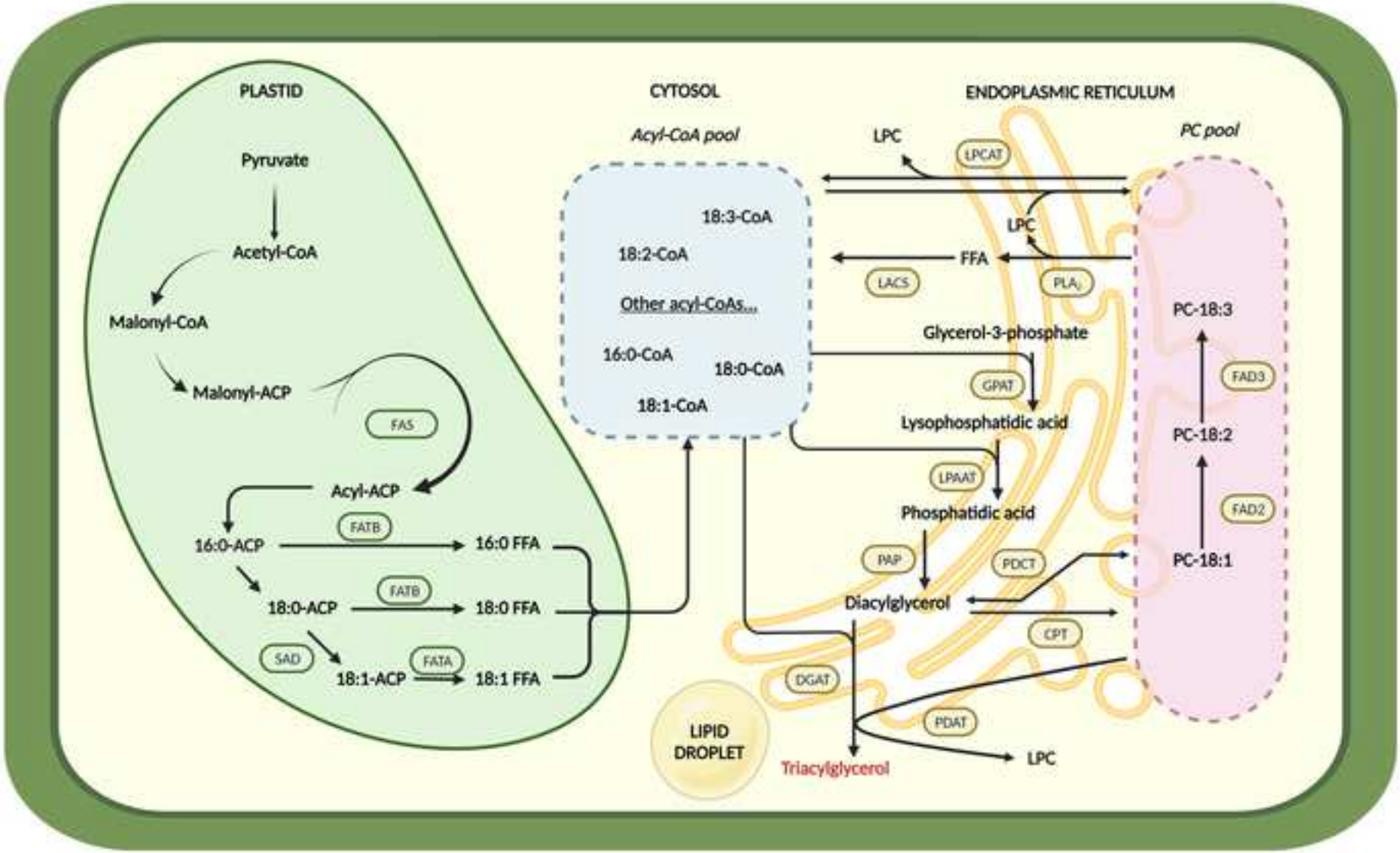
Rhodococcus opacus	Wild type	Glucose, fed-batch culture starting with pH shifting from 6.4 to 7.0	C15:0 (5.0g/L), C16:0 (26.5g/L), C16:1 (6.7g/L), C17:0 (4.2g/L), C17:1 (6.6g/L), C18:0 (4g/L), C18:1 (15g/L)	75.5%	(Kim et al., 2019)
Corynebacterium glutamicum	Expression of atf1, atf2, pgpB, tadA and engineering of lipid synthetic and turnover pathway	Glucose	C16:0 (46%), C18:1 (48%), C18:0 (6%)	17.8%	(Plassmeier et al., 2016)
Rhodococcus opacus	NADP+-dependent malic enzyme	Glucose	NA	46%	(Hernández & Alvarez, 2019)
Rhodococcus rhodochrous	Wild type	Glucose	C16:0 (35 %) and C18:0 (42 %)	50%	(Shields-Menard et al., 2015)

*Genetic manipulations and cultivation conditions summarized in this table do not include all specific details, but only those that are unique and of concern to this review. *Abbreviations:* ACC, acetyl-CoA carboxylase; ARE, sterol acyltransferase; CKB, regulatory subunits of casein kinase 2; DGA, diacylglycerol acyltransferase; GPD, glycerol-3-phosphate dehydrogenase; GSY, glycogen synthase; HXK1, hexokinase; OLE, Δ9 fatty acid desaturase; POX, acyl-coenzyme A oxidases; SCD, stearoyl-CoA desaturase; SNF, ADP-activated serine/threonine kinase; SUC2, invertase; TGL, triacylglycerol lipase; TKL, transketolase; XKS, xylulose kinase; XYL1, xylose reductase; XYL2, xylitol dehydrogenase; atf1 and atf2, *R. opacus* diacylglycerol acyltransferase; pgpB, *E. coli* phosphatidic acid phosphatase; tadA, *R. opacus* lipid droplet protein; TAG, triacylglycerol

new Figure 1

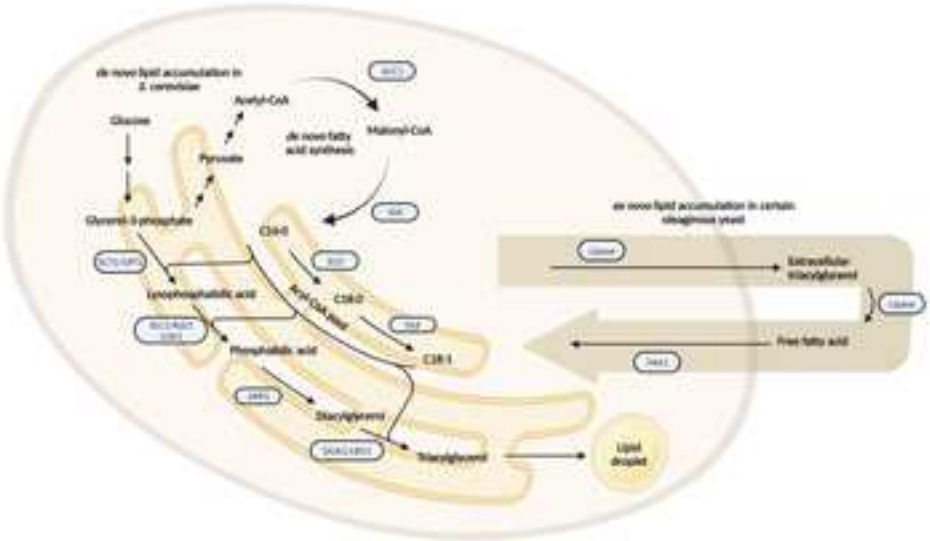

Lipid-based biofuels
Fatty acid alkyl esters (Biodiesel)
Hydrocarbons (Drop-in biofuels)
Fatty acid ethyl esters (FAEE)
Fatty acid methyl esters (FAME)
Gasoline equivalent
Diesel equivalent
Jet fuel equivalent

new Figure 2

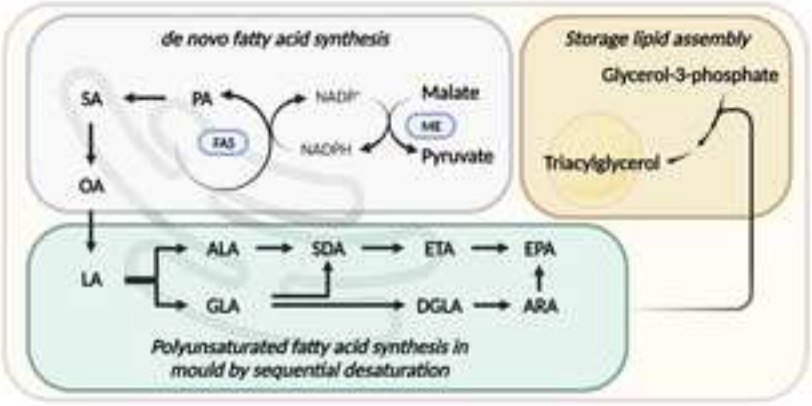

PLASTID
Pyruvate
Acetyl-CoA
Malonyl-CoA
Malonyl-ACP
FAS
Acyl-ACP
16:0-ACP
FATB
16:0 FFA
18:0-ACP
FATB
18:0 FFA
SAD
FATA
18:1-ACP
18:1 FFA
CYTOSOL
Acyl-CoA pool
18:3-CoA
18:2-CoA
Other acyl-CoAs...
16:0-CoA
18:0-CoA
18:1-CoA
ENDOPLASMIC RETICULUM
LPC
LPCAT
LPC
FFA
LACS
PLA2
Glycerol-3-phosphate
GPAT
Lysophosphatidic acid
LPAAT
Phosphatidic acid
PAP
PDCT
Diacylglycerol
CPT
DGAT
PDAT
LPC
Triacylglycerol
LIPID DROPLET
PC pool
PC-18:3
FAD3
PC-18:2
FAD2
PC-18:1

new Figure 3

A



B



C