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Microbial production of fatty acid-derived fuels and chemicals
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Fatty acid metabolism is an attractive route to produce liquid transportation fuels and commodity oleochemicals from renewable feedstocks. Recently, genes and enzymes, which comprise metabolic pathways for producing fatty acid-derived compounds (e.g. esters, alkanes, olefins, ketones, alcohols, polyesters) have been elucidated and used in engineered microbial hosts. The resulting strains often generate products at low percentages of maximum theoretical yields, leaving significant room for metabolic engineering. Economically viable processes will require strains to approach theoretical yields, particularly for replacement of petroleum-derived fuels. This review will describe recent progress toward this goal, highlighting the scientific discoveries of each pathway, ongoing biochemical studies to understand each enzyme, and metabolic engineering strategies that are being used to improve strain performance.

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Introduction
Production of fuels and petrochemicals from renewable resources (e.g. photosynthetically produced biomass in plants and algae) is an attractive strategy to meet growing demand while simultaneously curbing the net release of greenhouse gases. A common approach to implementing this strategy is to engineer the metabolism of a microorganism to convert biomass-derived sugars to desired products. Engineered strains can then be cultivated in industrial-scale bioreactors and the resulting products purified using traditional chemical and biochemical engineering techniques. Of the many metabolic pathways found in nature, fatty acid metabolism has received significant attention as a biological route to convert sugars to liquid transportation fuels and higher value oleochemicals (Figure 1). This review will focus on recent progress in developing microbes for converting renewable feedstocks to fuels and chemicals derived from fatty acid metabolism. It should be noted that other groups are pursuing the same goals using photosynthetic organisms and/or traditional catalytic technologies to produce fuels and chemicals [1–3].

Fatty acid metabolism is one of a handful of anabolic pathways capable of producing large (>6 carbon) hydrophobic metabolites that are similar to molecules found in high energy petroleum distillates. Other pathways, including isoprenoid and polyketide biosynthesis, have been described elsewhere [4–7]. Higher energy density distillates such as diesel and jet fuel are needed by the heavy transportation sector due to weight and range constraints. Unlike the case for personal vehicles, these constraints also functionally prohibit electricity as a power source, due to the energy storage limitations of modern batteries. Instead, microbially produced fatty acid derivatives have been viewed as a supplement to and potentially a replacement for petrodiesel and jet fuel. This potential has motivated research efforts to metabolically engineer microbes to approach theoretical yields from lignocellulosic feedstocks. Unlike short chain alcohols, the low water solubility of longer carbon chain-length hydrocarbons and esters should result in reduced recovery costs and reduced toxicity in the fermentation broth due to phase separation. In addition, fatty acid derivatives are more likely to be compatible with existing engines as well as transport and storage infrastructure.

In addition to fuels, fatty acid metabolism is a route to many oleochemicals that are currently made from fossil fuels or plant oils. These compounds include oils, waxes, free fatty acids (FFA), fatty alcohols, and bioplastics, which are used as surfactants, lubricants, and materials in many consumer products. Conversion of biomass-derived sugars to these compounds is economically attractive because the ability of microbial catalysts to control product chain length, add precise modifications, and use non-plant oils as feedstocks. Oleochemicals are sold at higher prices than fuels and therefore commercial processes that leverage microbial catalysts will likely target non-fuel applications first.

While the ability of microbes to produce hydrocarbons was discovered decades ago, many of the biochemical pathways for converting intermediates in fatty acid metabolism to downstream products have only been
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Sustainable production of fatty acid-derived fuels and chemicals. The process begins with the conversion of photosynthetic biomass (e.g., plants or algae) to fermentable intermediates that can be catabolized by cells to generate energy and the building blocks (e.g. acetyl-CoA) required to synthesize anabolic products, including fatty acids. Alternatively, these same chemicals can be derived directly from photosynthesis and carbon fixation. Acetyl-CoA is the direct precursor to fatty acid biosynthetic intermediates that are the starting point for a wide range of chemical products. These products can be used as fuels or chemicals. Upon combustion, the fatty acid-derived products release energy and carbon dioxide that can be recycled to close the carbon cycle.

recently elucidated. Pathways for producing fatty acid-derived chemicals start from fatty acyl–acyl carrier protein (ACP) thioesters (in fatty acid biosynthesis), fatty acyl-coenzyme A (CoA) thioesters (in fatty acid catabolism), or free fatty acids (from thioesterase cleavage of acyl-thioesters). The remainder of the review will focus on each product group, recent discoveries, and current challenges to commercial deployment. In general, current research is focusing on identifying homologous pathways in various organisms, characterizing the enzymes that comprise each pathway, and applying metabolic engineering strategies to maximize yields of each product. The major challenges to commercialization lie in increasing yields of products such that they approach the theoretical limits (~0.25–0.40 g/g glucose), engineering strategies for controlling product chain length, and developing strains that can process lignocellulosic hydrolysates.

Biodiesel
Biodiesel is a mixture of fatty acid methyl esters (FAMEs) that is currently being produced from plant oils, animal fats, and/or FFA using traditional catalysis [8]. Escherichia coli has also been engineered to produce FAME in situ by heterologous expression of a fatty acid O-methyltransferase (FAMT) from Mycobacterium marinum or similar homologues (Figure 2) [9]. The tested enzymes were most active on C9–C12 saturated and 3-hydroxylated FFA and used S-adenosylmethionine (SAM) as the methyl donor. In this study, the supply of SAM was increased by deletion of metI, a global regulator that feedback inhibits SAM biosynthesis in response to elevated concentrations of SAM. Yields and titers were very low, with the bottleneck hypothesized to be the lack of thioesterase activity for producing 3-hydroxydecanoic acid, the preferred substrate of the FAMT from M. marinum. Achieving higher yields of FAME will likely require a combination of protein engineering (improving thioesterase and FAMT activities against various fatty acids) and metabolic engineering (optimizing the recycling of SAM).

Fatty acid ethyl esters (FAEEs), which are chemically similar to current biodiesel, can be produced from acyl-CoAs and ethanol using a promiscuous wax ester synthase/acyl-CoA:diacylglycerol acyltransferase (WS/DGAT) first described by the AtfA from Acinetobacter sp. ADP1 (Figure 2) [10**]. Homologues of AtfA have been identified in other γ-proteobacteria and actinomycetes and exhibit different substrate specificities [11–13], including an isoprenoid wax ester synthase in Marinobacter hydrocarbonoclasticus that could be used to make highly branched esters with reduced cloud points [14]. To produce FAEEs, fatty acids and ethanol must be fed to a strain harboring AtfA or the atfA expressing strain must be engineered to produce both metabolites. In E. coli, ethanol production can be increased by heterologous expression of pyruvate decarboxylase (Pdc) and alcohol dehydrogenase (AdhB) from the ethanologen Zymomonas mobilis [15]. When E. coli expressing atfA, pdc, and adhB
on a plasmid was fed oleate in the fermentation broth, up to 1.28 g/L of FAEE was produced [16]. Similarly, when \( a彷 \) was expressed in engineered ethanol producing \( S.a pneumoniae cersuis \), \( S.a pneumoniae cersuis \) was produced when cells were fed fatty acids [17]. Expression of \( a彷 \) in a FFA producing E. coli with exogenous feeding of ethanol produced a titer of 0.4 g/L of FAEE [18*]. The first demonstration of FAEE synthesis without feeding used three plasmids to overexpress \( a彷 \) (on two separate plasmids), \( pdc \), \( adhB \), \( tesX \) and \( fadD \) (to increase acyl-CoA synthesis from FFA). When E. coli (DH1 \( S.fadE \)) harboring these plasmids was cultivated in broth overlaid with dodecane, the culture produced \( \approx 0.7 \) g/L of FAEE, representing approximately 7% of the maximum theoretical yield [18*]. Fed batch cultivation of a similar strain increased production to 0.922 g/L at the expense of yield (reported as 0.025 g FAEE g\(^{-1}\) glucose) [19]. A similar strain was used to produce biodiesel from a minimal media formulated with ionic liquid pretreated switchgrass, albeit at low rates (100 mg/L in \( \approx 200 \) hours) [20]. It should be noted that this strain coexpressed genes for producing ethyl esters and cellulosases to release sugars from the pretreated biomass, a first step toward consolidated bioprocessing to produce advanced biofuels.

These initial demonstrations confirmed the ability of E. coli to produce biodiesel de novo from renewable carbon sources. While promising, three challenges remain: firstly increasing yields to approach theoretical limits; secondly attaining these yields from biomass-derived feedstocks; and finally tailoring FAME/FAEE composition to meet desired fuel properties (e.g. energy density and melting point, illustrated in Figure 3, and cetane number [ASTM > 40]). In the latter challenge, the preferred chain lengths, modifications, and degree of homogeneity (i.e. single component or blends) will depend on the demands of the final application. Product yields are a critical issue that will determine if microbial hydrocarbons will be competitive economically. At current prices, sugar (~$0.16–0.20 lb\(^{-1}\)) will contribute $3.00–4.50 per gallon of FAEE, if produced at 95% of theoretical yield (Figure 4). The theoretical yield (TY) is defined here as the maximum stoichiometric yield of product given the known metabolic network of the cell, in addition to any heterologously introduced pathways. Significant efforts to decrease the cost of sugars are underway, but feedstock costs are likely to remain a major percentage of final fuel costs, as it is the case with current petroleum derived fuels. The challenges to achieving theoretical yields of FAEE include deregulating control and maximizing flux through fatty acid biosynthesis (FAB), balancing the rates of fatty acid synthesis, acyl-CoA synthesis, ethanol production, and FAEE synthesis, and developing cultivation strategies to minimize the amount of feedstock that is consumed for cell growth and maintenance. A recent synthetic biology study aimed at balancing the supply...
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The properties of fatty acid derivatives depend on the length of the acyl chain. For example, the molar energy content increases with each additional carbon, but the volumetric energy density plateaus after 12–14 carbons near the density of petrodiesel (36 MJ/L). Similarly, the melting temperatures of alkanes, olefins, and esters corresponding to the common membrane fatty acids (C16, C18) are above 0 °C, which complicates their use as neat (i.e. single component) fuels in cold climates. These examples illustrate the need to control chain length in order to obtain useful chemical products. The properties of fatty acids are also influenced by incorporation of branches and other chemical modifications. Data were collected from CRC Handbook of Chemistry and Physics, NIST WebBook, and Materials Safety Data Sheets.

of ethanol and acyl-CoA succeeded in improving FAEE titer (1.5 g/L) and yield (reported as 28% of TY) [21**]. Substrate supply was controlled using a dynamic sensor-regulator system that comprised two FadR-regulated promoters. DNA binding by FadR is modulated by the presence of acyl-CoA. Therefore, the presence of excess acyl-CoA led to derepression of promoters controlling transcription of genes involved in ethanol synthesis and conversion of acyl-CoA to FAEE enabling ethanol synthesis and conversion of acyl-CoA to FAEE (consumption of acyl-CoA). The final strain incorporated engineered FadR-responsive promoters that optimized FAEE yields, albeit with significant room for additional improvement.

Alkanes, fatty aldehydes, and fatty alcohols

Bacteria have been known to synthesize hydrocarbons for many decades [22] and the search for enzymes responsible has been fraught with difficulties [23]. However, recent work has uncovered several pathways for producing long-chain (C10 < x < C20) and very-long chain (C20+) alkanes. For example, a bioinformatic comparison of cyanobacteria capable of producing and not producing alkanes identified two genes that were later shown to encode enzymes that produce fatty aldehydes and convert them to alkanes by removing the carbonyl carbon [24]. The first enzyme was proven to be an acyl-ACP reductase that cleaves the thioester bond and uses NADPH to reduce the fatty acid to an aldehyde (Figure 5). Subsequent work re-classified the second enzyme as an aldehyde deformylase that uses oxygen and a protein reductive system (NADPH, ferridioxin, ferridioxin reductase) to generate an alkane, formate, and water [25,26]. When the acyl-ACP reductase and aldehyde deformylase were heterologously expressed in E. coli, up to 300 mg/L of pentadecane, pentadecene, and heptadecene was produced. The alkane composition matched the predominant fatty acids (16:0, 16:1, and 18:1) found in E. coli. The successful demonstration of alkane production motivates further metabolic engineering to improve yields. Microbially derived hydrocarbons possess higher energy densities and higher cloud points than current biodiesel blends, enabling year-round usage in all climates [27,28]. To leverage these advantages, challenges including a large NADPH requirement, balancing the protein reductive system for the aldehyde deformylase, and potential toxicity caused by aldehyde intermediates will need to be solved. Inhibition of aldehyde deformylase by hydrogen peroxide (with respect to O₂) was also recently reported [29]. Performance was improved in vitro by expression of a fusion protein of aldehyde deformylase and catalase.

Fatty aldehydes (and theoretically alkanes) can also be produced by acyl-CoA reductases, which have been identified in many species [30–32,33]. In this pathway (Figure 5), substrate specificity is provided by acyl-ACP thioesterases (individual thioesterases target a wide range of chain lengths [34]) which produce FFA that can be converted to acyl-CoAs by acyl-CoA synthetases (e.g. E. coli FadD). When acyl-CoA reductases are expressed in
E. coli in the presence of acyl-CoAs, a mixture of fatty aldehydes and fatty alcohols is detected, suggesting either spontaneous oxidation of aldehydes to fatty alcohols or the existence of other reductive enzymes capable of acting on long chain substrates. Fatty alcohols have higher selling prices than diesel, are widely used in industry, and are currently derived from plant oils or petrochemical sources. These reasons motivate metabolic engineering efforts to construct fatty alcohol producing strains. Of the efforts to characterize acyl-CoA reductases, only a handful reported titers higher than 10 mg/L of fatty alcohol. Overexpression of TesA', overexpression of FadD, and heterologous expression of acr1, an acyl-CoA reductase from Acinetobacter calcoaceticus, in an E. coli ΔfadE strain resulted in production of approximately 100 mg/L of C_{16} and C_{18} fatty alcohols [18*]. Swapping the acyl-ACP thioesterase from TesA' to those from Umbellularia californica or Cuphea hookeriana shifted the predominant chain length to C_{12} and C_{14}, respectively, but with unspecified titers. In order to further increase yields, it is likely that all of the conversion steps after FAB will need to be carefully balanced to avoid the accumulation of intermediates, analogous to FAEE synthesis. Optimization of expression levels of the U. californica thioesterase, fadD, and an acyl-CoA reductase more than doubled fed-batch titers in a recent study from 210 to 449 mg/L of C_{12}–C_{14} alcohols [35]. Recently, an NADPH-dependent fatty alcohol reductase has been isolated from Marinobacter aquaeolii VT8 which possesses both acyl-CoA reductase and aldehyde reductase activity [33*,36]. This enzyme could be used to prevent accumulation of toxic aldehydes in a fatty alcohol producer. During the preparation of this review, a carboxylic acid reductase from M. marinum was found to exhibit substantial activity toward reduction of medium-chain and long-chain length FFAs to the corresponding fatty aldehydes [37]. When coupled with a native aldehyde reductase from E. coli (YjgB) or aldehyde dehydrogenase, titers of over 350 mg/L of fatty alcohols and 2 mg/L of alkanes were achieved.

**α-Olefins**

In addition to alkanes, several bacterial species produce α-olefins, hydrocarbons with terminal double bonds, (Figure 6). This class of compound could be blended with diesel fuels but would have higher value as chemical building blocks. Native olefins are typically either one carbon shorter or one carbon longer than the dominant fatty acids found in the cell membrane. Synechococcus sp. PCC 7002, a cyanobacteria, produces two α-olefins, 1-nonadecene and 1,14-nonadecadiene, that are one carbon larger than the largest fatty acids [22,38]. A gene, called *ols* (for *olefin synthase*), encoding a large protein sharing homology with type I polyketide synthases was recently shown to be responsible for producing these two olefins [38]. Ols has a domain architecture that suggests that an
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Figure 6

Biosynthesis of olefins. (a) In cyanobacteria, olefins are formed via an elongation–decarboxylation mechanism catalyzed by a type I megasynthase. Synthesis begins when a fatty acyl chain is transferred from a discrete acyl–acyl-carrier protein (ACP) to the ACP1 domain. A typical polyketide extension module consisting of ketosynthase, acyltransferase, and ketoreductase domains extends the acyl-chain by two carbons donated from malonyl-CoA and reduces the beta-ketogroup to a hydroxyl. This position is activated by transfer of sulfate group from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) via the sulfotransferase domain. The terminal thioesterase (TE) domain catalyzes release of carbon dioxide, a sulfate ion and the olefin product. (b) Alternatively, terminal double bonds are introduced by decarboxylation catalyzed by cytochrome P450 (OleT) leading to olefins one carbon shorter than their substrates. (c) Internal olefins and ketones are produced by a set of enzymes that operate on acyl-thioesters. The pathway begins by OleA catalyzed condensation of two acyl-chains. The remaining enzymes are responsible for reducing and dehydrating the OleA product. Alternatively, spontaneous decarboxylation can produce ketones.

elongated β-hydroxyacyl chain is generated by standard polyketide enzymology. A predicted sulfotransferase domain is hypothesized to activate the β-hydroxy group by addition of sulfate from an activated intermediate (e.g. 3′-phosphoadenosine 5′-phosphosulfate). Sulfation would provide the activation necessary to allow subsequent dehydration and decarboxylation to yield 1-nonadecene. While titers from PCC 7002 were increased 2-fold to 5-fold for 1-nonadecene and 1,14-nondecadiene, respectively, by replacement of the native als promoter with a stronger promoter, titers remain in the sub μg/mL range. Heterologous expression of als was recently demonstrated after significant optimization, but alkene products were not detected in E. coli [39]. Engineering of the als gene (e.g. replacing the loading domain) could lead to a set of pathways for producing a wide range of α-olefins. Unfortunately, expression of polyketide synthases in E. coli is not facile and efforts may need to shift to other hosts (e.g. Actinobacteria).

A second route for terminal alkene production, identified in a Jeotgalicoccus species, is catalyzed by a cytochrome P450 enzyme named OleT (Figure 6) [40]. When OleT was heterologously expressed in E. coli, 1-pentadecane and 1,10-heptadecadiene (one carbon less than the dominant fatty acids in E. coli) were produced at unreported titers. Similarly, 1-heptadecene was produced when stearic acid (18:0) was fed to E. coli expressing OleT and in vitro reactions using purified OleT. These data suggest that OleT can utilize both acyl-ACPs and FFA as substrates. Combining expression of thioesterases with OleT could lead to efficient production of medium chain length α-olefins as long as high levels of functional OleT expression (P450 expression is notoriously difficult) can be achieved in a facile host such as E. coli.

Oils, waxes, long chain olefins, and ketones
While not likely to be used directly as fuels, high molecular weight oils and waxes could be synthesized by microorganisms and used as feedstocks for producing fuels and chemicals via traditional refining methods. These compounds also have a variety of industrial applications as lubricants, food additives, and plasticizers. Many oleaginous bacteria and fungi accumulate triacylglyceride (TAG) oils at high yields [41] and metabolic engineering has succeeded increasing production from these strains (e.g. Yarrowia lipolytica [42]). The physical properties of oils can be modulated by introducing acyl chains of various lengths. For example, a diacylglycerol acyltransferase from Euonymus alatus (burning bush) incorporates an acetyl group in place of the third acyl chain to produce a low viscosity oil [43]. Heterologous expression of oil synthesis pathways in oleaginous microbes is therefore a promising strategy to produce oils from biomass feedstocks in high yield.
Waxes have also been produced in *E. coli* using a wax ester/diaclyglycerol acetyltransferase (WS/DGAT, e.g. AtfA) [44]. Similar long-chain (C20+) alkenes (with internal double bonds, Figure 6) and ketones are produced in several bacteria by a set of enzymes named OleABCDE [45]. While the details of the OleABCDE pathway are not completely elucidated, it has been shown that OleA catalyzes the head-to-head condensation of two acyl-thioesters (between the carbonyl of one acyl chain and the alpha carbon of the second) and OleD catalyzes the reduction of an internal ketone that is essential for the subsequent dehydration (hypothetically catalyzed by OleC and/or OleB) that yields the final alkene [45–47]. Heterologous expression of OleA from *Micrococcus luteus* in a FFA producing *E. coli* (DH1 ΔfadE, plasmid based ‘TesA’), generated small quantities of long-chain length (C27–C29) monoketones [48] that are hypothesized to be spontaneous decarboxylation products [46]. Similarly, when the three gene operon (homologs of OleB and OleC are fused in *M. luteus*) was expressed in the same FFA producing *E. coli*, four internal alkene species (C27–C29 in size with 2 or 3 desaturations) were observed. As in the case of α-olefins, identification and characterization of the OleABCDE pathway enable metabolic engineering efforts to produce ketones and olefins. Unlike the case for α-olefins, the enzymes involved are reasonably sized, express well in *E. coli*, and have minimal cofactor requirements.

**Free fatty acids**

While not useful as fuels, free fatty acids (FFAs) are commodity chemicals that are commonly derived from plant oils such as coconut (for C12–C14) and palm (for C16–C18). FFAs are feedstocks for chemicals used in cleaning products, agrochemicals, biocidal agents, textile processing agents, and polymer additives and can also be used as a feedstock for *ex vivo* production of biodiesel. FFAs are the most direct product that can be derived from FAB and yields of over 70% of theoretical have been achieved [49]. Introduction of an efficiently expressed acyl-ACP thioesterase deregulates FAB and results in greatly increased carbon flux through the pathway. Several recent reviews have highlighted thioesterase expression and additional efforts that increase flux through FAB to FFA [50,51].

**Methyl ketones and polyesters**

Methyl ketones are another class of fatty acid-derived products, which could have value as biofuels or chemical feedstocks. Recent work has demonstrated production of C11–C15 methyl ketones in a strain of *E. coli* engineered to produce FFA and guide them through β-oxidation to

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**Figure 7**

Synthesis of chemicals via intermediates in β-oxidation. Fatty acids generated by thioesterase (TE) cleavage or scavenged from the environment enter β-oxidation as acyl-CoA synthesized by acyl-CoA synthetases. Oxidation of acyl-CoA by FadE yields enoyl-CoA that can be hydrated by enoyl-CoA hydratase (FabB or PhaJ) to generate 3-hydroxyacyl-CoAs of the (S)-stereochemistry and (R)-stereochemistry, respectively. 3-(R)-hydroxyacyl-CoA can be polymerized (red highlights) by polyhydroxyalkanoate (PHA) synthases (PhaC) to yield medium chain length polyester (mcl-PHA). 3-(S)-hydroxyacyl-CoA is further oxidized by FabD to a 3-ketoacyl-CoA that is ordinarily cleaved by FadA to generate an acyl-CoA and acetyl-CoA. Alternatively, ketoacyl-CoA can be hydrolyzed by FabM to yield a keto-acid that spontaneously decarboxylates to yield a methyl ketone (blue highlights). To facilitate the synthesis of mcl-PHA and methyl ketones, it is necessary to knockout competing pathways at branch points (FabD for mcl-PHA, FadA for methyl ketone).
β-ketoacyl-CoA thioesters (Figure 7) [52]. This was accomplished by replacing FadE activity with a soluble acyl-CoA oxidase from M. jannaschii, overexpressing FadB (generates β-ketoacyl-CoA from enoyl-CoA), and deleting fadB (used to produce acetyl-CoA and a shorter acyl-CoA from β-ketoacyl-CoA). Additional overexpression of a poorly characterized acyl-CoA thioesterase, FadM, was found to significantly increase titer to approximately 50 mg/L, suggesting its role is as a β-ketoacyl-CoA thioesterase. Released β-ketoacids were hypothesized to spontaneously decarboxylate to yield methyl ketones. Use of a decane overlay further improved titers to 380 mg/L [52].

A final category of fatty acid-derived products are bioplastics generated from polymerizing hydroxylated acyl-CoAs to form polyhydroxalkanoates with pendant medium-chain length alkyl groups (Figure 7). This class of bioplastics has been generated by feeding vegetable oils to bacterial cultures, which enter β-oxidation to generate polymerizable 3-hydroxyacyl-CoA monomers [53]. However, the iterative nature of β-oxidation generates a spectrum of monomer chain lengths, yielding undefined heteropolymers. Alternative strategies disrupt normal β-oxidation while taking advantage of an acyl-ACP thioesterase to generate more uniform acyl chain lengths (mostly C12) [54-56].

Concluding remarks
Fatty acid metabolism offers the means to produce a wide range of high value chemicals and energy dense transportation fuels from renewable feedstocks. With the recent discovery of the genes and enzymes involved, attention can turn to engineering strains to produce target compounds in high yield. Balancing the supply of substrates and cofactors has proven to be important in improving yields, but identifying and circumventing the remaining barriers (regulation of fatty acid biosynthesis, product toxicity, and maximizing carbon flux through the engineered pathway) will be critical to achieve industrially viable yields. Furthermore, understanding the mechanisms by which these enzymes operate will lead to opportunities to engineer or evolve variants that produce products of non-native chain length at higher rates. Lastly, identifying and engineering hosts that can simultaneously produce fatty acid derived products and provide advantages when using lignocellulosic feedstocks (lignotoxin tolerance, consolidated bioprocessing, thermal tolerance, solvent resistance, acid tolerance, generally regarded-as-safe) will be essential for biotechnology to play a leading role in a biomass-based chemical economy.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

11. This manuscript describes the discovery and initial engineering of enzymes capable of conferring FAME production to E. coli.

This manuscript demonstrates successful production of FFA, FAEE, and fatty alcohols in engineered E. coli using laboratory media and a renewable feedstock (xylan).


This article demonstrates how an acyl-CoA sensor can be used to balance FAEE synthesis activity with the supply of ethanol and acyl-CoA. A synthetic biology strategy was used to convert FadR-responsive promoters into a sensor for optimizing FAEE synthesis in E. coli. The resulting strain generated the highest reported titers of FAEE to date.


This manuscript details a bioinformatic study that led to the identification of enzymes that catalyze the production of alkanes in cyanobacteria.


This article describes the characterization of an acyl-CoA reductase that catalyzes both the reductive cleavage of the acyl-CoA thioester and the reduction of the fatty aldehyde to yield a fatty alcohol. This enzyme was discovered in a marine, oil-degrading bacterium and offers the ability to channel flux from acyl-CoA to fatty alcohol without releasing large amounts of fatty aldehyde.


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This article describes metabolic engineering of E. coli for production of methyl ketones. The strategy involves genetic manipulations of beta-oxidation and overexpression of FadM, which is hypothesized to be a beta-ketoacyl-CoA thioesterase.


