Engineered fatty acid catabolism for fuel and chemical production

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Abstract

Fatty acid oxidation pathways are attractive for metabolic engineering purposes due to their cyclic nature as well as their reactions that allow for the selective functionalization of alkyl chains. These characteristics allow for the production of various chemicals, such as alcohols, alkanes, ketones and hydroxyacids, in a wide range of carbon numbers. To this end, the α -, β -, and ω -oxidation pathways have been engineered for use in various hosts. Furthermore, the β -oxidation pathway has been engineered to operate in reverse, resulting in a promising carbon chain elongation platform. This review will describe the recent progress in metabolic engineering strategies for the production of chemicals through these fatty acid oxidation pathways.

Introduction

Bio-based renewable chemical production is becoming an increasingly important process for the production of industrial bulk and specialty chemicals due to concerns over finite nature of petroleum and other fossil-based resources, as well as the potential net greenhouse gas emissions resulting from the use of these feedstocks, which are thought to have an adverse effect on global climate [1]. Potential advantages related to process safety and green chemistry as well as opportunities to develop value added chemicals not readily obtainable via traditional petrochemical processes are also propelling these trends [2–4].

Feedstocks for current biocatalytic processes are either C5-C6 sugars, obtained from hydrolytic degradation of starch, cellulose, and hemicellulose, or oils and fats extracted from vegetables and animals. Extensive studies have focused on the development of biochemical

pathways to convert sugars into fuels and chemicals. Some products such as hydrogen, ethanol, lactic acid, and succinic acid can be produced directly or be readily synthesized from common compounds within central metabolism, and recent reviews cover these achievements [5,6]. More elaborate pathways such as the fatty acid biosynthesis (FAB) [7], isoprenoid [8], polyketide [9], and shikimate [10] pathways have also been employed to produce various value-added chemicals ranging from antibiotics to jet fuels.

Fatty acids, obtained from the splitting of triglycerides found in biological fats and oils, are also an important renewable feedstock for the chemical industry [11]. Biological platforms can utilize fatty acid oxidation pathways to obtain various chemicals. These pathways contain enzymes that allow for the selective functionalization of fatty acids, such as generation of C-C double bonds, additional reactions on the C-C double bond, and functionalization of C-H bond in the alkyl chain [12]. Three fatty acid oxidation routes have been elucidated, α -, β -, and ω -oxidation, and each pathway has potential to produce different products [13]. This review highlights the production of fuels and chemicals by engineering these metabolic pathways (Table 1).

Engineered β -oxidation pathway for the use of fatty acids as feedstock

The β -oxidation pathway (Figure 1) is the major route for fatty acid catabolism and is near ubiquitous in living organisms. The pathway is composed of the following iterative steps [13]: (i) activation of fatty acid to fatty acyl-CoA by acyl-CoA ligase/synthetase; (ii) conversion of fatty acyl-CoA to 2-enoyl-CoA by acyl-CoA oxidase or acyl-CoA dehydrogenase; (iii) hydration of enoyl-CoA to 3-hydroxyacyl-CoA by enoyl-CoA hydratase; (iv) reduction of 3-hydroxyacyl-CoA to 3-oxoacyl-CoA;

(v) thiolysis of 3-oxoacyl-CoA to acetyl-CoA and fatty acyl-CoA shortened by two carbons. The β -oxidation pathway can shorten the carbon chain, modify the β -carbon, and supply acetyl-CoA, which is a common building block for cell growth and the synthesis of many products. Thus, engineering β -oxidation provides the possibility for conversion of fatty acid feedstock to numerous products.

β -oxidation has been engineered to shorten long chain fatty acid feedstocks into more useful medium chain carboxylic acids [14], which have a variety of applications in industry in their native form or as derivatives, such as alcohols, methyl-esters or amides [15]. A strain of *Saccharomyces cerevisiae* was engineered for the replacement of its endogenous acyl-CoA oxidase POX1 with *Yarrowia lipolytica* long chain preferring acyl-CoA oxidase POX2. With additional overexpression of carnitine O-octanoyltransferase, which exports medium chain acyl-CoA from the peroxisome to prevent accumulation, titers of C₈-C₁₂ fatty acids increased by 3.3-fold compared to the wild type [16].

Engineered β -oxidation was also utilized in conversion of fatty acids to aliphatic methyl ketones which can have various applications in industry such as flavoring in dairy products, diesel fuel blending agents and pharmacological syntheses [17,18].

The microbial production of C₁₁-C₁₅ methyl ketones from fatty acid, which is intracellularly synthesized from carbon source glucose, has been achieved in *Escherichia coli* by the deletion of thiolase FadA, expression of heterologous acyl-CoA oxidase Mlut_11700, overexpression of native enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase FadB, and overexpression of thioesterase FadM [18]. Further optimizations to fatty acid supply from glucose and to expression of β -oxidation enzymatic components, along with the removal of acetate synthesis,

improved the titer of methyl ketones to 3.4 g/L using the engineered *E. coli* [19].

3-hydroxyacids are important building blocks for the synthesis of valuable compounds such as antibiotics [20,21] and polyhydroxyalkanoates (PHA), a class of biodegradable polymers [20] and they are another example of products generated through the engineered β -oxidation of fatty acids. The common strategy for the production of PHAs involves the deletions of the host thiolase(s) and 3-hydroxyacyl-CoA dehydrogenase(s). This accumulates 3-hydroxyacyl-CoAs, which are polymerized into PHAs by PHA synthase. Additionally, when PHA synthase was deleted, and a thioesterase was overexpressed for hydrolysis of 3-hydroxyacyl-CoA, non-polymerized medium-chain-length 3-hydroxyalkanoic acids, which are potentially valuable in pharmaceutical industry, were produced [22,23].

In *E. coli*, constitutive expression of β -oxidation by mutations in regulators FadR and AtoC along with expression of engineered native and heterologous fermentative pathways functional under respiratory conditions, enabled microbial productions of fermentative products like biofuels butanol and ethanol and chemicals acetate, acetone, succinate, isopropanol and propionate using fatty acid as the feedstock [24]. Biofuel and biochemical synthesis from fatty acid-rich feedstocks through this engineered β -oxidation has the advantage of high carbon efficiency because fatty acids can be converted to acetyl-CoA at 100% carbon yield.

Engineered β -oxidation reversal for the use of non-fatty acid feedstocks

Dellomonaco *et al.* [25] reported that it is possible to operate the β -oxidation cycle in the reverse direction (r-BOX), initiated by thiolase-catalyzed non-decarboxylative Claisen

condensation between two acetyl-CoAs. One complete cycle of this pathway consists of four core enzymes (Figure 2): (i) a thiolase (THL) that catalyzes the condensation of acetyl-CoA, which serves as the extender unit for carbon chain elongation, with an acyl-CoA, which serves as the primer, yielding a 3-ketoacyl-CoA; (ii) a hydroxyacyl-CoA dehydrogenase (HACD) that reduces the 3-ketoacyl-CoA to 3-hydroxyacyl-CoA; (iii) an enoyl-CoA hydratase (ECD) that converts the 3-hydroxyacyl-CoA to trans-2-enoyl-CoA; (iv) a trans-enoyl-CoA reductase (TER) that generates acyl-CoA two carbon longer than the initial acyl-CoA from enoyl-CoA. Furthermore, because the thiolase reaction is considered a potential pathway bottleneck due to unfavorable thermodynamics ($\Delta_r G_m^\theta = 5.3$ kcal/mol) [25,26], the levels of intracellular acetyl-CoA should be high (and/or the levels of 3-ketoacyl-CoA low) to drive the reaction in the condensation direction. This was accomplished in *E. coli* by deleting the enzymes that allow for the diversion of acetyl-CoA to byproducts such as lactate, pyruvate, acetate and ethanol [25]. The following sections describe products whose synthesis has been achieved through the r-BOX platform.

Aliphatic compounds

Aliphatic carboxylic acids are used as food/feed supplements, flavorings and pharmaceuticals and their derivatives are broadly used in fragrances and drug synthesis [27] and more recently as biofuels such as n-alcohol and alkanes [7].

The r-BOX platform has been used to produce butyric acid, a product obtainable via thioester bond cleavage of butanoyl-CoA by thioesterases (TEs) [28] and multiple deletions in fermentative genes to reach 9.7 g/L of butyric acid [29]. At the same time, efforts have been made to diversify the chain length of produced CAs. For example, deletion of endogenous thioesterases such as *yciA* or *tesB* caused a significant decrease in butyric acid generation,

while leading to an increase in the ratio of medium chain CAs produced by *E. coli* [29,30]. Furthermore, an r-BOX design consisting of BktB (THL), PhaB (HACD), PhaJ4b (ECH), Td/EgTer (TER) or AtoB/FadA (THL), FadB, EgTer led to hexanoic acid as the major product [31,32].

Aliphatic alcohols are used as solvents (C1~C6), plasticizers (C6~C11) and detergents (C12~C18) [33]. n-Butanol is naturally produced, along with acetone and ethanol, by *C. acetobutylicum* as part of its native r-BOX-like pathway consisting of *thl* (THL), *hbd* (HACD), *crt* (ECH) and *bcd* (butyryl-CoA dehydrogenase) [34]. Various attempts have been made to transfer this r-BOX platform to other hosts such as *E. coli* [35], *Saccharomyces cerevisiae* [36], and *Lactobacillus brevis* [37] to improve industrial productivity.

Strategies similar to those used for carboxylic acid production have been used to engineer r-BOX for alcohol production, with the main difference being the cycle termination enzymes. cACRs from *Clostridium* (*adhE1*, *adhE2*, and *ald*) have been extensively studied to produce butanol in various strains hosting the r-BOX platform [36–41]. MhpF from *E. coli*, EutE from *Salmonella typhimurium*, AcrM from *Acinetobacter* sp, and Maqu2507 from *Marinobacter aquaeolei* were proven to have the ability to produce medium chain primary alcohols via the r-BOX platform in *E. coli* [30].

The r-BOX pathway can be used for the production of alkanes in the gasoline range (C5 ~ C12) since this pathway can efficiently generate acyl-CoAs from short to medium (C4~C10) length. Currently, the most common approach uses aldehyde deformylating oxygenase (ADO) for the microbial synthesis of alkanes[42–47]. ADO Pmt1231 from *P. marinus* has been widely employed owing to its broad range of n-aldehyde specificity [48]. Menon *et al.* reported propane production (3.5mg/L) via butylaldehyde, which was generated from butyryl-CoA by acyl-CoA reductase, AdhE2 [49], whereas Sheppard *et al.* used both a

thioesterase and a carboxylic acid reductase to generate hexanal from hexanoyl-CoA, which subsequently converted to pentane (1.5mg/L) [31].

Functionalized compounds

Besides straight-chain aliphatic compounds, r-BOX is also utilized for productions of functionalized compounds, which contain functional groups not formed by cycle termination reactions in the carbon chain. The functionalization is achieved through cycle termination at different r-BOX intermediates with different β -reduction degrees (see below) or usage of functionalized primers or extender units [50].

The first intermediate generated in the r-BOX cycle is a 3-ketoacyl-CoA, a product of the condensation reaction between acetyl-CoA and an acyl-CoA. Cleavage of the thioester bond of 3-ketoacyl-CoA followed by decarboxylation can lead to synthesis of methyl ketones. Lan *et al.* reported 0.7 g/L of acetone and 0.24 g/L of 2-pentanone via an r-BOX platform consisting of BktB, Hbd, Crt and Ter, where carboxylic acid generation from ketoacyl-CoA intermediates in r-BOX was achieved by using 3-oxoadipate CoA-succinyl transferase (PcaIJ from *Pseudomonas putida*) and acetate CoA-transferase (AtoDA from *E. coli*) [51].

The second intermediate in r-BOX is 3-hydroxyacyl-CoA, which can be converted into 3-hydroxy carboxylic acids (HCA) by TEs. Clomburg *et al* reported 2.5 g/L of (S)-3-hydroxybutyric acid production via r-BOX pathway in *E. coli* using endogenous thioesterases as a cycle terminator [32]. When the target is PHA, a different set of r-BOX enzymes is required because the key r-BOX enzymes exclusively generate (S) stereoisomers, whereas PHA synthase has activity only on the (R) stereoisomer of a 3-hydroxyalkanoate. Therefore, 3-ketoacyl-CoAs need to be stereoselectively reduced to (R)-3-hydroxyacyl-CoA. Variants of

acetoacetyl-CoA reductase, either from *R. eutropha* or *Halomonas boliviensis*, have been adapted for this purpose [52,53], and several studies reported production of (R)-3HB using an endogenous thioesterase II of *E. coli* (*tesB*) to cleave the thioester bond of (R)-3-hydroxybutyryl-CoA [54–56]. Guevara-Martinez *et al.* reported production of 6.8 g/L (R)-3HB through phosphate limited fed-batch fermentation [54], while Zuang *et al.* successfully generated 11.2 wt% polyhydroxybutyrate using short chain PHA synthase from *R. eutropha* [57].

Cycle termination at the 3-hydroxyacyl-CoA node also can lead to the production of 1,3-butanediol (1,3-BDO), commonly used as an organic solvent for food flavoring and as a copolymer for polyurethane and polyester resins [58]. Gulevich *et al.* reported the production of 9 mg/L of 1,3-BDO using aldehyde dehydrogenase (*mhpF*) from *E. coli* to reduce 3-hydroxybutyryl-CoA into 3-hydroxybutyaldehyde, followed by the use of alcohol dehydrogenase (*fucO* or *yqhD*) to yield 1,3-BDO [59].

Recent reports showed that the r-BOX pathway can also utilize acyl-CoAs with ω or ω -1 functionalization as primers, leading to the production of ω or ω -1 functionalized products [60]. McMahon *et al.* reported production of 4-methylvalerate through an r-BOX pathway consisting of BktB, PhaB, PhaJ4 (ECH) and TdTer along with the transferase Pct from *Megaphaera elsdenii* for the activation of supplemented precursor isobutyrate to isobutyryl-CoA [61]. Another variant of the r-BOX pathway composed of PaaJ (THL), PaaH1 (HACD) or Hbd, Crt or Ech (ECH), and EgTer can use ω -carboxylated succinyl-CoA as the primer. This, along with Ptb (phosphate butyryltransferase) and Buk1 (butyryl kinase) for acid-forming termination, realized the full biosynthesis of adipic acid (639 μ g/L) in *E. coli* [62]. A more recent study reported a modified version of r-BOX capable of using different ω - and ω -1-

functionalized primers and α -functionalized extender units in combination with various termination pathways [63]. The authors used 7 different primers and 3 different extender units to demonstrate the synthesis of 18 products in the following 10 classes: phenylalkanoic, dicarboxylic, ω -hydroxy, 4-oxo, 4-methyl, 2-methyl, 2-methyl-2-enolic and 2,3-dihydroxy acids, β -hydroxy- ω -lactones and 4-methyl alcohols [63]. Of special interest is the synthesis of α -functionalized products by using propionyl-CoA as extender units in place of acetyl-CoA (Table 1).

ω -oxidation

ω -oxidation (Figure 3a) can act upon both alkanes and long-chain fatty acids and generate both dicarboxylic acids and ω -hydroxy fatty acids, which are versatile raw materials for the oleochemical industry [64,65]. ω -oxidation consists of three key steps carried out by cytochrome P450 alkane/fatty acid monooxygenase, alcohol oxidase and aldehyde dehydrogenase, which successively oxidize the methyl ends of alkanes or fatty acids to ω -hydroxyacids and ω -fatty acids. Production of ω -hydroxyfatty acids has been achieved from a range of fatty acid feedstocks by using *Candida tropicalis* engineered to have blocked β -oxidation and the additional deletion of endogenous diacid production genes consisting of six cytochrome P450s, four fatty alcohol oxidases and six alcohol dehydrogenases [66]. When selected P450 monooxygenases were restored, a total of 174g/L of 14-hydroxytetradecanoic acid was obtained from 200g/L of methyl tetradecanoate. Recently, an *Escherichia coli* strain was engineered to express heterologous ω -oxidation enzymes, blocked β -oxidation, and improve fatty acid uptake by deleting regulator FadR and expressing transporter

AlkL. This led to production of C₁₂ and C₁₄ dicarboxylic acids from corresponding fatty acids as well as mixed C₁₀-C₁₆ diacids from coconut oil hydrolysate, a mixture of C₈-C₁₈ fatty acids [67].

Clomburg *et al.* engineered an *E. coli* strain to have an r-BOX cycle comprised of BktB, FadB and EgTer along with thioesterase YdiI for cycle termination. This, along with alkane monooxygenase AlkBGT for ω -oxidation, yielded more than 0.8 g/L of C₆-C₁₀ ω -hydroxy acids from glycerol [68]. When overexpression of alcohol dehydrogenase ChnD and aldehyde dehydrogenase ChnE was added for further ω -oxidation, production of nearly 0.5 g/L of C₆-C₁₀ diacids was observed. In another study, Bowen *et al.* reported the production of C₁₂ and C₁₄ dicarboxylic acids and ω -hydroxy acids with controlled lengths from glucose in *E. coli* [69]. This was achieved through overexpression of thioesterases UcFatB2 and TesA, which preferentially release C₁₂ and C₁₄ fatty acids from the endogenous FAB pathway, respectively, along with cytochrome P450(CYP153A) from *Marinobacter aquaeolei* for ω -oxidation.

α -Oxidation

Analogous to β -oxidation and ω -oxidation, fatty acids can be degraded by oxidation at the α carbon. α -Oxidation pathways have been elucidated in plants [70] and mammals [71,72] and have also been hypothesized to exist in some prokaryotic organisms [73]. α -Oxidation (Figure 3b) is characterized by the shortening of fatty acids by one carbon, resulting in a fatty aldehydes. In plants, fatty acids are converted to 2-hydroperoxy fatty acids by an α -dioxygenase, which can either spontaneously decarboxylate to form the one carbon shortened fatty aldehyde or be converted into a 2-hydroxy fatty acid [70]. In mammals, a fatty acid is

first hydroxylated at the α -carbon by a fatty acid α -hydroxylase to give 2-hydroxy fatty acid. The acid is then converted to the corresponding 2-hydroxy fatty acyl-CoA and subsequently cleaved into the one carbon shortened fatty aldehyde and formyl-CoA by a thiamine pyrophosphate dependent enzyme called 2-hydroxyacyl-CoA lyase [72]. The resulting fatty aldehydes can be converted back to fatty acids for further rounds of degradation.

The reactions found in α -oxidation have been used in conjunction with carbon chain elongation pathways such as FAB and r-BOX to achieve the synthesis of other products. Thus far, major targets from the utilization of α -oxidation have been odd chain length products, primarily by expression of α -dioxygenase from *Oryza sativa* (rice). Kaehne *et al.* engineered an *E. coli* whole cell biocatalyst expressing α -dioxygenase, and demonstrated its ability to convert fatty acids (C10 – C18) into corresponding one carbon shortened fatty aldehydes [74]. Cao *et al.* went further by engineering *E. coli* to use α -dioxygenase in combination with FAB and an aldehyde reductase to produce about 100 mg/L odd chain fatty alcohols from glycerol [75]. In a related effort, Jin *et al.* engineered *Saccharomyces cerevisiae* to utilize the same odd chain fatty alcohol production pathway to produce approximately 20 mg/L fatty alcohols in flask cultures [76].

In addition to odd chain products, one can envision the application of α -oxidation to the production of α -functionalized products [77].

Concluding remarks

As summarized in Table 1, fatty acid oxidation pathways have enabled the synthesis of various chemicals, which can be used for the manufacture of products such as fuels, paints, food additives, resins, foams, lubricants, plasticizers, and cosmetics. Bio-based chemicals, however, suffer from economic competition from much cheaper counterparts derived from conventional fossil-based routes. Although studies of bioconversion processes to add

functionality to oils and fats have shown promising results in terms of both titer and yield, production on an industrial scale is limited due to use of feedstocks that are often competing with food production. On the other hand, commercialization of bio-chemical production using feedstocks derived from cellulosic materials has faced challenges due to low productivity. Nevertheless, economical industrialization of bio-based chemical production that have no petrochemical counterparts, such as alkylpolyglucoside and PLA polymers, highlights the importance of bio-based approaches to developing newly functionalized chemicals. While the fatty acid oxidation platforms covered in the present review are expected to play a crucial role in this effort, one of the major challenges is the selective production of functionalized chemicals with a specific carbon chain length. Fatty acid oxidation pathways (and their reversal) use an iterative mechanism to shorten (or elongate) alkyl chains, which in turn can lead to the production of molecules with the same functional groups but various carbon chain lengths (Table 1). The synthesis of a single product with a specific chain length (as opposed to a mixture of products with various chain lengths) will require the engineering of enzymes with high chain-length specificity, including thiolases, thioesterases, and acyl-CoA or acyl-ACP reductases. In addition, the efficient operation of platforms like the β -oxidation reversal will also require the engineering of pathways involved in central metabolism to achieve a balanced supply of primers, extender units, and reducing equivalents from a single carbon source.

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313 **Figure captions.**

314 **Figure 1.** Biochemical production from fatty acids via the β -oxidation pathway. Solid and
315 dashed line arrows indicate single and multiple reactions, respectively. Boxed products
316 represent those whose synthesis has been demonstrated through the use of a β -oxidation
317 pathway.

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319 **Figure 2.** Biochemical production via a reversed β -oxidation (r-BOX) pathway, an
320 engineered iterative pathway using acetyl-CoA as extender unit and acyl-CoA as primer.
321 Solid and dashed line arrows indicate single and multiple reactions, respectively. Boxed
322 products are those whose synthesis has been demonstrated from r-BOX pathway. The core of

the r-BOX pathway consists of four enzymes: (i) thiolase (THL); (ii) 3-hydroxyacyl-CoA dehydrogenase (HACD); (iii) enoyl-CoA hydratase (ECH); and (iv) trans-enoyl-CoA reductase (TER). The four acyl-CoA intermediates constitute the complete cycle of r-BOX and cycle termination with thioesterases results in carboxylic acid production, while termination with reductases yields aldehydes, which can be further converted into alkanes, ketones and alcohols.

Figure 3. Biochemical production via (a) ω -oxidation pathway, a minor fatty acid metabolism route found in yeast and plants capable of utilizing both alkanes and long-chain fatty acids as sole carbon source, and (b) α -oxidation pathway, an important route for the degradation of 3-methylbranched fatty acids that shortens fatty acids by one carbon. Solid and dashed line arrows indicate single and multiple reactions, respectively. Boxed products are those whose synthesis has been demonstrated from ω - and α -oxidation pathways.

Table

Summary of biochemical production through fatty acid oxidation pathways						
Product	Route	Host	Titer	Substrate	Cultivation	Ref.
Carboxylic acid						
Butyric acid	r-BOX	<i>Eco</i>	9.7 g/L	Glucose/CM	Batch	[29]
Hexanoic, Octanoic acid	r-BOX	<i>Eco</i>	2.2 g/L	Glucose/CM	Batch	[29]
Octanoic ~ Dodecanoic acid (even)	β	<i>Sce</i>	0.5 mg/L	Oleic acid/CM	Batch	[16]
4-methylpentanoic acid	r-BOX	<i>Eco</i>	0.6 mg/L	Glucose+isobutyric acid/CM	Batch	[61]
Dicarboxylic acid						
Adipic acid	r-BOX	<i>Eco</i>	2.5 g/L	Glycerol/CM	Batch	[63]
Dodecanedioic acid	ω	<i>Eco</i>	0.57 g/L	Glucose/MM	Fed-batch	[69]
Adipic ~ Sebacic acid (even)	r-BOX + ω	<i>Eco</i>	0.49 g/L	Glycerol/MM	Batch	[68]
Alcohol						

1,3-butanediol	r-BOX	<i>Eco</i>	27 mg/L	Glucose/MM	Batch	[59]
Butanol	r-BOX	<i>Cac</i>	14.9 g/L	Glucose/CM	Batch	[34]
Ethanol ~ Octanol (even)	r-BOX	<i>Eco</i>	2.3 g/L	Glycerol+glucose/ CM	Batch	[35]
Undecanol ~ Pentadecanol (odd)	α	<i>Eco</i>	1.95 g/L	Glycerol/CM	Fed- batch	[75]
4-methylpentanol	r-BOX	<i>Eco</i>	90 mg/L	Glucose/CM	Batch	[60]
2-Ketone						
Acetone	r-BOX	<i>Eco</i>	0.7 g/L	Glycerol+glucose/ CM	Batch	[51]
Pentanone	r-BOX	<i>Eco</i>	0.24 g/L	Glycerol+glucose/ CM	Batch	[51]
Undecanone ~ Pentadecanone (odd)	β	<i>Eco</i>	3.4 g/L	Glucose/MM	Fed- batch	[19]
Hydroxyacid						
(R)-3-hydroxybutyric acid	r-BOX	<i>Eco</i>	6.8 g/L	Glucose/MM	Fed- batch	[54]
(R)-3-hydroxyhexanoic ~ tetradecanoic acid	r-BOX	<i>Eco</i>	0.44 g/L	Glucose/CM	Batch	[57]
(R)-3-hydroxydecanoic acid	β	<i>Pen</i>	1.75 g/L	Decanoic acid/CM	Batch	[22]
(R)-3-dodecanoic acid	β	<i>Ppu</i>	7 g/L	Dodecanoic acid/CM	Fed- Batch	[23]
(R)-3-hydroxytetradecanoic acid	β	<i>Pen</i>	6.65 g/L	Tetradecanoic acid/CM	Batch	[22]
ω -hydroxyhexanoic ~ decanoic acid	r-BOX + ω	<i>Eco</i>	0.82 g/L	Glycerol/MM	Batch	[68]
ω -hydroxytetradecanoic acid	ω	<i>Cat</i>	174 g/L	Methyl- tetradecanoate + glucose + glycerol/MM	Fed- Batch	[66]
3,4-dihydroxybutyric acid	r-BOX	<i>Eco</i>	0.70 g/L	Glucose/MM	Batch	[56]
n-Alkane						
Propane	r-BOX	<i>Eco</i>	3.4 mg/L	Glucose/CM	Batch	[49]
Pentane	r-BOX	<i>Eco</i>	1.6 mg/L	Glucose/CM	Batch	[31]
Dodecane, Tetradecane	α	<i>Eco</i>	8 mg/L	Glycerol/CM	Batch	[75]
α-functionalized carboxylic acids						
(E)-2-methyl-2-petenoate	r-BOX	<i>Eco</i>	0.1 g/L	Glycerol/CM	Batch	[63]
Tiglic acid	r-BOX	<i>Eco</i>	2.5 g/L	Glycerol/CM	Batch	[63]
n-Aldehyde						
Pentadecanal	α	<i>Eco</i>	1.1 g/L	Hexadecanoic acid/MM	Cell- free	[74]
Fermentative products						
Ethanol	β	<i>Eco</i>	~20 g/L	hexadecanoic acid/MM	Fed- batch	[24]

Butanol	β	<i>Eco</i>	2.05 g/L	hexadecanoic acid/MM	Fed-batch	[24]
Acetic acid	β	<i>Eco</i>	35 g/L	hexadecanoic acid/MM	Fed-batch	[24]

The abbreviation of species is as follows: *Eco*, *Escherichia coli*; *Sce*, *Saccharomyces cerevisiae*; *Cae*, *Clostridium acetobutylicum*; *Pen*, *Pseudomonas entomophila*; *Ppu*, *Pseudomonas putida*; *Cat*, *Candida tropicalis*.

CM, complex medium; MM, minimal medium.

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Figure 1.

Figure 3.

Graphical Abstract

- Raw materials from renewables

- Selective functionalization of alkyl chains
 - Generation of C-C double bond
 - Additional reaction on C-C double bond
 - Functionalization of C-H bond

- Functional products
- Platform chemicals
- Intermediates