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Advanced metabolic Engineering strategies for the sustainable production of free fatty acids and their derivatives using yeast

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Abstract
The biological production of lipids presents a sustainable method for generating fuels and chemicals. Recognized as safe and enhanced by advanced synthetic biology and metabolic engineering tools, yeasts are becoming versatile hosts for industrial applications. However, lipids accumulate predominantly as triacylglycerides in yeasts, which are suboptimal for industrial uses. Thus, there have been efforts to directly produce free fatty acids and their derivatives in yeast, such as fatty alcohols, fatty aldehydes, and fatty acid ethyl esters. This review offers a comprehensive overview of yeast metabolic engineering strategies to produce free fatty acids and their derivatives. This study also explores current challenges and future perspectives for sustainable industrial lipid production, particularly focusing on engineering strategies that enable yeast to utilize alternative carbon sources such as CO_2, methanol, and acetate, moving beyond traditional sugars. This review will guide further advancements in employing yeasts for environmentally friendly and economically viable lipid production technologies.

Keywords Free fatty acid, Carbon sources, Metabolic engineering, Yeast

Introduction

The relentless expansion of petroleum-based industries has exacerbated greenhouse gas emissions, contributing significantly to environmental issues such as climate change. This ongoing environmental degradation highlights the urgent necessity for a shift toward more sustainable industrial practices to mitigate their adverse effects on our lives. Advancements in metabolic engineering and synthetic biology have led to the rise of microorganism-based industries as practical alternatives. These technologies have led to the inception of the production of diverse industrial products by converting greenhouse gases and C1 compounds such as CO_2, CH_4, and green methanol. This approach not only leverages bioconversion technologies but also plays a crucial role in mitigating environmental greenhouse gas emissions, addressing key challenges in sustainability [1, 2].

Free fatty acids (FFAs) are critically important across various industries, serving as the preferred precursors for synthesizing a range of fatty acid derivatives. A notable characteristic of FFA is their straightforward conversion into a diverse spectrum of biofuels, which further underscores the role of FFA in advancing sustainable energy solutions. Biodiesel, the most well-known and commercially produced biomass-derived diesel fuel, consists of mono-alkyl esters of long-chain fatty acids. Traditionally, biodiesel is synthesized from plant oils via chemical

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transesterification—a process that is becoming problematic for large-scale commercial viability due to the cost and availability of feedstocks. Moreover, a surplus of alcohol is frequently necessary to drive the reaction towards near completion, further increasing production costs. In response, recent efforts have focused on directly producing fatty acid ethyl esters (FAEEs) in vivo to create more sustainable forms of biodiesel [3–5].

Yeast engineering has extended efforts to produce other FFA-based products, such as fatty alcohols (FAs) and fatty alkanes (FALKs) directly in vivo. FAs, long-chain hydrocarbons with over ten carbon atoms and a terminal –OH group, are utilized in various applications, including lubricants, surfactants, cosmetics, pharmaceuticals, agricultural chemicals, plastic polymerization agents, textile coatings, personal care commodities, mineral processing substances, and fuels [6]. Another vital class of hydrocarbons is FALKs, which are utilized as primary liquid fuels for transportation and in the manufacturing of plastics, being key components of petrol, diesel, and jet fuel. Terminal alkenes, also known as olefins, possess a high energy density and exhibit comparable storage, transportation, and combustion properties to current liquid transportation fuels, rendering them advantageous for synthesizing polyethylene, lubricants, and detergents [7].

Yeast-based platforms have attracted significant attention due to progress in metabolic engineering and synthetic biology, along with the designation of several yeast species under the Generally Recognized as Safe (GRAS) status. Among various yeast species, *Saccharomyces cerevisiae* and *Yarrowia lipolytica* have been widely studied for producing fatty acid-derived hydrocarbons. *S. cerevisiae* is valued for its robustness, capable of thriving in low pH and challenging environmental conditions, and is well-equipped with genetic tools that facilitate metabolic engineering [8, 9]. Numerous studies with *S. cerevisiae* engineering have been conducted to produce biofuels such as bio-ethanol. *Y. lipolytica*, known for oleaginous yeast, has attracted significant interest due to its ability to accumulate high lipid content [10]. Recently, *Rhodosporidium toruloides*, another oleaginous yeast, has shown promising results in the production of FFA and FAEE [11, 12]. Additionally, the ability of methylotrophic yeasts to metabolize methanol has opened new avenues for research, with multiple studies exploring the potential of engineered yeasts to transform methanol and CO_2 into lipids [13, 14]. *Pichia pastoris* and *Ogataea polymorpha*, methylotrophic yeasts, have demonstrated their efficacy in producing fatty acid-derived hydrocarbons from sustainable one-carbon (C1) feedstock methanol [15–17]. The use of yeast platforms for synthesizing fatty acids and their derivatives thus holds significant promise for advancing sustainable industrial processes.

This review comprehensively examines the recent advancements in metabolic engineering strategies designed to enhance the biosynthesis of fatty acids and their derivatives (FA, FAEE, and FALK) in yeast. Additionally, this review aims to introduce strategies for metabolizing CO_2 and methanol in yeast and to discuss lipid metabolism approaches. It will also present effective strategies for future research on lipid production based on C1 compounds, illustrating the potential advancements in yeast-based biotechnological applications contributing to an environmentally friendly and renewable energy future.

Metabolic engineering strategies for free fatty acid (FFA) production

In microbial systems, lipids are typically stored as triacylglycerides (TAGs), which limits their direct usability. However, compared to TAGs, FFAs are critical precursors for the synthesis of a wide variety of compounds for extensive industrial applications. Therefore, biologically deriving FFAs presents a highly feasible and economically viable method. This chapter summarizes the metabolic engineering strategies for producing FFAs in yeast (Fig. 1; Table 1).

Enhancement of acetyl-CoA and malonyl-CoA pools

Acetyl-CoA and malonyl-CoA are crucial intermediates in the biosynthetic pathway of fatty acids (Fig. 1). A pyruvate dehydrogenase (PDH) complex plays a role in converting pyruvate to acetyl-CoA [18, 19]. The conversion of acetyl-CoA to malonyl-CoA, catalyzed by acetyl-CoA carboxylase *(*ACC), marks the beginning of fatty acid synthesis. Malonyl-CoA acts as the two-carbon donor in the chain-elongation process of fatty acid synthesis, which continues until the desired chain length is achieved. Zhang et al. (2020) demonstrated that introducing the cytosolic pyruvate dehydrogenase (*cPDH*) complex from *Enterococcus faecalis* into *S. cerevisiae* significantly enhanced the cytosolic acetyl-CoA pool, resulting in an enhanced FFA production. In this study, while a parental strain of *S. cerevisiae* (Δ*FAA1/4*, Δ*POX1*, Δ*HFD1*) produced FFA at 458.9 mg/L, the introduction of the PDH protein complex increased the FFA titer to 512.7 mg/L [20]. Additionally, *ACC1* overexpression can enhance malonyl-CoA pools and subsequently increase FFA titers in yeast [21–24]. In an engineered strain of *Y. lipolytica* (Δ*GPD1*, Δ*GUT2*, Δ*PEX10*), the initial FFA production was quantified at a level of 382.8 mg/L. However, the overexpression of *ACC1* significantly increased the titer to 1436.7 mg/L, representing a 3.7-fold enhancement in comparison to the parental strain [23]. Zhou et al. (2016b) replaced the native promoter of the *ACC1* gene with the strong TEF1 promoter in *S. cerevisiae*, resulting in the production of 10.4 g/L of FFAs, while the parental

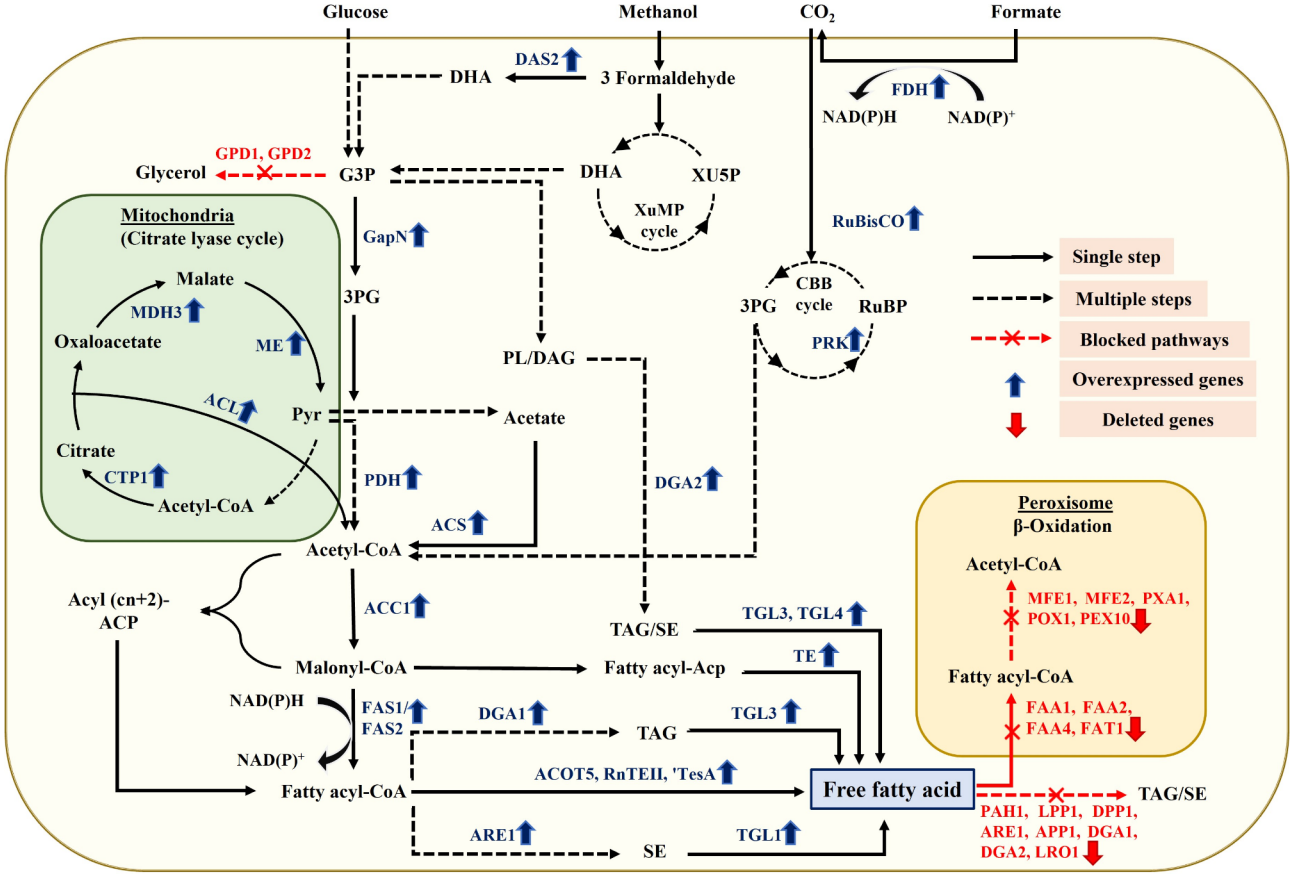

Fig. 1 A schematic overview of metabolic engineering strategies for producing FFAs in yeasts. Glucose, methanol, CO_2, and its derivative formate represent the initial carbon source. Overexpressed genes and knocked-out genes are shown in blue and red, respectively. Abbreviations: ACC1, acetyl-CoA carboxylase; ACL, ATP: citrate lyase; ACS, acetyl-CoA synthetase; ARE, sterol acyltransferases; CBB cycle, Calvin-Benson-Bassham cycle; CTP, citrate transporter; DAG, diacylglycerol; DAS, dihydroxyacetone synthase; DGA, diacylglycerol acyltransferases; DHA, dihydroxyacetone; FAA/FAT, fatty acyl-CoA synthetases; FAS, fatty acid synthetases; FDH, formate dehydrogenase; G3P, glyceraldehyde 3-phosphate; GapN, glyceraldehyde-3-phosphate dehydrogenase; GPD, glycerol-3-phosphates; LRO, diacylglycerol acyltransferase; MDH, malate dehydrogenase; ME, malic enzyme; MFE, multifunctional enzymes; 3PG, 3-phospho-glycerate; PAH/LPP/DPP/APP, phosphatidate phosphatases; PDH, pyruvate dehydrogenase; PEX10, peroxisome synthetase; PL, phospholipid; POX, peroxisomal acyl-CoA oxidase; PRK, phosphoribulokinase; PXA, peroxisomal acyl-CoA transporter; Pyr, pyruvate; SE, sterol esters; RuBisCO, ribulose 1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate; TAG, triacylglycerol; TE/ACOT5/RnTEII/'TesA, thioesterases; TGL, triacylglycerol lipases; Xu5P, xylulose 5-phosphate; XuMP cycle, xylulose monophosphate cycle

strain (Δ*POX1*, Δ*Faa1/4*, Δ*HFD1*, '*TesA*↑, *RtFAS*↑) produced only 7.0 g/L [22].

Directing fatty acyl-CoA to FFAs production with the inhibition of TAG, SE, and phospholipid synthesis

The conversion of malonyl-CoA to fatty acyl-CoA is a critical step in fatty acid synthesis, where each elongation cycle adds two carbons to the growing fatty acyl chain (Fig. 1). The fatty acid synthetase (*FAS1* and *FAS2*) elongates this carbon chain, ultimately forming fatty acyl-CoA, a direct precursor for FFAs. Heterologous type-I FAS from *Brevibacterium ammoniagenes* (*baFAS*) (Eriksen et al., 2015), *Rhodosporidium toruloides* FAS (*RtFAS1* and *RtFAS1*) [22] or endogenous *FAS1*/*FAS2* from *S. cerevisiae* [25] were employed to enhance fatty acyl-CoA production. For instance, the expression of *baFAS* in the *ΔFAS1 S. cerevisiae* strain resulted in a 2.75-fold increase in palmitic acid production compared to the parental strain [26].

Thioesterases play a pivotal role by converting fatty acyl-CoA into FFAs, thereby inhibiting their storage as TAGs or sterol esters (SEs). A truncated version of acyl-CoA thioesterase (*Acot5s*) from *Mus musculus* was expressed in the cytoplasm of *S. cerevisiae*, resulting in improved FFA synthesis, achieving up to 500 µg/mL in batch cultivation [21]. The overexpression of *E. coli* acyl-ACP thioesterase 'TesA in *S. cerevisiae* resulted in the production of 5 mg/L of FFAs, an 8-fold increase in comparison to the background strain [25]. Zhou et al., (2016b) also overexpressed *'tesA in S. cerevisiae*, which resulted in the production of 0.67 g/L FFA [22]. In *Y. lipolytica*, coupling the overexpression of *FAS1* with thioesterase from *E. coli* led to production levels reaching 1.3 g/L in shake flasks and up to 9 g/L in bioreactors [24].

Table 1 Summary of metabolic engineering strategies for free fatty acid production in yeasts

Strain	Metabolic engineering strategies	Medium and carbon sources	Results	Fatty acid composition (%)	References
Yarrowia lipolytica	Overexpression of *DGA2*, *TGL4* and *KlTGL3*; deletion of *FAA1* and *MFE1*	YNB, glucose	2.8 g/L (batch), 10.4 g/L (fed-batch)	C16:0, C16:1, C18:0, C18:1, C18:2	[27]
	Overexpression of *RnTEII*; deletion of *DGA1*, *DGA2*, *LRO1*, *ARE1*, *FAA1 and MFE1*	YNB, glucose	3 g/L (batch)	C16:0, C16:1, C18:0, C18:1, C18:2	[27]
	Overexpression of truncated *hFAS-EcTesA'*	YNB, glucose	1.3 g/L (batch), 9.67 g/L (fed-batch)	C12:0 (7.5%), C14:0 (29.2%), C16:0, C16:1, C18:0, C18:1, C18:2	[24]
	Overexpression of *ACC1*; deletion of *GPD1*, *GUT2* and *PEX10*	YNB, glycerol and glucose	2 g/L (batch)	C16:0 (9.6%), C16:1 (9.8%), C18:0 (6.9%), C18:1 (46.7%), C18:2 (20.4%), C20:0 (0.7%), C22:0 (0.7%), C24:0 (4.1%)	[23]
	Overexpression of *MaC16E*, *CDS1*, *PSD1*, *CHO2*, *OPI3*, and *CpFAH12*; deletion of *MEF1*, *PEX10*, *FAD2*, *PAH1*, *APP1*, *MHY1*, and *DGA1*	YNBR, glucose	2 g/L (batch)	C18:1-OH, (74%), C18:2 (11%), C16:0, C16:1, C18:0, C18:1 (15%)	[28]
Saccharomyces cerevisiae	Overexpression of *ACC1*, *FAS1*, *FAS2* and *'TesA*; deletion of *PXA2*, *POX1*, *FAA1* and *FAA4*	MM, glucose	400 mg/L (batch)	C12:0 (2.7%), C14:0 (9.4%), C16:0 (47.0%), C16:1 (19.3%), C18:0 (10.4%), C18:1 (10.7%)	[25]
	Overexpression of *ACOT5*; deletion of *FAA1* and *FAA4*	YNBD, glucose	500 µg/ml (batch)	C16:0, C16:1, C18:0, C18:1	[21]
	Overexpression of *DGA1* and *TGL3*; deletion of *FAA1*, *FAA2*, *FAA4*, *FAT1*, *PXA1* and *POX1*	YPD, glucose	2.2 g/L (batch)	C16:0, C16:1, C18:0, C18:1	[29]
	Overexpression of *CTP1*, *RtME*, *MDH3*, *MmACL*, *RtFAS*, *ACC1* and *'TesA*; deletion of *HFD1*, *FAA1*, *FAA4* and *POX1*	MM, glucose	1 g/l (batch), 10.4 g/l (fed-batch)	C16:0, C16:1, C18:0, C18:1	[22]
	Overexpression of the *cPDH* and *GapN*; and deletion of *GPD1*, *GPD2*, *PAH1*, *LPP1*, *DPP1* and *ARE1*	MM, glucose	840.5 mg/L (batch)	C16:0, C16:1, C18:0, C18:1	[20]
	Overexpression of *FDH* and CBBm	MM, CO_2, formate, and glucose	10.1 g/L (fed-batch)	C16:0, C16:1, C18:0, C18:1	[30]
	Overexpression of *ACS* and *FDH*	MM, acetate, glucose and formate	6.6 g/l (fed-batch)	C16:0, C16:1, C18:0, C18:1	[31]
Starmerella bombicola	Deletion of *FAA1* and *MFE2*	Lang production media, glucose	0.933 g/L (batch)	C16:0 (13.2%), C18:0 (40%), C18:1 (43.8%)	[32]
Pichia pastoris	Overexpression of *MmACL*, *DAS2*, *XFPK*, *ScIDP2* and *PTA*; deletion of *FAA1* and *FAA2*	MM, methanol	5.1 g/L (batch), 23.4 g/L (fed-batch)	C16:0, C16:1, C18:0, C18:1, C18:2	[15]
Ogataea polymorpha	Overexpression of *FBP1*, *RPE*, *MmACL*, *ZWF1*, *ScIDP2*, *AOX1*, *DAS* and *DAK*; deletion of *FAA1*, *LPL1* and *IZH3*	MM, methanol	15.9 g/L (fed-batch)	C16:0 (30–40%), C16:1 (< 5%), C18:0 (< 5%), C18:1 (20–30%), C18:2 (30–40%)	[16]

An engineered strain of *Y. lipolytica* (Δ*ARE1*, Δ*DGA1*/*2*, Δ*LRO1*, Δ*FAA*, Δ*MFE1*), lacking neutral lipid synthesis pathways (TAG/SE), significantly increased FFA production from 730 mg/L to 3 g/L upon overexpressing a cytosolic thioesterase from *Rattus norvegicus* (*RnTEII*) [27].

In *Y. lipolytica*, the production of ricinoleic acid (RA) via the cytidine diphosphate diacylglycerol (CDP-DAG) pathway was achieved by regulating lipid flux towards the phosphatidylcholine (PC) and oleic acid (OA) pool. This enhancement began with the overexpression of the *CpFAH12* encoding fungal Δ12 oleate hydroxylase from *Claviceps purpurea*, combined with the deletion of the TAG synthesis pathway (Δ*DGA1*). Further amplification of the phospholipid pool was achieved by overexpressing several key genes: *CDS1* (phosphatidate cytidylyltransferase), *PSD1* (phosphatidylserine decarboxylase), *CHO2* (phosphatidylethanolamine N-methyltransferase), and *OPI3* (phosphatidyl-N-methylethanolamine N-methyltransferase). Finally, the overexpression of fatty acid elongase from *Mortierella alpine* (*MaC16E*) led to 2.061 g/L RA acid production [28].

FFAs can also be generated by remodeling TAGs, where triacylglycerol lipases (*TGL*) break down TAGs into FFAs. In *S. cerevisiae*, a genetically modified strain

co-overexpressing *TGL3* and *DGA1* produced up to 2.2 g/L of extracellular FFAs [29]. Similarly, in *Y. lipolytica*, employing a comparable engineering strategy involving the overexpression of *TGL3*, *TGL4*, and *DGA2* achieved a FFAs production level of 2.8 g/L [27].

Additionally, the complete elimination of phospholipid synthesis from FFAs through the deletion of phosphatidate phosphatase genes (*PAH1*, *APP1*, *DPP1*, *LPP1*) further enhanced FFA production [20, 28].

Inhibition of beta-oxidation

Blocking competing metabolic pathways is a general approach to direct and enhance the carbon flux towards desired products. Thus, one of the critical strategies for FFA production involves eliminating the β-oxidation pathway, which naturally degrades fatty acids into acetyl-CoA in the peroxisome, thereby preventing the potential recycling of FFAs into unwanted metabolic products (Fig. 1). In the β-oxidation cycle, the peroxisomal acyl-CoA transporter (*PXA1*), *POX1*, and multifunctional enzymes (encoded by *MFE1*, *MFE*2) have been mainly targeted for deletion. Additionally, inhibiting peroxisome synthesis through the deletion of peroxisome synthetase (*PEX10)*, which is crucial for peroxisome biogenesis, prevents FFAs from being converted into β-oxidation products [23, 28]. Further measures include disrupting fatty acyl-CoA synthetases, namely *FAA1*, *FAA2*, *FAA4*, and *FAT1*. These genes are responsible for converting FFAs back into fatty acyl-CoA, and their inhibition is vital for ensuring that fatty acids are not recycled in the β-oxidation cycle but instead accumulate as FFAs [20–22, 24, 27, 29–32]. Deleting these enzymes makes it possible to prevent the reconversion process of fatty acid, thus promoting the accumulation of FFAs instead of their re-utilization as fatty acyl-CoA. However, the reduction of the β-oxidation was synergetic when it was applied to other FFA enhancement strategies. Thus, this strategy is not usually used solely.

Enhancing cofactor (NADPH) supply

The synthesis of fatty acids in yeast critically relies on an adequate supply of NADPH, which acts as a reducing equivalent. This cofactor is pivotal for the reductive steps that transform acetyl-CoA and malonyl-CoA into longer-chain fatty acids. Specifically, NADPH supplies the necessary electrons for the reduction reactions catalyzed by the fatty acid synthase complex (FAS). This complex condenses acetyl-CoA and malonyl-CoA into acyl-CoA. Each step in this elongation process requires two molecules of NADPH to reduce the carbonyl group of the acyl intermediates, thereby enabling further chain extension. Consequently, a deficiency in NADPH can significantly hinder the production of fatty acids [10, 33, 34]. Thus, various NADPH-dependent enzymes involved in lipid synthesis have been employed for metabolic engineering. Chen et al. (2016) overexpressed $NADP^+$-dependent aldehyde dehydrogenase to enhance the cellular pool of NADPH. Indeed, this augmentation supports fatty acid synthesis by providing a robust supply of reducing equivalents [9]. The introduction of $NADP^+$-dependent glyceraldehyde-3-phosphate dehydrogenase (*GapN*) from *Streptococcus mutans* enabled the irreversible conversion of glyceraldehyde-3-phosphate to 3-phosphoglycerate, thereby producing NADPH. This modification enhanced the production of FFA and FAEE while reducing glycerol synthesis [20, 35]. Another strategic modification in *S. cerevisiae* involved redirecting carbon flux towards glutamate biosynthesis by deleting the NADPH-dependent glutamate dehydrogenase (*GDH1*). This change significantly improves NADPH availability, increasing the FFA pool for FA synthesis [36]. In *Y. lipolytica*, the use of a microbial electrosynthesis (MES) system proved effective in converting electrons directly into NADPH, resulting in a 2.79-fold increase in the NADPH/$NADP^+$ ratio and enhancing the production of FAs from acetate [37].

Metabolic engineering strategies for FFA-derived products

We have introduced various metabolic engineering strategies aimed at augmenting the production of FFAs. Moving forward, this chapter will discuss diverse engineering strategies that have been implemented to synthesize fatty alcohols, fatty alkyl ethyl esters, and fatty alkanes in yeast in vivo, using FFAs or fatty acyl-CoA as the starting substrates (Fig. 2; Table 2).

Fatty alcohol (FA)

The production of FAs typically begins with the precursors, fatty acyl-CoA or fatty acyl-ACP, which are converted by fatty acyl-CoA reductases (FAR) (Fig. 2). Indeed, the heterologous overexpression of *FAR* from *Marinobacter aquaeolei* successfully enhanced FA production in *Y. lipolytica* (5.8 g/L), *L. starkeyi* (770 mg/L), *P. pastoris* (2 g/L), and *R. toruloides* (8 g/L) [11, 15, 38, 39]. Additionally, the heterologous expression of *FAR* from *Mus musculus* effectively converted fatty acyl-CoA into FAs, leading to production levels of up to 6.0 g/L in *S. cerevisiae* with endoplasmic reticulum localization [36]. Similarly, the expression of *TaFAR1* from *Tyto alba* facilitated the production of hexadecanol from glucose in *S. cerevisiae* and *Y. lipolytica*, achieving yields of 655 mg/L and 636 mg/L, respectively [40, 41].

An alternative two-enzyme pathway for FA production involves initially reducing FFAs to the fatty aldehyde using carboxylic acid reductase (CAR), followed by conversion to FA via endogenous aldehyde reductases (ALR) or alcohol dehydrogenases (ADH) [6]. This pathway was successfully operated with *CAR* from *Mycobacterium*

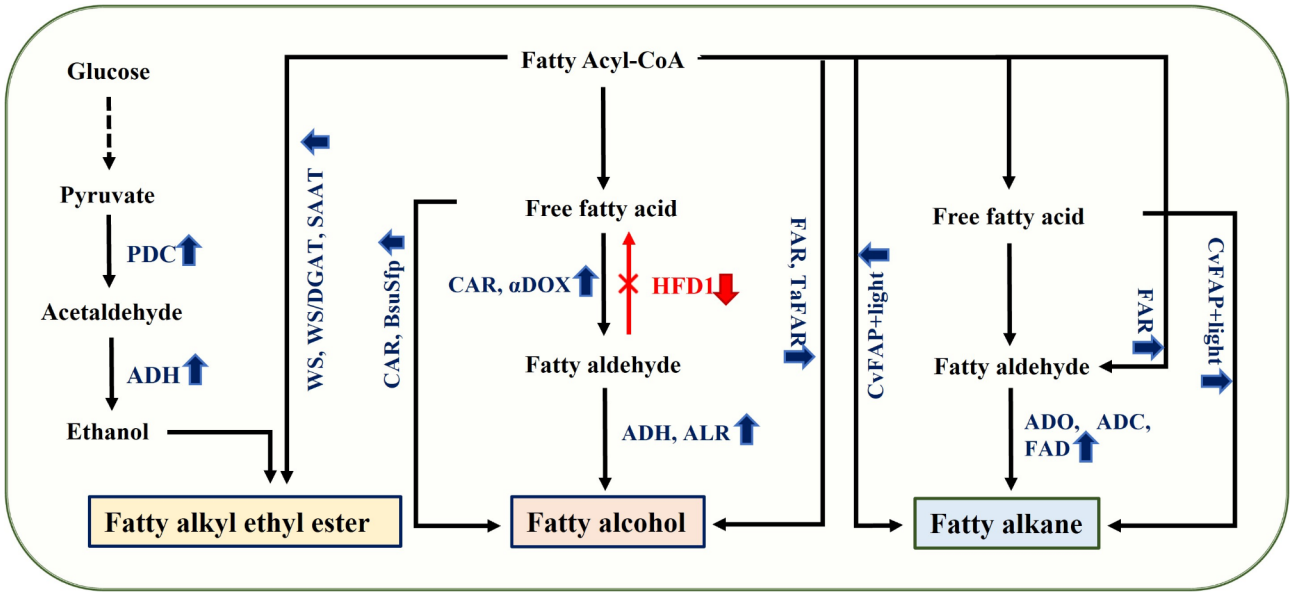

Fig. 2 A schematic overview of metabolic engineering strategies for producing FA, FAEE, and FALK in yeasts. Overexpressed genes and knocked-out genes are shown in blue and red, respectively. Abbreviations: ADC, aldehyde decarbonylase; ADH, alcohol dehydrogenases; ADO, aldehyde deformylating oxygenase; ALR, aldehyde reductases; BsuSfp, phosphopantetheinyl transferase; CAR, carboxylic acid reductase; CvFAP, fatty acid photodecarboxylase; DGAT, acyl-CoA-diacylglycerol acyltransferase; FAD, fatty aldehyde decarbonylase; FAR/TaFAR, fatty acyl-CoA reductases; HFD1, aldehyde dehydrogenase; PDC, pyruvate decarboxylase; SAAT, alcohol acyltransferase; WS, wax ester synthase; αDOX, α-dioxygenase

marinum and endogenous *ALR* or *ADH* in *S. cerevisiae* [22, 42–44]. Furthermore, it was also effective in blocking the reversible reaction of fatty aldehyde to FFA by deleting *HFD1* [15, 17, 22, 36, 42, 44–46].

FA production primarily derives from FFA or fatty acyl-CoA. Therefore, strategies that enhance fatty acid and fatty acyl-CoA pools are critical alongside those directly aimed at producing FAs. Thus, it is required to inhibit pathways that reactivate FFAs to fatty acyl-CoA (Δ*FAA1*, Δ*FAA4*), initiate β-oxidation (Δ*PXA1*, Δ*POX1*, Δ*PEX10*), or utilize fatty acyl-CoA to synthesis sterol ester (Δ*ARE1*, Δ*ARE2*) and triacylglycerol (Δ*DGA1*, Δ*LRO1*). These modifications enhance the production of FFA and subsequently increase FA synthesis [22, 36, 42, 43, 47, 48]. The combined deletion of *HFD1* and *ADH6*, along with the inhibition of fatty acid degradation to fatty acyl or acetyl-CoA (Δ*POX1*, Δ*FAA1/4*), co-expression of *ADH5*, *FAR*, and *CAR*, achieved production levels of up to 1.5 g/L FA under glucose-limited fed-batch cultivation in *S. cerevisiae* [22]. By expressing *CAR* combined with an acyl carrier protein activation module, phosphopantetheinyl transferase (*BsuSfp*) from *Bacillus subtilis*, the direct conversion of FFAs into FAs was achieved in a modified strain of *S. cerevisiae* (Δ*FAA1/4*, Δ*DGA1*, *ACOT*↑), producing 31.2 mg/L [47] In a FFA overproducing strain (Δ*FAA1/4*, TE↑) of *S. cerevisiae*, the overexpression of rice α-dioxygenase (*αDOX*) converted intracellular even-chain-length FFAs into odd-chain-length fatty aldehydes through oxidative decarboxylation. These fatty aldehydes are subsequently reduced to FAs by endogenous NAD(P)H dependent *ADH*, producing 20 mg/L of FAs (Jin et al., 2016).

Fatty alkyl ethyl ester (FAEE)

FAEEs are synthesized through a transesterification reaction that converts fatty acyl-CoA with endogenous or exogenous ethanol, catalyzed by wax ester synthase (WS) (Fig. 2) [49]. Several studies demonstrated the production of FAEE in *S. cerevisiae* and *Y. lipolytica* by overexpressing *WS2* from *Marinobacter hydrocarbonoclasticus* [50–53]. To improve endogenous ethanol production for FAEE synthesis, pyruvate decarboxylase (*PDC*) and *ADH* from high ethanol-producing strains such as *Z. mobilis or S. cerevisiae* have been overexpressed [50–52, 54]. Additionally, adding external ethanol also increases FAEE production [51, 52, 55]. For instance, the overexpression of *WS2* with two heterologous genes, *PDC1* and *ADH1*, from *S. cerevisiae* and adding of 2% exogenous ethanol resulted in an FAEE titer of 360.8 mg/L in *Y. lipolytica* [52].

Furthermore, in order to augment the amount of fatty acyl-CoA, many studies blocked the β-oxidation and TAG/SE synthesis pathways utilizing fatty acyl-CoA as a substrate [24, 50, 51, 53, 54, 56, 57]. In *Y. lipolytica*, overexpressing acetyl-CoA synthetase (*ACS2*), *ACC1*, and ATP-citrate lyase (*ACL1*, *ACL2*) enhanced metabolic flow towards acetyl-CoA. Moreover, deleting *PEX10* and *DGA1* restricted the β-oxidation and TAG production pathways. This strategy, combined with *WS2*, *PDC1*, *ADH4* overexpression, and the addition of 5%

Table 2 Summary of metabolic engineering strategies for fatty alcohol, fatty acid ethyl ester, fatty alka(e)ne production in yeasts

Strain	Metabolic engineering strategies	Medium and carbon sources	Results	Fatty acid composition (%)	References
Fatty alcohol (FA)					
Yarrowia lipolytica	Overexpression of *ACC1* and *TaFAR1*; deletion of *DGA1* and *FAO1*	SD, glucose	690.21 mg/L (batch)	C:16:0	[41]
	Overexpression of *FAR* and *FATcpa*; deletion of *PEX10*	Low Nitrogen media, glucose	550 mg/L (batch)	C10, C16:0, C16:1, C18:0, C18:1, C18:2	[78]
	Overexpression of *FAR* and *EcfadD*	MM, glucose	205.4 mg/L (batch), 2.15 g/L (fed-batch)	C16:0 (23.82%), C18:0 (60.63%), C18:1 (15.55%)	[24]
	Overexpression of *MaFAR*	Mineral media, glucose	167 mg/L (batch)	C16:0 and C18:0 (85–88%), C18:1 (12–15%), C20:0 (2.5%)	[38]
	Overexpression of *FAR* with the promoter PFBAin, coordination with glycolysis	YPD, glucose	5.75 g/L (batch)	Not applicable	[79]
	Overexpression of *MhFAR*	YNB, glucose	1.5 g/L (fed-batch), 5.8 g/L (fed-batch)	C16:0 (19.1%), C17:0 (1.3%), C18:0 (30.5%), C18:1 (45.2%), C18:2 (2.9%)	[39]
	Overexpression of *GPD1*, *BlucFAR1* and *DGA2*; deletion of *TGL4*, POX1, POX2, POX3, POX4, POX5 and POX6	MedA+, glycerol	166.6 mg/L (batch)	C18:0, C20:0, C22:0, C24:0	[80]
	NHEJ strategy, overexpression of *FAR*; deletion of *PEX10*	YPD, glucose	255.3 mg/L (batch)	Not applicable	[81]
	Overexpression of *ackA-pta* and employment of MES system	YPA41, glucose	83.8 mg/g dry cell (batch)	Not applicable	[37]

Table 2 (continued)

Strain	Metabolic engineering strategies	Medium and carbon sources	Results	Fatty acid composition (%)	References
Saccharomyces cerevisiae	Overexpression of *FAR*; deletion of *DGA1*	MM, galactose	98 mg/L (batch)	C16:1, C18:1	[82]
	Overexpression of *ACC1*, *TaFAR*, *ADH2*, *ALD6*, *ACS* and *ACL*; deletion of *RPD3*, *POX1* and *POT1*	MM, glucose	655 mg/L (batch), 1.1 g/L (fed-batch)	C16:0	[40]
	Overexpression of *PTE1*, α*DOX* and *ADH*; deletion of *FAA1* and *FAA4*	YPD, glucose	20.3 mg/L (batch)	C12:0, C13:0, C14:0, C14:1, C15:0, C15:1	[43]
	Expression of *XR*, *XDH*, *XKS*, *TAFAR*, *ACC1*, *ACL1* and *ACL2*; deletion of *RPD3*	SC, xylose	0.79 g/L (batch), 1.2 g/L (fed-batch)	C16:0	[83]
	Overexpression of *ADH5* and *FaCoAR*, genomic integration of *MmCAR*; deletion of *POX1*, *FAA1*, *FAA4*, *HFD1* and *ADH6*	MM, glucose	0.12 g/L (batch), 1.51 g/L (fed-batch)	Not applicable	[22]
	Overexpression of *TaFAR*, *ACC1*, *PEX7*, *PEX3* and *PEX19*	N_2 limited SC, glucose	817.9 mg/L (batch), 1.3 g/L (fed-batch)	C10:0 (6.9%), C12:0 (27.5%), C14:0 (2.9%), C16:0 (62.7%)	[84]
	Overexpression of peroxisomal *FaCoAR*; deletion of *ARE1*, *ARE2*, *DGA1*, *LRO1* and *POX1*	MM, glucose	193 mg/L (batch)	Not applicable	[48]
	Overexpression of *MaFAldhR*, *ACC1* and *ELO2*; deletion of *ELO3*	MM, glucose	7.84 mg/g cell dry weight (batch)	C16:0, C16:1, C18:0, C18:1, C20:0, C20:1, C22:0, C22:1	[85]
	Overexpression of *MmCAR* and *ADH5*	MM, glucose	52 mg/L (batch)	C10:0 (3.50%), C:12: (8.8%), C14:0 (10.81%), C16:0 (45.81%), C16:1 (11.37%), C18:0 (15.29%), C18:1 (4.40%)	[42]
	Overexpression of *MmFAR1*, *ACC1*, *OLE1*, *FAS1* and *FAS2*; deletion of *GDH1*, *DGA1*, *HFD1* and *ADH6*	YPD, glucose	1.2 g/L (batch), 6.0 g/L (fed-batch)	C12:0 (3%), C14:0 (3%), C16:0 (87%), C18:0 (7%)	[36]
	Overexpression of *CAR*, *SFP*, and *ACOT*; deletion of *FAA1* and *FAA4*	YNB, galactose	31.2 mg/L (batch)	C8:0 (1.1%), C10:0 (2.3%), C12:0 (2.1%), C14:0 (11.3%), C16:0 (15.4%), C16:1 (45.3%), C18:0 (10.5%), C18:1 (12%)	[47]
	Overexpression of *ACC1*, *ELO1*, *ELO2*, *AtFAR* and introduction of *MbFASI* system; deletion of *ELO3*	MM, glucose, and galactose	83.5 mg/L (batch)	C26:0	[86]
	Overexpression of *MDH3*, *RTME*, *MMACL*, *AnACL* and *CTP1*, optimization of *MmCAR*; deletion of *TPO1*	MM, glucose	252 mg/L (batch)	C8:0, C10:0, C12:0 (59%) and C14, C16:0, C16:1, (41%)	[44]
	Overexpression of *MaFAR1* into 30 neutral sites; deletion of *HFD1*	MM, glucose	110 mg/L (batch)	Not applicable	[46]
	Overexpression of *MaFAR1*, *PXA1*, *PXA2*, *FAA2*, *RTME*, *IDP2*, *IDP3*, *PEX7* and *PEX28*; deletion of *HFD1*	Delft MM, glucose	282 mg/L (batch)	Not applicable	[45]
Rhodosporidium toruloides	Expression of *FAR*	YPD, sucrose	2 g/L (batch), 8 g/L (fed-batch)	C16:0 (19%), C18:0 (24%), C18:1 (57%)	[11]
Pichia pastoris	Overexpression of *FaCoAR*; deletion of *HFD1*	MM, methanol	233 mg/L (batch), 2.0 g/L (fed-batch)	C16:0 (36%), C18:0 (26%), C18:1 (36%)	[15]
Ogataea polymorpha	Overexpression of *DAS2*, *ScIDP2*, *PYC1*, *MDH3*, *RtME1*, *TaFAR1* and *ScADH5*; deletion of *ARE*, *HFD1*, *LPL1* and *IZH3*	MM, methanol	3.6 g/L (fed-batch)	C16:0 (52%), C18:0 (43%), C18:1 and C18:2 (5%)	[17]
Lipomyces starkeyi	Expression of *MaFAR*	Mineral media, glucose	770 mg/L (batch)	C:16:0, C18:0 and C18:1	[38]
	Expression of *mFAR1*	YPD, glucose	1.7 g/L (batch)	C16:0 (74%), C18:0 (15%), C18:1 (11%)	[87]
Fatty acid ethyl ester (FAEE)					

Table 2 (continued)

Strain	Metabolic engineering strategies	Medium and carbon sources	Results	Fatty acid composition (%)	References
Yarrowia lipolytica	Expression of *AbAtfA* to ER and overexpression of *perCat2*	YNB, glucose	142.5 mg/L (batch)	C15:0, C16:0, C16:1, C18:0, C18:1, C18:2, C20:0	[24]
	Overexpression of *ACL1*, *ACL2*, *ACS2*, *ACC1* and *MhWS*; deletion of *PEX10*	YPD, glucose and exogenous ethanol	1.18 g/L (batch)	C14:0, C16:0, C16:1, C18:0, C18:1, C18:2	[51]
	Overexpression of *WS2*, *PDC* and *ADHB*; deletion of *GPD1*, *SCT1*, *DGA1*, *IDH1*, *MFE1* and *PEX10*	YPD, glucose and vegetable cooking oil	82 mg/L (batch)	C16:0, C16:1, C18:0, C18:1, C18:2	[54]
	Co-overexpression of *PDC1* and *ADH1*, overexpression of *MhWS* and *GAPDH*	YPD, glucose and exogenous ethanol	360.8 mg/L (batch)	C4:0 (10.9%), C10:0 (0.4%), C:12:0 (1.2%), C14:0 (1.9%), C16:0 (56.4%), C16:1n-7 (5.6%), C18:0 (20.2%), C18:1n-9 (2.3%), C18:2n-6 (1.1%)	[52]
	Overexpression of *Lip2*	YP, sunflower seed oil and exogenous ethanol	13.5 g/L (batch)	C16:0, C16:1, C18:0, C18:1, C18:2	[55]
Saccharomyces cerevisiae	Overexpression of *WS/DGAT*, *GCY1*, *GUP1*, *DAK1*; deletion of *GPD2* and *FPS1*	YNB, glycerol	0.52 g/L (batch)	Not applicable	[88]
	Overexpression of *WS2*; deletion of *DGA1*, *LRO1*, *ARE1*, *ARE2* and *POX1*	SD, glucose	17.2 mg/l (batch)	C14:0, C16:0, C16:1, C18:0, C18:1	[57]
	Chromosomal delta-integration of *WS2*, overexpression of *ACB1* and *GAPN*	SD, glucose	34 mg/L (batch)	Not applicable	[35]
	Overexpression of *ACS1*, *ACS2*, *KS*, *KR*, *HTD*, *TER*, *EEB1* and *EHT1*; deletion of *ADH1*, *ADH2*, *ADH3*, *ADH4*, *GPD1* and *GPD2*	SCD-Ura, glucose	0.75 g/L (batch)	Not applicable	[89]
	Overexpression of *MhATF1*; deletion of *FAA2*, *PXA2* and *ACB1*	SC-URA, glucose	2.1 mg/L (batch), 25 mg/L (fed-batch)	C10:0, C12:0, C14:0, C15:0, C16:0, C16:1, C18:0, C18:1	[56]
	Overexpression of *ws2*, *ADH2*, *ALD6*, *ACS*, *ACC1* and *ACB1*	SD, glucose	4.4 mg/L (batch)	C14:0, C16:0, C16:1, C18:0, C18:1	[50]
	Overexpression of *ALD6*, *ACS1*, *ACC1*, *FAS1*, *FAS2* and *SAAT*	Corn mash media, ethanol	EH (7.53 mg/L), EO (13.65 mg/L) and ED (13.87 mg/L)-(batch)	C6:0, C8:0, C10:0	[58]
	Integration based overexpression of *WS2*, *ALD6*, *ACS1* and *AnACLa*; deletion of *POX1*, *DGA1*, *LRO1*, *ARE1*, *ARE2*, *PXA1*, *PXA2* and *ADH1*	YEPD, glucose	1 g/L (batch), 5 g/L (fed-batch)	C12:0, C14:0, C16:0, C16:1, C18:0, C18:1	[53]
Rhodotorula toruloides	Overexpression of *AbWS/DGAT*	YPD, glucose	4.03 g/L (batch), 9.97 g/L (fed-batch)	C14:0, C16:0, C18:0, C18:1, C18:2	[12]
Fatty alka(e)nes (FALK)					
Yarrowia lipolytica	Overexpression of *MmCAR*, *BsuSfp* and *PmADO*	YNB, glucose	23.3 mg/L (batch)	C13:0, C15:0, C15:1, C17:0, C17:1	[24]
	Overexpression of *ACC*, *GPD1*, *DGAT*1, *TGLI-CAR-ADC* to the ER and LB with *CAR-ADC* to peroxisome; deletion of *GUT2* and *MFE1*	YNBD, glucose	372.4 mg/L (batch)	Not applicable	[90]
	Expression of *CvFAP* with LED light 467 nm	YSM, glycerol	10.9 mg/L (batch), 58.7 mg/L (fed-batch)	C15:0, C17:0, C17:1, C17:2	[61]
	Overexpression of *CvFAP* with blue light; deletion of *FAA1*, *ALK1* and *ALK2*	YNB, glucose	1.47 g/L (fed-batch)	C15:0, C17:0, C17:1, C17:2	[62]

Table 2 (continued)

Strain	Metabolic engineering strategies	Medium and carbon sources	Results	Fatty acid composition (%)	References
Saccharomyces cerevisiae	Overexpression of *SeFAR* and *SeFADO*, expressing an *EcFdx/EcFpr* from *E. coli*; deletion of *HFD1*	MM, glucose	22.0 μg/g DCW (batch)	C13:0, C15:0, C17:0	[59]
	Overexpression of *aDOX* and *cADO*; deletion of *FAA1* and *FAA4*	MM, glucose and galactose	42.4 μg/L tetradecane, 31.1 μg/L hexadecane (batch)	C14:0, C16:0	[91]
	Overexpression of *MmCAR*, *NpgA* and *NpADO*; deletion of *POX1*, *HFD1* and *ADH5*	MM, glucose	0.82 mg/L alkanes (batch)	C13:0, C15:0, C15:1, C17:0, C17:1	[22]
	Peroxisomal targeting of *MmCAR*, *SeADO* and *PEX34*; deletion of *HFD1*, *POX1*, *PEX31* and *PEX32*	MM, glucose	3.55 mg/L (batch)	C15:1, C15:2, C17:1, C17:2	[48]
	Co-expression of *SeADO* and *CwADO*; deletion of *POX1*, *HFD1* and *ADH5*	MM, glucose	1.14 mg/L (batch)	C11:0, C13:0, C15:0, C15:1, C17:0, C17:1	[92]
	Co-overexpression of *maFACR* and *cADO*; deletion of *ADH4*, *ADH5*, *ADH6*, *ADH7*and *OPI1*	YNB-URA, glucose and galactose	1.54 mg/L (batch)	C13:0, C15:0, C15:1, C17:0, C17:1	[60]

exogenous ethanol, resulted in a FAEE production level of 1.18 g/L [51]. Similarly, by overexpressing *WS2*, *PDC*, and alcohol dehydrogenase II (*ADHB*) while inhibiting key competitive metabolic pathways, such as TAG synthesis (Δ*GPD1*, Δ*SCT1*, Δ*DGA1*), β-oxidation (Δ*PEX10*, Δ*MFE1*), and the TCA cycle (Δ*IDH1*), there was a substantial increase in the fatty acyl-CoA pool in *Y. lipolytica*. This approach was further supported by adding vegetable cooking oil, successfully producing 82 mg/L of FAEE [54]. In *S. cerevisiae*, enhancing the pool toward FFA by deactivating fatty acid utilization pathways, such as triacylglycerol synthesis (Δ*DGA1*, Δ*LRO1*), sterol ester synthesis (Δ*ARE1*, Δ*ARE2*), and beta-oxidation (Δ*POX1*), coupled with the overexpression of *WS2*, achieved a titer of 17.2 mg/L FAEE [57]. Simultaneously, eliminating the above competitive pathways while overexpressing *ALD6*, *ADH2*, and ACS increased the acetyl-CoA and the cofactor NADPH. This strategy was further complemented by upregulating ACC1 and acyl-CoA binding protein (*ACB1*), boosting the acyl-CoA pool, resulting in 4.4 mg/L FAEE through the catalytic activity of *WS2* [50]. Adopting a similar strategy to enhance both the acetyl-CoA and acyl-CoA pools and utilizing strawberry alcohol acyltransferase (*SAAT*) to improve alcohol acyltransferase activity enabled *S. cerevisiae* to produce 7.53 mg/L of ethyl hexanoate (EH), 13.65 mg/L of ethyl octanoate (EO), and 13.87 mg/L of ethyl decanoate (ED) [58].

Fatty alkane (FALK)

Engineered yeast can produce FALKs through two distinct pathways (Fig. 2). The first involves the conversion of fatty acyl-ACP or fatty acyl-CoA into fatty aldehydes, which are subsequently decarbonylated to form alkanes (two-step). The second pathway converts FFAs or fatty acyl-CoA into alkanes via a photodecarboxylation process (single-step). Specifically, in the engineered *Y. lipolytica*-producing FFAs, FALKs were synthesized through a two-step enzymatic process. Initially, fatty acids were converted into fatty aldehydes and subsequently decarboxylated into alkanes by aldehyde deformylating oxygenase (ADO). This was achieved through the cytosolic expression of *CAR*, *BsuSfp*, and *PmADO* from *Prochlorococcus marinus*. This approach successfully produced FALKs of about 23.3 mg/L [24]. In a genetically optimized FFA-producing strain of *S. cerevisiae*, co-expression of *CAR* and its activator 4′-phosphopantetheinyl transferase (*NpgA*) from *Aspergillus nidulans* along with *NpADO* from *Nostoc punctiforme*, and the elimination of competing fatty alcohol synthesis pathways (Δ*ALR*, Δ*ADH*), resulted in only 0.82 mg/L of FALK [22]. However, the peroxisomal overexpression of *CAR*, *NpgA*, *Synechococcus elongates SeADO*, and *PEX34*, coupled with the deletion of *PEX31* and *PEX32*, enhanced alkane production to 3.55 mg/L in *S. cerevisiae* [48]. By employing

the cyanobacterial fatty acyl-CoA-derived pathway, which utilizes a fatty acyl-ACP/CoA reductase (*SeFAR*) and aldehyde deformylating oxygenase (*SeADO*) from *Synechococcus elongatus*, alkane/alkene production in *S. cerevisiae* reached 22 µg/g DCW and 1.54 mg/L [59, 60].

A single-step process has also been employed to produce alkane/alkene directly from fatty acids. In *Y. lipolytica*, utilizing the fatty acid photodecarboxylase from *Chlorella variabilis* (CvFAP) enabled the light-dependent synthesis of FALKs from either FFAs or fatty acyl-CoA. With this approach, 58.7 mg/L of FALKs was achieved directly from FFAs [61]. Li et al. (2020) further identified that fatty acyl-CoAs are more efficient substrates than FFAs for CvFAP in the photodecarboxylation reaction, leading to a substantial increase in FALKs production (1.47 g/L) directly from fatty acyl-CoA [62].

Utilizing non-conventional carbon sources for lipid production

CO_2 and its derivatives, including formate, acetate, and methanol, serve as sustainable feedstocks for the production of FFAs in yeast. Specifically, acetate can be converted into acetyl-CoA, while formate acts as an energy source by formate dehydrogenase (FDH), generating NAD(P)H [63, 64]. Utilizing formate for energy and acetate for carbon sources, combined with the overexpression of *FDH* and *ACS*, resulted in the production of 6.6 g/L of FFAs in *S. cerevisiae* [31]. Another study demonstrated that overexpressing *FDH* along with key enzymes from the Calvin-Benson-Bassham pathway, specifically phosphoribulokinase (PRK) and ribulose bisphosphate carboxylase oxygenase (RuBisCO), and their molecular chaperones (GroES and GroEL), in *S. cerevisiae* led to the production of 10.1 g/L of FFAs. This increase was facilitated by the development of a CO2 fixation pathway and enhanced utilization of formate [30].

With advancing technology for converting CO_2 into methanol, the utilization of methanol in yeast has gained increasing attention. Methylotrophic yeasts such as *Ogataea polymorpha* and *P. pastoris* can metabolize methanol via the DAS pathway in the peroxisome. Several studies demonstrated that methanol can potentially serve as an alternative carbon source for FFAs production [15, 16, 32]. Methanol metabolism in *O. polymorpha* was significantly improved by adjusting the expressions of aldehyde oxidase (*AOX*1), dihydroxyacetone synthase (*DAS*), and dihydroxyacetone kinase (*DAK*) along with the overexpression of ribulose-phosphate 3-epimerase (*RPE*), fructose-1,6-bisphosphatase (*FBP1*). Additionally, the acetyl-CoA pool was increased via ACL overexpression. Glucose-6-phosphate dehydrogenase (*ZWF1*) and isocitrate dehydrogenase (*ScIDP2*) were overexpressed to enhance the NADPH supply. Concurrently, the putative lipase (*LPL1*) and membrane protein associated with zinc metabolism (*IZH3*) were deleted to optimize cell survival by reinstating phospholipid metabolism, thereby enhancing resistance to methanol toxicity and streamlining metabolic flux towards FFA production, resulting in 15.9 g/L FFAs using methanol [16]. Adopting a similar methanol-utilizing strategy in *Ogataea polymorpha* also achieved a FA production level of 3.6 g/L. For FA production, the malate cycle (pyruvate carboxylase *PYC1*↑, malate dehydrogenase *MDH3*↑, malic enzyme *RtME1*↑) was improved, and *TaFAR1* and *ADH5* were overexpressed, coupled with the deletion of *HFD1* [17]. In *P. pastoris*, overexpressing *MmACL* from *Mus musculus*, along with *DAS2* to enhance formaldehyde assimilation, phosphoketolase (*XFPK*), and phosphotransacetylase (*PTA*) to improve the acetyl-CoA supply, and further overexpression of *ScIDP2*, resulted in the production of FFAs at a concentration of 23.4 g/L from methanol. A high FA titer of 2.0 g/L was achieved in this FFA-overproducing strain of *P. pastoris* with a methanol consumption rate of 1.2 g/L/h, through the simultaneous restoration of *FAA1* and *FAA2* to reactivate FAA along with the expression of *FAR* and deletion of *HFD1* [15].

Challenges and perspectives

The biological production of industrial chemicals and fuels continues to attract ongoing interest due to its potential for environmental sustainability. Particularly in industrial production, the stability of bioprocesses must be ensured [65]. Research utilizing oleaginous yeast *Y. lipolytica*, a Generally Recognized as Safe (GRAS), has been extensive due to its robust lipid accumulation, typically in the form of triacylglycerols (TAG). However, for direct industrial use, TAG must be converted back into fatty acids, a process that incurs additional energy and costs. Consequently, recent focus has shifted towards directly producing FFAs from organisms [66, 67]. Although various studies explored yeast-based production of fatty acids and their derivatives, the titer remains low for industrial applications (Tables 1 and 2). In *Y. lipolytica*, the intense flux toward TAG production should be redirected to enhance FFA [68]. In *S. cerevisiae*, a major barrier is redirecting its strong ethanol production flux towards FFA production [69]. Furthermore, the toxicity of FFAs within cells can make high-level production challenging. Thus, in addition to metabolic engineering, the optimization of a bioprocess is required to elevate fatty acid production to industrial levels [66, 70].

Typically, obtaining biomass in bioprocesses is based on lignocellulosic glucose. Most studies reviewed in this study also mainly utilize glucose for lipid production. However, for bioproducts such as biofuels that require mass industrial production, the volatility of sugar prices poses significant economic challenges [71, 72]. As highlighted in this review, research is increasingly exploring

non-conventional carbon sources such as CO_2, methanol, and acetate, which do not rely on conventional sugars (Fig. 1). Recent advancements include studies on converting methylotrophic yeasts such as *P. pastoris* to autotrophic yeasts. The CO_2 conversion was achieved by introducing a CO_2-fixation pathway into the peroxisomes employing the heterologous Calvin-Benson-Bassham (CBB) cycle [73]. The engineered autotrophic *P. pastoris* strain was also employed to produce organic acid from CO_2 [74]. In addition, Mitic et al. (2023) successfully constructed an oxygen-tolerant reductive glycine pathway for CO_2 utilization [75]. The assimilation of low-carbon compounds such as CO_2, methanol, and acetate also required NAD(P)H. However, as NADH is also competitively utilized for lipid synthesis, careful consideration of energy supply dynamics is required [14].

Additionally, the potential applications of fatty acids and their derivatives are intrinsically influenced by factors such as chain length, structural type, and the degree and distribution of saturation or unsaturation [76, 77]. While most studies on yeast-based lipid production have primarily focused on increasing overall lipid production and analyzing fatty acid compositions (Tables 1 and 2), efforts to engineer and regulate specific fatty acid profiles remain limited. Optimizing microbial platforms for the synthesis of FFAs and their derivatives necessitates a deeper focus on tailoring chain length to enhance their functionality and suitability for downstream applications.

Conclusion

The biological production of FFAs and their derivatives, such as fatty alcohols and alkanes, is essential for sustainable industrial processes. Metabolic engineering of yeasts has already achieved notable successes in producing FFAs, highlighting the importance of tailored metabolic engineering strategies for each yeast strain. Additionally, the utilization of low-carbon compounds such as CO_2 and methanol is increasingly vital for sustainable industrial production. Therefore, refining metabolic pathways to convert these compounds into FFAs is crucial. Ongoing advancements in synthetic biology, omics analysis, and systems metabolic engineering will enable sustainable and large-scale industrial production of FFAs and their derivatives.

Abbreviations

ACB	Acyl-CoA binding protein
ACC	Acetyl-CoA carboxylase
ACL	ATP: citrate lyase
ACP	Acyl carrier protein
ACS	Acetyl-CoA synthetase
ADC	Aldehyde decarbonylase
ADH	Alcohol dehydrogenases
ADO	Aldehyde deformylating oxygenase
ALD	Acetaldehyde dehydrogenase
ALK	Diacylglycerol transferases
ALR	Aldehyde reductases
ARE	Sterol acyltransferases
BsuSfp	Phosphopantetheinyl transferase
CAR	Carboxylic acid reductase
CBB	Calvin-Benson-Bassham
CDS	Phosphatidate cytidylyltransferase
CDP-DAG	Cytidine diphosphate diacylglycerol
CHO	Phosphatidylethanolamine N-methyltransferase
CpFAH12	Δ12 oleate hydroxylase
CTP	Citrate transporter
CvFAP	Fatty acid photodecarboxylase
DAG	Diacylglycerol
DAK	Dihydroxyacetone kinase
DAS	Dihydroxyacetone synthase
DGA	Diacylglycerol acyltransferases
DGAT	Acyl-CoAdiacylglycerol acyltransferase
DHA	Dihydroxyacetone
EcfadD	Fatty acyl-CoA synthetase
EcFdx	E. coli ferredoxin
EcFpr	E. coli ferredoxin reductase
ED	Ethyl decanoate
EEB1/EHT1	Acyl-CoA: ethanol O-acyltransferases
EH	Ethyl hexanoate
ELO	Fatty acid elongases
EO	Ethyl octanoate
FAA/FAT	Fatty acyl-CoA synthetases
FAD	Fatty aldehyde decarbonylase
FA	Fatty alcohol
FAEE	Fatty acid ethyl esters
FALK	Fatty alkane
FAR/TaFAR	Fatty acyl-CoA reductases
FAS	Fatty acid synthetases
FBP1	Fructose-1,6-bisphosphatase
FDH	Formate dehydrogenase
FFA	Free fatty acid
G3P	Glyceraldehyde 3-phosphate
GapN	Glyceraldehyde-3-phosphate dehydrogenase
GCY	Glycerol dehydrogenase
GDH	Glutamate dehydrogenase
GPD	Glycerol-3-phosphates
GRAS	Generally Recognized as Safe
GUP	Glycerol uptake protein
GUT	Glycerol 3-phosphate dehydrogenase
HFD	Aldehyde dehydrogenase
HTD	β-hydroxyacyl-CoA dehydratase
KS	β-ketoacyl-CoA synthase
KR	β-ketoacyl-CoA reductase
IDH	Isocitrate dehydrogenase
IDP	Isocitrate dehydrogenase
IZH	Membrane protein associated with zinc metabolism
LPL	Putative lipase
LRO	Diacylglycerol acyltransferase
Lip2	Lipase
MaC16E	Fatty acid elongase
MDH	Malate dehydrogenase
ME	Malic enzyme
MES	Microbial electrosynthesis
MFE	Multifunctional enzymes
MHY	C_2H_2-type zinc finger protein
NAD	Nicotinamide adenine dinucleotide phosphate
NpgA	4'-phosphopantetheinyl transferase
OA	Oleic acid
OLE	Δ9-fatty acid desaturase
OPI	Phosphatidyl-N-methylethanolamine N-methyltransferase
3PG	3-phospho-glycerate
PAH/LPP/DPP/APP	Phosphatidate phosphatases
PC	Phosphatidylcholine
PDC	Pyruvate decarboxylase
PDH	Pyruvate dehydrogenase
PSD	Phosphatidylserine decarboxylase
PEX	Peroxisome synthetase

PL	Phospholipid
POX	Peroxisomal acyl-CoA oxidase
PRK	Phosphoribulokinase
PTA	Phosphotransacetylase
PXA	Peroxisomal acyl-CoA transporter
PYC	Pyruvate carboxylase
Pyr	Pyruvate
RA	Ricinoleic acid
RPD	Histone deacetylase
RPE	Ribulose-phosphate 3-epimerase
RuBP	Ribulose 1,5-bisphosphate
RuBisCO	Ribulose 1,5-bisphosphate carboxylase/oxygenase
SAAT	Alcohol acyltransferase
SCT	Glycerol-3-phosphate O-acyltransferase 1
SE	Sterol esters
TAG	Triacylglycerol
TCA	Tricarboxylic acid cycle
TE/ACOT5/RnTEII/'TesA	Thioesterases
TER	trans-2-enoyl-CoA reductase
TGL	Triacylglycerol lipases
TPO	Medium-chain fatty acids exporter
WS	Wax ester synthase
XDH	Xylitol dehydrogenase
XFPK	Phosphoketolase
XKS	Xylulose kinase
XR	Xylose reductase
Xu5P	Xylulose 5-phosphate
XuMP	Xylulose monophosphate
ZWF	Glucose-6-phosphate dehydrogenase
αDOX	α-dioxygenase

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not Applicable. This is a review paper and does not involve direct research on humans or animals.

Consent for publication
"Not applicable" as this manuscript does not contain data from any individual person.

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The authors declare no competing interests.

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