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Current Opinion in Biotechnology

Review

# Advances in metabolic engineering for the production of sustainable aviation fuels

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Sustainable aviation fuels (SAF) are critical for decarbonising the hard-to-abate aviation sector, which significantly contributes to global $CO_2$ emissions. Conventional SAF production routes, such as Hydroprocessed Esters and Fatty Acids, Fischer-Tropsch and Alcohol-to-Jet, offer drop-in compatibility but are constrained by feedstock availability, high costs and environmental impacts. This review highlights, as promising alternatives, microbial bioproduction via precision fermentation of SAF-relevant compounds from low-cost feedstocks, with reduced land use and enhanced circularity. Here, we focus on microbially derived SAF precursors such as alcohols, terpenes, fatty acid ethyl esters, methyl ketones and saturated hydrocarbons, as well as recent advances in host engineering, pathway design, and bioprocess optimisation.

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**Current Opinion in Biotechnology** 2026, **100**:103523

This review comes from a themed issue on **Energy Biotechnology**

Edited by **Joshua Yuan** and **Zhihua Liu**

Available online 3 June 2026

https://doi.org/10.1016/j.copbio.2026.103523

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## Introduction

The aviation sector remains difficult to decarbonise due to its reliance on high-energy-density fuels. This industry contributes roughly 2% of global anthropogenic CO2 emissions, with demand expected to increase [1]. Aircraft require fuels with high gravimetric and volumetric energy density, properties provided by the C10-C16 kerosene fraction in Jet A/Jet A-1, which exhibits favourable cold-flow properties, thermal stability and compatibility with existing engine designs and infrastructure [2]. Alternatives must match these properties to ensure safety. Hydrogen, batteries, and other non-drop-in solutions (i.e. fuels or precursors requiring substantial modification to aircraft, engines or infrastructure) face energy-density, storage, or mass constraints, restricting them to short-range niches [3]. Consequently, only very few pathways provide scalable, near-term substitutes for energy-dense liquid hydrocarbons in medium- and long-haul aviation [4].

Sustainable aviation fuels (SAF) represent the most realistic near-term alternative to lowering aviation's life-cycle emissions. Defined as renewable or waste-derived fuels that achieve reduced life-cycle greenhouse gas emissions compared to conventional jet fuel and must meet established jet fuel specifications (e.g. ASTM D7566) [5], SAF must exhibit compatible physico-chemical properties with traditional fossil-derived fuels to enable drop-in use (i.e. direct utilisation in existing aircraft and fuel infrastructure without modification). Within current frameworks, SAF are primarily deployed as blended fuels, whereby synthetic components are incorporated into conventional jet fuel at specific volumetric limits. Such compatibility facilitates integration into existing global infrastructure, positioning SAF as the primary scalable, low-emission solution [6].

Currently, SAFs are produced via different routes that convert renewable feedstocks into drop-in fuels. The Hydroprocessed Esters and Fatty Acids (HEFA) [7], Fischer-Tropsch (FT) synthesis [8] and Alcohol-to-Jet (ATJ) [9] pathways enable upgrading of lipids, biomass-derived syngas, and bio-alcohols into jet-compatible hydrocarbons. Although these routes support early SAF adoption, scalability is limited by feedstock constraints, environmental concerns, and high operational costs, emphasising the need for novel approaches [10].

Microbial bioproduction via precision fermentation is one such alternative, using engineered microorganisms to produce SAF precursors from diverse, sustainable substrates such as sugars, lignocellulosic biomass, or even captured $CO_2$ [11]. This approach may reduce reliance on arable land, lowers water and fertiliser requirements, and enables circular economies through waste valorisation [12]. Moreover, metabolic engineering allows targeted pathway optimisation and yield increase through, for example, heterologous gene expression, overexpression or deletion of native genes and pathways, and protein engineering to increase precursor supply and direct flux towards desired products, concomitantly reducing burden. These strategies allow the bioproduction of compounds with desirable carbon chain lengths, branching and functional groups aligned with jet fuel properties. However, challenges related to substrate and product toxicity, metabolic burden, environmental stress responses, and process scalability remain [11].

This review examines current technologies for SAF production, with a focus on key compound classes that can be produced microbially, including alcohols, terpenes, fatty acid ethyl esters (FAEEs), methyl ketones and saturated hydrocarbons, all of which can be upgraded into drop-in hydrocarbons or directly blended [13]. It focuses on recent advances in host engineering, pathway design and bioprocess optimisation for each of these compound classes, highlighting promising metabolic engineering targets and briefly discussing current barriers and solutions for industrial commercialisation.

## Current state of sustainable aviation fuel technology

Thermochemical routes, which upgrade biomass or fossil feedstocks into jet-range hydrocarbons, currently dominate the production of SAF. Five main pathways have reached ASTM approval for up to 50% blends: FT synthesis of synthetic paraffinic kerosene (FT-SPK), HEFA, FT-SPK with aromatics (FT-SPK/A), ATJ, and catalytic hydrothermolysis jet fuel (CHJ) [14]. Among these, HEFA is the most technologically mature and commercially deployed pathway for SAF, hydrotreating and hydrocracking triglycerides or free fatty acids from plant oils, animal fats, and waste lipids into paraffinic hydrocarbons [14,15]. These products consist mainly of n- and iso-alkanes, which together form a major portion of conventional Jet A fuels.

HEFA is expected to remain the main contributor to SAF supply through 2030. However, its scalability is constrained by the limited availability and fluctuating costs of lipid feedstocks [15]. In response to these constraints, efforts have focused on diversifying lipid sources. HC-HEFA, a pathway relying specifically on hydrocarbons from *Botryococcus braunii*, was the first pathway approved in 2020 for up to 10% blend through the fast-track process [16].

New conversion schemes based on lignin and C1 feedstocks are emerging. For example, catalytic strategies have been proposed to elongate lignin-derived fragments into C8-C16 intermediates suitable for hydrodeoxygenation upgrading [17]. $CO_2$-to-jet technologies are also being explored, either through gasification to syngas or via methanol intermediates coupled to modified FT or methanol-to-olefin [18]. Nonetheless, catalyst stability, selectivity, and several conversion steps remain major hurdles to commercial viability [18].

In parallel, the Direct Sugar to Hydrocarbons (DSHC) pathway provides a compelling blueprint for biologically tailored SAF molecules. In this process, starch-based or lignocellulosic sugars are directly fermented into the sesquiterpene farnesene, a suitable jet fuel component [14]. Although current commercial deployment is limited by process energy efficiency and an ASTM blend allowance capped at 10%, DSHC illustrates the potential of engineered microbial systems to deliver high-value, drop-in hydrocarbons without extensive upgrading. A timeline overview of SAF development and major milestones is shown in Figure 1.

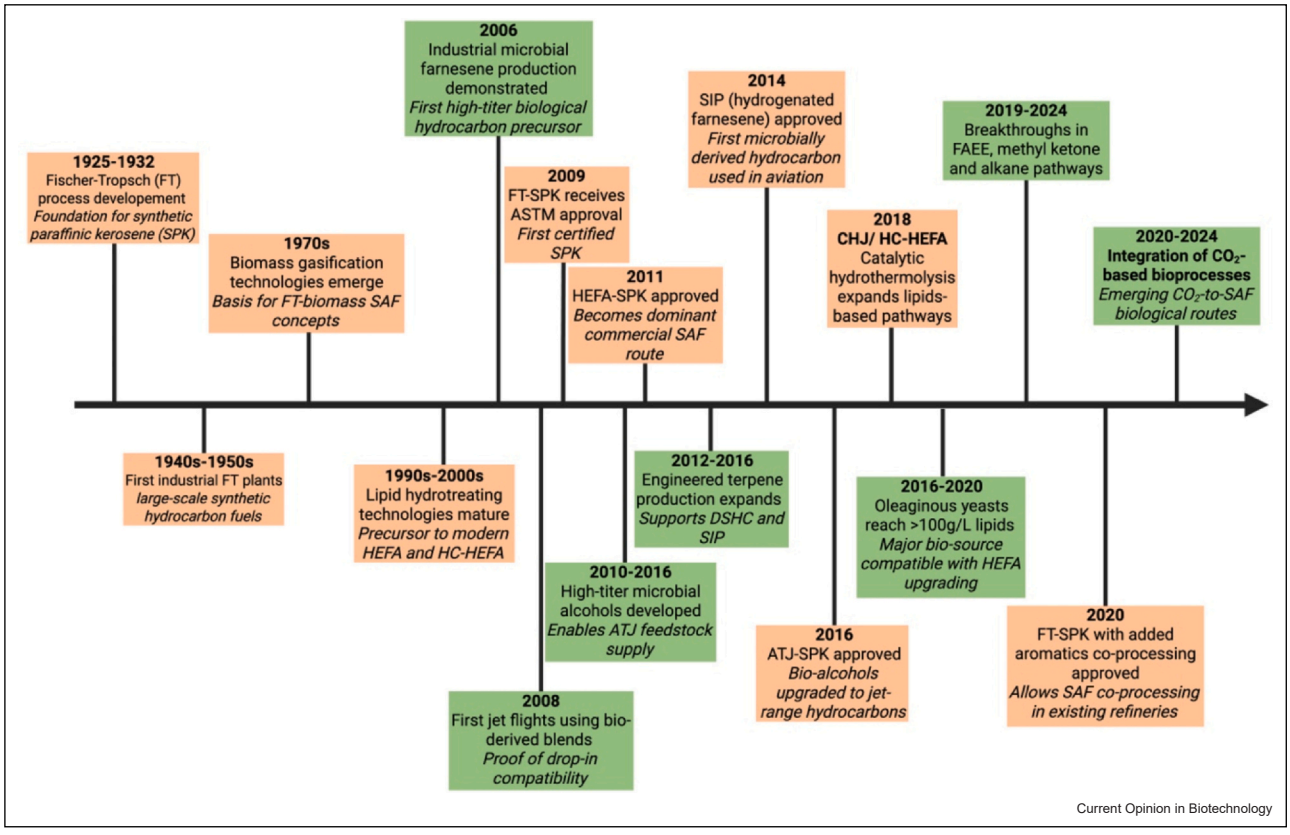
Within this evolving technological landscape, recognising that thermochemical routes alone may not satisfy future SAF demand, the integration of engineered microbial platforms with subsequent catalytic upgrading is increasingly viewed as crucial for diversifying the routes and molecular products available for SAF [16]. Microorganisms can convert renewable carbon sources into a variety of hydrocarbon precursors, including free fatty acids compatible with HEFA upgrading and drop-in molecules such as alcohols, terpenes, FAEEs, methyl ketones and saturated hydrocarbons [19,20]. Microbial pathways offer unique advantages, including control over product structure, integration with circular carbon streams such as organic waste or industrial $CO_2$, and the potential to bypass energy-intensive upgrading steps.

## Alcohols

A schematic overview of metabolic synthetic pathways and derived SAF precursors and possible feedstocks are summarised in Figure 2 and Table 1. Alcohols (C2-C8) are versatile, well-established microbially produced SAF precursors that can be upgraded, mainly via Alcohol-to-Jet pathways, to hydrocarbons compatible with jet fuel specifications [15]. Short-chain alcohols like ethanol and butanol isomers are particularly amenable to microbial fermentation, as many established industrial hosts natively produce them via short, high-flux branches of the central carbon metabolism that efficiently regenerate NAD+ under oxygen-limited and anaerobic conditions [21].

Ethanol (C2), the most mature biofuel, is produced at industrial scales in *Saccharomyces cerevisiae* (*S. cerevisiae*) and

**Figure 1**



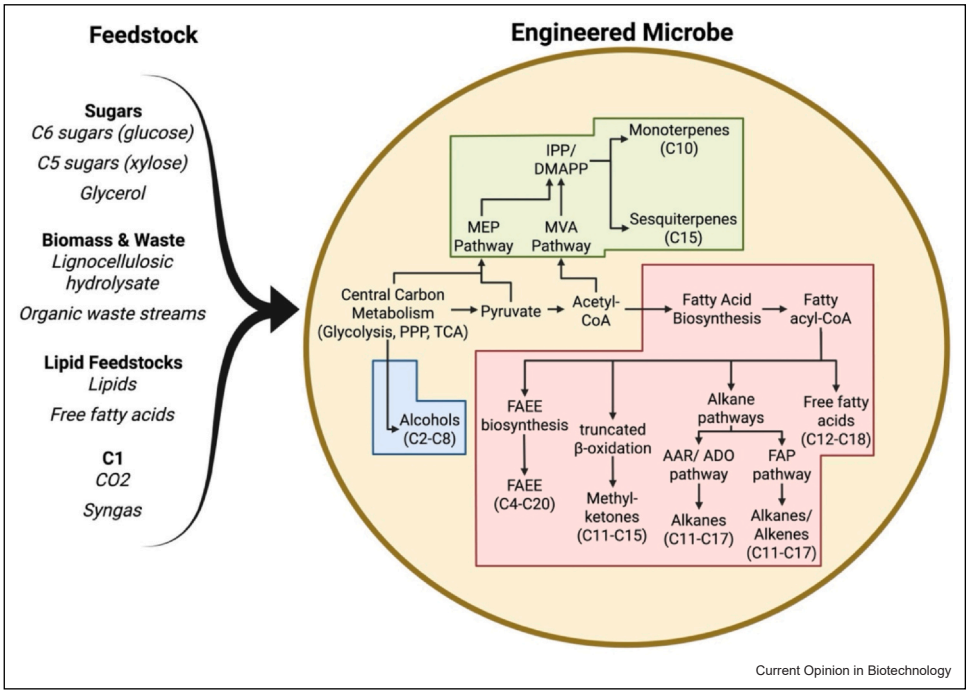

Timeline of SAF development and milestones. Broad overview of important SAF development milestones reached until to date. *Orange*: key SAF thermochemical milestones, including FT, HEFA, SIP, ATJ, CHJ and FT-SPK/A approvals. *Green:* major metabolic engineering advances, including microbial farnesene, high-titre alcohol and terpene production, oleaginous yeast lipid platforms, engineered C8-C16 hydrocarbons, FAEE/methyl ketone/alkane pathways, and emerging $CO_2$-based bioprocesses. (Image created in http://BioRender.com).

*Zymomonas mobilis* (*Z. mobilis*), achieving titres exceeding 100 g/L in fed-batch fermentations [22,23]. Their historic role in ethanol fermentation has led to a deep understanding of their ethanol-producing metabolism, enabling precise engineering of flux distributions, feedstock flexibility, and robustness under industrial conditions [24]. Recent metabolic engineering strategies have significantly expanded on both feedstock range and fermentation robustness, enabling the efficient conversion of lignocellulosic hydrolysate. Key advances in *S. cerevisiae* and *Z. mobilis* include the introduction of xylose-utilisation pathways such as the xylose reductase (XR)/xylitol dehydrogenase (XDH) or xylose isomerase pathway, enhancement of the non-oxidative pentose-phosphate flux and deletion of byproduct-forming genes (e.g. GRE3, PHO13), collectively delivering high ethanol yields on C5/C6 sugar feedstocks [25,26]. Complementary strategies focused on enhanced cellular robustness and tolerance of high ethanol and inhibitor concentrations and osmotic pressure. Targeting membrane and cell-wall biosynthesis pathways, combined with adaptive laboratory evolution (ALE), has repeatedly selected for mutations in regulators such as UTH1, SSD1 and components of the CWI/HOG stress-response networks [26]. Nonetheless, ethanol's short chain demands extensive dehydration and oligomerization, increasing downstream processing costs [9].

Butanol (C4) and its isomers require fewer upgrading steps, making them attractive SAF precursors. *Clostridia*, native butanol producers, remain key n-butanol producers, with high titres achieved in *Clostridium acetobutylicum* through implementing fermentation strategies such as gas stripping [27], while integrated metabolic-evolutionary engineering in *Clostridium cellulovorans* increased production from $< 0.025$ to 3.47 g/L from lignocellulosic substrate [28]. Engineered microbial platforms, such as *Escherichia coli (E. coli)*, enable robust expression of CoA-dependent butanol pathways through chromosomal integration and targeted elimination of competing fluxes [29], and engineered keto-acid pathways enable high isobutanol production with *in situ* removal [30].

Higher alcohols (C5-C8, e.g. isopentanol, 1-octanol) provide direct mid-chain SAF precursors requiring even

**Figure 2**




Schematic overview of microbial feedstocks and main metabolic pathways for SAF precursor bioproduction. Schematic overview of carbon feedstocks and drop-in fuel precursors bioproduced in engineered microorganisms. Diverse substrates, including C5/C6 sugars, glycerol, lignocellulosic hydrolysates, organic waste streams, lipids, free fatty acids, $CO_2$ and syngas, are assimilated into the central carbon metabolism. Carbon is subsequently partitioned via key metabolic nodes, notably pyruvate and acetyl-CoA, into modular bioproductive pathways yielding alcohols (blue), terpenes (mono- and sesquiterpenes via the MVA and MEP pathways, green), and fatty-acid-derived products, including fatty acid ethyl esters, methyl ketones, alkanes/alkenes, and free fatty acids (red). In brackets, [Cn], the carbon length range of the respective SAF precursors is shown. The figure highlights major pathway branches and product classes relevant to sustainable aviation fuel applications while abstracting detailed enzymatic steps. (Image created in http://BioRender.com).

fewer upgrading steps. Historically, production relied on redirecting carbon through branched-chain amino acid pathways, via the Ehrlich or the keto-acid pathways, in *E. coli* and yeasts [31]. Engineering the branched-chain biosynthesis pathway (AlsS-IlvC-IlvD-LeuABCD) and optimising the keto-acid decarboxylase (Kivd) and alcohol dehydrogenase (ADH2) modules enable gram-titre production of 3-methyl-1-butanol in two-phase *E. coli* bioreactors [32]. For longer chain targets, thioesterase-based routes have proven effective; expression of a high flux acyl-ACP-thioesterase-aldehyde reductase/alcohol dehydrogenase enabled 1-octanol titres of 1.3 g/L from glycerol by improving acyl-CoA availability and β-oxidation [33]. Yeast hosts combine relatively high solvent tolerance with well-characterised Ehrilch- and keto-acid pathways, and recent work has focused on tuning precursor supply, redox balance, and key Ehrlich-pathway enzymes to control higher alcohol production [34].

## Terpenes

Assembled from isoprenoid (C5) building blocks, especially mono-(C10) and sesquiterpenes (C15) have become of great interest, as these can be upgraded via hydrogenation and/or dimerisation to achieve jet-range properties and be used in SAF blends. Naturally, they are produced through the mevalonate (MVA) or the methylerythritol phosphate (MEP) pathways. The MVA pathway, mainly found in the cytoplasm of eukaryotic microorganisms, higher plants and archaea, uses Acetyl-CoA as a precursor to generate isopentenyl pyrophosphate (IPP) via a 6-step enzymatic process, which will be reversibly isomerised to dimethylallyl diphosphate (DMAPP) by IPP isomerase (IDI). The rate-limiting step and therefore a key metabolic engineering target is catalysed by HMG-CoA reductase (HGMR), converting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonate. The MEV pathway, mainly found in bacteria, cyanobacteria, green algae and plastids of higher plants, uses pyruvate and glycerol-3-phosphate as precursors, converting them directly to IPP and DMAPP alike, with ratios balanced by IDI. Here, 1-deoxy-D-xylulose-5-phosphate synthase (DXS) and 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) are the rate-limiting enzymes and thus key metabolic engineering targets. Together, IPP and DMAPP serve as isoprenoid

**Table 1**

**Summary of mentioned microbial production of SAF–precursors.**

| Molecule produced | Microbial host | Feedstock | Titre | Scale/notes | **Reference** |
|---|---|---|---|---|---|
| Ethanol | *Zymomonas mobilis* | Biogas slurry | 113.4 g/L | 5 L Bioreactor, fed-batch | Duan et al., 2019 [23] |
| Ethanol | *Saccharomyces cerevisiae* | Corn stover hydrolysate + glucose | ~111 g/L | Shake flask, batch, 92.9% of theoretical maximal yield achieved | Zhao et al., 2024 [22] |
| 3-Methyl-1-butanol (isopentanol) | *Escherichia coli* | Glucose | 9.5 g/L; yield 0.11 g/g glucose (60 h) | Shake flask, batch. Two-phase fermentation to mitigate toxicity | Connor et al., 2010 [32] |
| Isobutanol | *Escherichia coli* (JCL260) | Glucose | > 50 g/L (72 h) | 1 L bioreactor, batch; in situ product removal by gas stripping | Baez et al., 2011 [30] |
| Isobutanol | *Escherichia coli* | Glucose | 20 g/L | 7.5 L stir tank with 4 L working volume, batch | Dong et al., 2017 [29] |
| n-Butanol | *Clostridium acetobutylicum* JB200 | Glucose | 19.1 g/L (no gas stripping); 150.5 g/L butanol in condensate (intermittent gas stripping) | Fibrous bed bioreactor, fed-batch; intermittent gas stripping concentrates product stream | Xue et al., 2012 [27] |
| n-Butanol | *Clostridium cellulovorans* | Lignocellulosic substrate | 3.47 g/L | 3 L bioreactor, batch | Wen et al., 2019 [28] |
| 1-Octanol | *Escherichia coli* | Glycerol | 1.3 g/L | Shake flask, batch; high-flux thioesterase route | Hernández Lozada et al., 2020 [33] |
| Farnesene | *Saccharomyces cerevisiae* | Glucose | > 130 g/L | Industrial fermentation (Amyris) | Meadows et al., 2016 [40] |
| Limonene | *Escherichia coli* | Glycerol | 7.3 $g \cdot L_{org}^{-1}$; 3.6 g/L (aqueous production phase) | 3.1 L stirred-tank reactor, fed-batch; in situ extraction | Rolf et al., 2020 [37] |
| Nerolidol | *Yarrowia Lipolytica* | Glucose | 11.1 g/L | 5 L Bioreactor, Fed-batch | Liu et al., 2022 [42] |
| α-Pinene | *Escherichia coli* | Glycerol | 1035.1 mg/L | 1.3 L Bioreactor, fed-batch | Zhou et al., 2023 [38] |
| β-Myrcene | *Saccharomyces cerevisiae* | Glucose | 142.64 mg/L | 5 L Bioreactor, fed-batch | Shu et al., 2024 [39] |
| (*E*)-α-Bisabolene | *Rhodotorula toruloides* | Corn stover hydrolysate | 20.8 g/L | Microtitre plate | Adamczyk et al., 2025 [41] |
| Free fatty acids (FFAs) | *Yarrowia lipolytica* | Glucose | Up to 120% DCW | 5 L Bioreactor, batch, alkene overlay | Ledesma-Amaro et al., 2016 [44] |
| Short-chain FFAs (C6–C8) | *Saccharomyces cerevisiae* | Glucose | 72 mg/L (C6); 245 mg/L (C8) | Shake flask, batch | Gajewski et al., 2017 [46] |
| FAEEs | *Escherichia coli* (Microdiesel strain) | Glucose + ethanol | 1.28 g/L | Shake flask fed-batch, > 25% biomass dry weight | Kalscheuer et al., 2006 [48] |
| FAEEs | *Saccharomyces cerevisiae* | Glucose | 17.2 mg/L | Shake flask, batch | Valle-Rodríguez et al., 2014 [51] |
| FAEEs | *Synechococcus elongatus* PCC 7942 | $CO_2$ | 10.0 ± 0.7 $mg/L_{OD730}$ | Flask, continuous fluorescent light | Lee et al., 2017 [52] |
| FAEEs | *Yarrowia lipolytica* | Glucose + 5% vol exogenous ethanol | ~1.18 g/L | Shake flask, batch | Gao et al., 2018 [50] |
| Methyl ketones (C11–C15) | *Escherichia coli* | Glucose | ~380 mg/L (total methyl ketones) | Tubes; two-phase (decane overlay) used to enhance recovery/production | Goh et al., 2012 [54] |
| Methyl ketones | *Escherichia coli* | Glucose | 3.4 g/L (fed-batch, ~45h) | 2 L bioreactor, fed-batch; ~40% of maximum theoretical yield reported | Goh et al., 2014 [55] |
| Methyl ketones | *Pseudomonas putida* KT2440 | Lignocellulosic hydrolysate | ~700 mg/L | Shake flask | Dong et al., 2019 [57] |

**Table 1** (*continued*)

| Molecule produced | Microbial host | Feedstock | Titre | Scale/notes | Reference |
|---|---|---|---|---|---|
| Methyl ketones (C11–C17) | *Pseudomonas taiwanensis* VLB120 | Glucose | ~9.8 g/L aqueous (~69 g/L extraction phase) | 1.3 L Bioreactor, fed-batch | Nies et al., 2020 [56] |
| Alka(e)nes (AAR/ADO) | *Cupriavidus necator* H16 | Fructose (heterotrophic) & $CO_2$ (autotrophic) | 435 mg/L heterotrophic; 4.4 mg/L autotrophic | 6 L Bioreactor, N-limited biphasic fed-batch (heterotrophic); 1.4 L Bioreactor (autotrophic) | Panich et al., 2021 [60] |
| Saturated alka(e)nes | *Yarrowia lipolytica* (CvFAP) | Glucose | ~1.47 g/L and ~89% acyl-CoA conversion | Shake flask, fed-batch | Li et al., 2020 [63] |
| Alka(e)nes (FAP route) | *Rhodotorula toruloides* | Glucose/lignocellulosic hydrolysate | ~1.73 g/L (glucose), 0.94 g/L (hydrolysate) | Shake flask, fed-batch, N deficiency | Yu et al., 2025 [64] |

The table compiles quantitative studies cited in the text, grouped by the produced molecule, and covers all microbial hosts discussed, including bacteria, yeasts, cyanobacteria, and oleaginous organisms. Reported examples cover key SAF precursor classes (alcohols, terpenes, fatty-acid-derived esters, methyl ketones, and saturated hydrocarbons) along with the corresponding hosts, feedstocks, titres, and cultivation scales.

building blocks, undergoing sequential condensation reactions to form SAF-relevant terpene precursors geranyl pyrophosphate (GPP) and farnesyl pyrophosphate (FPP). Alternatively, isoprenoid building blocks can be obtained through expression of bypass pathways such as the isopentenol utilisation pathway (IUP), whereby exogenous isopentenol is phosphorylated by choline kinase (CK) and isopentenyl phosphate kinase (IPK) to generate IPP and DMAPP. Expression of the IUP pathway in a *Yarrowia lipolytica (Y. lipolytica)* has been reported to increase IPP and DMAPP pools by 15.7-fold compared to the endogenous MVA pathway [35].

Monoterpenes are derived from GPP and provide direct precursors for high-density SAF. Metabolic engineering strategies encompass increasing isoprenoid precursor supply and flux through the MVA (e.g. overexpression of HMGR) or MEP (e.g. overexpression of DXS and DXR) pathways, optimising terpene synthase performance, rewiring central carbon metabolism to enhance acetyl-CoA or pyruvate availability, eliminating competing pathways to minimise by-product formation, and implementing toxicity-mitigation approaches such as efflux systems, membrane engineering and two-phase extraction [35,36]. In *E. coli*, balancing MEP-derived precursor supply with high-activity limonene synthase variants, combined with two-phase extraction to alleviate product inhibition, has enabled gram-scale limonene production [37]. Moreover, identifying the rate-limiting enzymes and tailoring the terpene synthase terminal region has enabled efficient production of α-pinene in *E. coli* [38]. In yeast, myrcene production has been achieved by systematic engineering of precursor supply, optimisation of synthase expression and reducing competing fluxes [39].

Sesquiterpenes are derived from FPP and have strong potential as SAF precursors due to their inherent drop-in compatibility [11]. Farnesene, the only terpene currently certified for jet fuel (as hydrogenated farnesane), has been produced at an industrial scale in engineered *S. cerevisiae*, where extensive MVA-pathway engineering, central-carbon rewiring, and chassis optimisation enabled titres exceeding 130 g/L [40]. Similar engineering strategies have expanded production to non-conventional hosts: bisabolene reached 20.8 g/L in *Rhodosporidium toruloides* (*R. toruloides*) grown on lignocellulosic hydrolysates [41], while nerolidol achieved 11.1 g/L in *Y. lipolytica* through increased precursor supply and optimised synthase expression [42]. Even though microbial terpene production has been shown to be viable at an industrial scale, their production is hindered by cellular toxicity from product accumulation, pathway flux limitations due to feedback inhibition, and insufficient precursor supply in the MVA and MEP pathways [43].

## Free fatty acids

Free fatty acids (FFAs) are attractive as fuel precursors due to their high energy density and straightforward

conversion into a wide range of industrially relevant molecules [19]. FFA production can alleviate the intrinsic storage limitations associated with triacylglycerol (TAG) accumulation, particularly when secretion strategies are implemented, and provides a more direct path for downstream lipid recovery and biofuel synthesis [44,45].

Metabolic engineering to enhance FFA production was first established in *E. coli*, where carbon flux was redirected toward fatty acid biosynthesis through the heterologous expression of acyl-ACP thioesterases from plants, eradication of the beta-oxidative pathway, and optimisation of precursor supply (malonyl-CoA) [45].

In *S. cerevisiae*, enzyme engineering of the native fungal fatty acid synthase (FAS) complex has been shown to support short-chain fatty acid production via modulation of chain-length specificity, achieving extracellular titres of up to 72 mg/L (C6) and 245 mg/L (C8) [46].

Subsequent work has focused on *Y. lipolytica*, which naturally exhibits high capacity for lipid biosynthesis. In this host, similar strategies as those previously used for *E. coli* were compared with the overexpression of endogenous genes responsible for lipid body formation and endogenous and exogenous lipases that promote FFA excretion into the culture medium [19,44]. The best performing engineered *Y. lipolytica* strains were reported to secrete fatty acids at levels reaching up to 120% of the dry cell weight with the addition of an alkane layer during fermentation, significantly simplifying downstream recovery [44]. Despite these advances, challenges such as FFA toxicity, metabolic burden, and the need for integrated bioprocess optimisation remain critical barriers to industrial deployment [19].

## Fatty acid ethyl esters

FAEEs derive directly from fatty-acid metabolism and can be upgraded into jet-range paraffinic hydrocarbons [16,19]. Foundational work in *E. coli* first demonstrated the production of microbial FAEE by combining heterologous ethanol biosynthesis (PDC/ADH from *Z. mobilis*) with a wax ester synthase (WS/DGAT from *Acinetobacter baylyi*), enabling esterification of intracellular fatty acyl-CoA [48] and achieving titres of approximately 1.3 g/L and up to 26% of cell dry weight as FAEEs in fed-batch fermentation [48].

Since then, metabolic engineering of *S. cerevisiae* and *Y. lipolytica* has focused on increasing ethanol availability and acyl-CoA supply, boosting fermentative flux through overexpression of pyruvate decarboxylase enzymes (PDC*)* and alcohol dehydrogenases (ADH*)* and reinforcing fatty-acid biosynthesis via acetyl-CoA carboxylase (ACC1) and ATP-citrate lyase (ACL*)* upregulation, while blocking competing triacylglycerol, sterol ester synthesis, and β-oxidation sinks [19,49]. Coupled with high-activity wax ester synthases such as WS2 from *Marinobacter hydrocarbonoclasticus* and ethanol supplementation, *Y. lipolytica* achieved FAEE titres of 1.18 g/L [50]. In *S. cerevisiae*, similar pathway configurations, though without ethanol feeding, yield lower titres of 17.2 mg/L [51].

FAEE biosynthesis has also been demonstrated in the photosynthetic cyanobacterium *Synechococcus elongatus* (*S. elongatus*) PCC 7942, by introducing a heterologous ethanol producing pathway and WS along with acetyl-CoA-enhancing phosphoketolase enabled direct conversion of $CO_2$ to FAEEs with titres of around 10 mg/L under a hexadecane overlay [52]. It demonstrates the feasibility of directly coupling solar $CO_2$ capture to fuel precursors in a photosynthetic chassis.

## Methyl ketones

Methyl ketones, derived from fatty acids, are another class of compounds with desirable jet-fuel properties [53]. Medium-chain methyl ketones have been produced in *E. coli* by rerouting fatty-acid metabolism through truncated β-oxidation and overexpression of the endogenous Long-chain acyl-CoA thioesterase III (*fadM*), enabling accumulation of C11-C15 ketones from glucose [54,55]. More recently, *Pseudomonas taiwanensis* VLB120 has emerged as a superior chassis for this pathway, where deletions of native acyl-CoA dehydrogenases combined with optimised fed-batch fermentation have enabled titres of 9.8 g/L, the highest reported for recombinant methyl ketone production [56]. Lignocellulosic-based production has also been demonstrated in *Pseudomonas putida*, where plant-derived amino acids in biomass hydrolysates stimulate methyl-ketone formation to 700 mg/L, reinforcing compatibility with low-cost feedstocks and biorefinery co-processing [57].

## Saturated hydrocarbons (alkanes and alkenes)

Saturated hydrocarbons derived directly from fatty acids already fall in the n- and iso-alkane classes that dominate Jet A and can reduce aromatic content while preserving or even improving energy density and combustion characteristics [16]. Two main metabolic engineering strategies are currently being pursued.

The first relies on the heterologous expression of the cyanobacterial acyl-ACP reductase (AAR), which reduces fatty acyl-ACPs into aldehydes, and aldehyde deformylating oxygenase (ADO), a ferredoxin-dependent enzyme that converts aldehydes to alkanes and formate [58]. Although ADO turnover numbers remain low, structure-based enzyme engineering has significantly increased *in vivo* flux [58,59]. A similar AAR/ADO module from *S.*

*elongatus* enabled production of 435 mg/L of alka(e)nes in the $CO_2$-fixing bacterium *Cupriavidus necator* heterotrophically and 4.4 mg/L in autotrophic conditions [60]. Fatty acid decarboxylases such as UndA/UndB and cytochrome P450 OleT also enable alkene formation from fatty acids, although with lower titres, up to 55 mg/L and 97.6 mg/L, respectively, and stronger dependence on H2O2 or redox partners [61].

The second, and currently more productive, strategy uses expression of fatty acid photodecarboxylases (FAPs), particularly *Chlorella variabilis* FAP (CvFAP), which decarboxylate fatty acids to C1-shortened alkanes in a blue-light-driven reaction [62]. Discovery of FAP activity in microalgae and thylakoid membranes provided the initial proof of concept for light-driven conversion of fatty acids into C15-C17 alka(e)nes. CvFAP has been heterologously expressed in *Y. lipolytica*, in which fed-batch cultivation and extensive host engineering yielded about 1.47 g/L alka(e)nes and 89% conversion of the intracellular acyl-CoA pool [63]. This study also showed that CvFAP operates preferentially on acyl-CoA rather than free fatty acids *in vivo*, suggesting that peroxisomal engineering and acyl-CoA trafficking are key design levers in oleaginous yeasts.

More recent work extended the FAP concept to the yeast *R. toruloides* and systematically compared FAP, AAR/ADO and UndA/UndB pathways performance [64]. By combining enzyme mining, pathway reconstitution through a polycistronic expression system, elimination of competing pathways (fatty acid synthesis), precursor supply enhancement, gene copy number optimisation and electron transfer system engineering, the FAP route delivered up to 1.73 g/L alka(e)nes from glucose and 938.7 mg/L from lignocellulosic hydrolysate, the highest reported titres for microbially produced alka(e)nes from this feedstock [64]. Complementary biocatalyst designs have explored three-liquid-phase systems in *E. coli*, where a lipase-CvFAP cascade operates in microdroplets that enhance mass transfer, light penetration, and enzyme stability, achieving > 90% conversion of fatty-acid substrates [65]. At the molecular level, spectroscopic studies of CvFAP have revealed unexpectedly high activity on C8 substrates and an autocatalytic effect of the n-heptane product [66].

Current bottlenecks include slow ADO catalysis, light-utilisation efficiency, product toxicity, and tailoring chain-length distributions to meet certified SAF blending requirements for iso- and cycloalkanes [16,61,65].

## Microbial sustainable aviation fuel barriers and solutions for industrial commercialisation

The choice of microbial chassis is a critical decision to successfully produce SAF precursors and increase productivity. Key host attributes include endogenous metabolic pathways strength, cell growth rate, tolerance to diverse culture conditions, feedstock flexibility, resistance to toxic compounds (final products, intermediate or side metabolites) and the availability of synthetic biology toolkits for genetic manipulation. *E. coli* and *Pseudomonas* spp. are well-characterised chassis with highly developed genetic toolkits and fast growth rates that reduce fermentation times. However, they are generally sensitive to organic solvents, and they lack a robust endogenous lipid metabolism, making them better hosts for shorter-chain compounds such as alcohols and saturated hydrocarbons [29,31,56,57]. *S. cerevisiae* benefits from GRAS status, an advanced genetic toolkit, high solvent tolerance, and remains the workhorse host for ethanol and terpene production; however, it has a limited native lipid accumulation capacity [23]. In contrast, the non-conventional yeast, *Y. lipolytica* and *R. toruloides* possess strong host endogenous mevalonate and lipid biosynthetic pathways, making them suitable for terpene, alkane and FA-based production [41,42]. Both are tolerant to inhibitory compounds and have a broad feedstock compatibility (particularly hydrophobic substrates). Finally, cyanobacteria such as *S. elongatus* can fixate $CO_2$ and use it as a substrate to convert into FAEE precursors and saturated hydrocarbons, though titres remain low, limiting a near industrial application [52].

Despite impressive lab-scale titres, only a few products, for example, ethanol and farnesene, have reached industrial viability. Microbially derived SAF precursors remain constrained by substrate cost, pathway burden, accumulation of toxic intermediates, and limited robustness of chemical, physical and reactor stresses. Emerging bioengineering strategies aim to close this gap; AI-guided pathway and chassis design, coupled to automated high-throughput DBTL cycles and Adaptive laboratory Evolution (ALE) under process-relevant conditions, can systematically identify and generate optimised strains [67–69]. Concomitantly, dynamically regulated pathways, including CRISPRi/a-based control of competing fluxes, inducible or growth-phase–dependent promoters, and metabolite-responsive biosensors that enable real-time adjustment of pathway activity and C1/waste-carbon assimilation are beginning to facilitate scale-up by improving carbon utilisation and maintaining stable, high-productivity fermentation under industrial conditions [70,71]. Furthermore, emerging co-culture strategies offer a complementary solution by distributing precursor generation and, for example, terpene biosynthesis across specialised strains, reducing metabolic burden, mitigating toxicity, and enabling modular optimisation of pathway fluxes [47,72].

## Conclusions

The aviation sector faces a decarbonisation challenge due to its dependence on high-energy-density liquid

fuels and projected traffic growth. SAF are essential to reduce emissions while maintaining infrastructure compatibility; however, current certified routes, led by HEFA and followed by FT-SPK, ATJ and DSHC, complement this landscape, offering advantages in feedstock flexibility, aromatic content or molecular tailoring, yet all remain constrained by cost, energy efficiency and feedstock availability.

The recent approval of HC-HEFA through ASTM's fast-track process highlights the industry's interest in expanding lipid sources, a trend that aligns with the potential of engineered microorganisms to supply tailored hydrocarbon precursors. Yeasts, bacteria, and cyanobacteria can now produce jet-relevant molecules, including alcohols, terpenes, fatty-acid-derived esters, methyl ketones and saturated hydrocarbons. These advances are enabled by metabolic engineering strategies that leverage pathways such as the Ehrlich and keto-acid routes, the MVA and MEP terpenoid pathways, FAEE transesterification modules, truncated β-oxidation and AAR/ADO or FAP-based alkane synthesis, allowing precise control over chain length and product structure.

The path forward requires broadening engineering targets beyond canonical precursor nodes and further integrating compartmentalisation, redox balancing, membrane and secretion engineering and division-of-labour strategies. Equally important is the integration of bioprocess optimisation, reactor design and downstream upgrading to ensure that pathway advances translate into commercial-scale titres, yields and productivities. Addressing these challenges will be critical for establishing microbial SAF precursors as reliable and scalable contributors to a more sustainable aviation sector.

## CRediT authorship contribution statement

**Nicolas Fink:** Conceptualization, Investigation, Writing – original draft, Visualization. **Laura de Freitas Naves:** Conceptualization, Investigation, Writing – original draft, Visualization. **Heber Gamboa-Melendez:** Supervision, Conceptualization, Writing – review & editing. **Rodrigo Ledesma-Amaro:** Supervision, Funding acquisition, Project administration, Writing – review & editing.

## Data Availability

No data were used for the research described in the article.

## Declaration of Competing Interest

We declare that we have no known financial, personal, or professional conflicts of interest that could have influenced the work, analysis, or conclusions presented in relation to COBIOT.

## Acknowledgements

R.L-A. received funding from BBSRC (BB/R01602X/1, BB/T013176/1, BB/T011408/1–19-ERACoBioTech-33 SyCoLim, BB/X01911X/1, BB/Y008510/1 – Engineering Biology Hub for Microbial Foods), EPSRC (AI-4-EB BB/W013770/1, and EEBio Programme Grant EP/Y014073/1), Yeast4Bio Cost Action 18229, European Research Council (ERC) (DEUSBIO - 949080), the Bio-based Industries Joint (PERFECOAT-101022370) under the European Union's Horizon 2020 research and innovation programme and the European Innovation Council (EIC) under grant agreement No. 101098826 (SKINDEV). Imperial College London UKRI Impact Acceleration Account (EPSRC –EP/X52556X/1, BBSRC -BB/X511055/1). Thanks to the Bezos Earth Fund through the Bezos Centre for Sustainable Protein (BCSP/IC/001). R.L-A., H.G-M., N.F., L. dF N. received funding from Yeast Aviation Fuels (YAF) Programme, which received funding from EPSCR (EP/Y031881/1) and Horizon Europe (HORIZON) Marie Skłodowska-Curie Actions Doctoral Networks (MSCA-DN) (101120389).

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