Biofuel Research Journal 40 (2023) 1974-1988

Biofuel Research Journal

Journal homepage: www.biofueljournal.com

Review Paper

Opportunities and challenges for n-alkane and n-alkene biosynthesis: A sustainable microbial biorefinery

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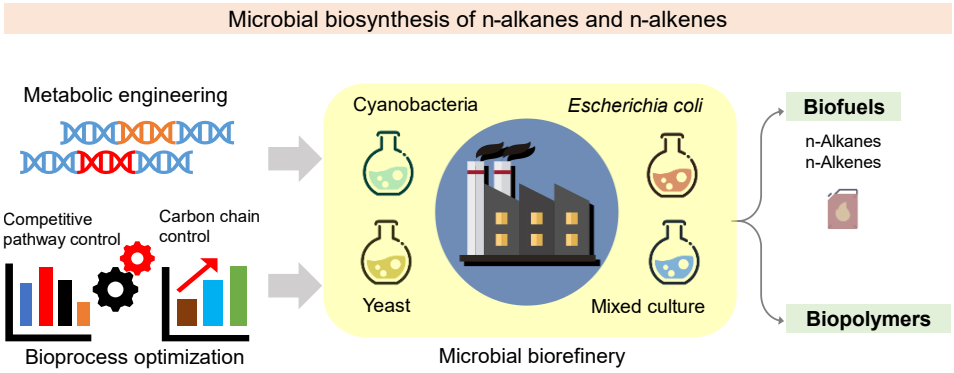
HIGHLIGHTS

- Major fatty acid biosynthetic pathways were reviewed and discussed.
- Alkanes and alkenes are valuable platform chemicals with diverse industrial applications and potential use in biofuels.
- Key enzymes and competitive pathways in microbes impact the yield of n-alkane/n-alkene biosynthesis.
- Genetic modification is crucial for large-scale n-alkane/n-alkene biosynthesis from CO_2.
- Detailed techno-economic analysis is necessary for mass production of n-alkane/n-alkene.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 July 2023
Received in revised form 26 October 2023
Accepted 2 November 2023
Published 1 December 2023

Keywords:
Microbial biosynthesis
Genetic engineering
n-alkane/n-alkene
Competing metabolic pathways
Aldehyde deoxylase
Biorefinery

ABSTRACT

Alkanes and alkenes are high-value platform chemicals that can be synthesized by microorganisms, utilizing organic residues from agri-food industries and municipalities, thereby offering an alternative opportunity in resource recovery. Current research and technological advancements for the biosynthesis of alkanes and alkenes are mainly impeded by low product titers, obstructing the bioprocess upscaling and large-scale applications. Thus, current scientific investigations aim to improve productivity by utilizing natural and engineered metabolic pathways in various microbial chassis to suppress competing metabolic pathways, coupled with bioprocess optimization. Additionally, to reduce costs, research is being conducted on utilizing inorganic carbon sources such as CO_2 to promote the green synthesis of alkanes and alkenes. Therefore, this review critically discusses the opportunities and challenges in alkane and alkene biosynthesis, aiming to examine the current technological advancements. In this review, the limitations of five major metabolic pathways for alkane and alkene biosynthesis are thoroughly discussed, highlighting their shortcomings. Additionally, various techniques, including metabolic engineering, autotrophic metabolic pathways, and new non-biosynthetic routes, are investigated as potential methods to enhance product titers. Furthermore, this review offers valuable insights into the economic and environmental aspects of alkane and alkene biosynthesis while also presenting perspectives for future research directions.

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Abbreviations

13-HPOD	13-Hydroperoxylinoleic acid	FFA	Free fatty acids
AAR	Acyl-acyl carrier protein reductase	FNR	Ferredoxin-NADP+ reductase
ACP	Acyl carrier protein	GmLox1	Soya lipoxygenase
AD	Aldehyde deformylase	HTDO	Hydrothermal deoxygenation
ADC	Aldehyde decarbonylase	KR	β-keto reductase
ADO	Aldehyde deformylating oxygenase	KS	3-β-keto-acyl synthase
AHR	Aldehyde reductases	MCCA	Medium-chain carboxylic acids
AT	Acyltransferase	MES	Microbial electrosynthesis
CAR	Carboxylic acid reductase	NADP+	Nicotinamide adenine dinucleotide phosphate
CBB cycle	Calvin-Benson-Bassham cycle	NADPH	Nicotinamide adenine dinucleotide phosphate
CER1	Aldehyde decarbonylase	OleA	Thiolytic enzyme
CvFAP	Carboxylic acid decarboxylase	OleB	α/β hydrolase
CYP4G	Cytochrome P450 aldehyde decarbonylase	OleC	AMP-dependent ligase/synthetase
DH	Dehydratase	OleD	Reductase
DOX	α-dioxygenase	OleTJE	Cytochrome P450 fatty acid decarboxylase
EF	Electro-fermentation	Ols	Olefin synthase
ER	Enoyl reductase	PKSs	Polyketide synthases
FA	Fatty acids	Pt/TiO_2	Platinum/titanium dioxide catalyst
FAP	Fatty acid photodecarboxylase	Ru/C	Ruthenium/carbon catalyst
FAR	Fatty acid reductase	SCCA	Short-chain carboxylic acids
FASI	Type I fatty acid synthase	TCA cycle	Tricarboxylic acid cycle
FASII	Type II fatty acid synthase	TE	Thioesterase
FBA	Flux balance analysis	TGL	Triacylglycerol lipase
Fd	Ferric oxide reducing proteins	UndA	Non-heme iron oxidase
fdh	Formate dehydrogenase gene	UndB	Membrane-bound fatty acid decarboxylase

1. Introduction

Alkanes and alkenes play a vital role in energy systems and are important components of fossil fuels such as gasoline, diesel, and jet fuel. In ecosystems, alkanes act as a protective layer for plant stems and leaves, preventing water loss, and alkanes have also been found to play a role in signaling and nerve protection for certain insects (Basri et al., 2020). The commercial production of alkanes consumes large amounts of non-renewable energy resources and generates large quantities of liquid and gaseous emissions, which not only increases production costs but also causes environmental pollution (Pradhan et al., 2015; Mokhtar et al., 2023). Consequently, there has been growing attention towards sustainable and environmentally friendly alkane biosynthesis methods. Initially, biofuels were produced using food materials like corn, sugarcane, wheat, and barley (**Fig. 1**). However, this approach raised concerns and controversies regarding potential food shortages (Pradhan et al., 2015; Kang and Nielsen, 2017; Pradhan et al., 2022; Wang et al., 2022).

To address the problem of alkane production from food materials, more efficient and environmentally friendly production methods are being explored. These methods primarily focus on two aspects of metabolic engineering for alkane biosynthesis using different microorganisms. The first aspect involves optimizing autotrophic metabolic pathways that can utilize CO_2 to synthesize alkanes and alkenes. Scientists have employed various strategies to enhance the autotrophic alkane biosynthesis route, such as modifying enzymes, blocking competitive pathways, or introducing

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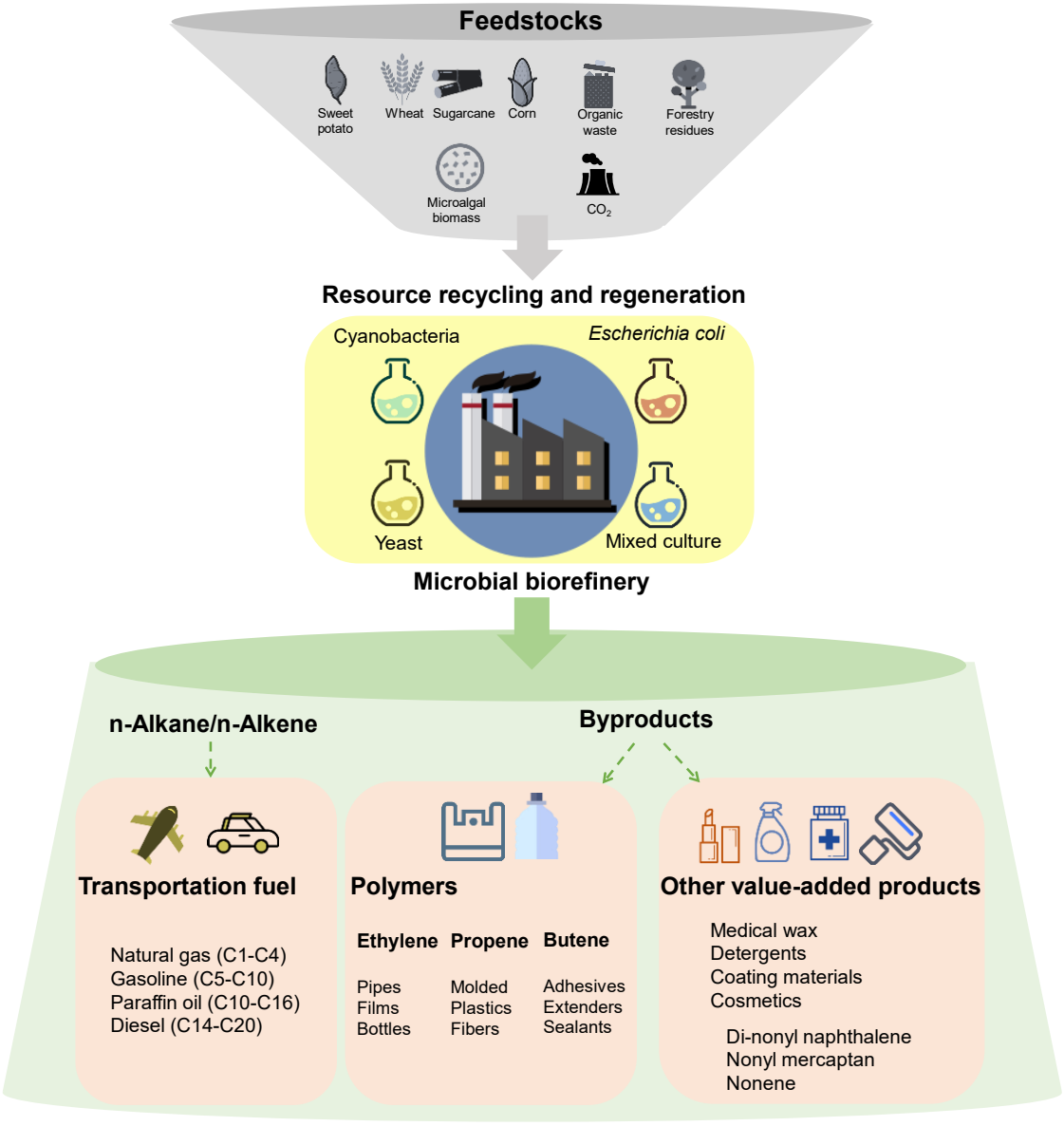

Fig. 1. Microbial alkane and alkene biosynthesis using diverse feedstocks and microbial platforms for the production of primary alkane and alkene products, along with the generation of various commercially valuable byproducts

extracellular organics (Okoro et al., 2022). The second aspect revolves around transforming heterologous microorganisms, which are incapable of synthesizing alkanes, through genetic modifications using recombinant DNA technology and promoter engineering techniques. This involves introducing the genes of key enzymes from the biosynthetic pathway into the target microorganisms to enable alkane biosynthesis (Knoot and Pakrasi, 2019).

Microbial alkane and alkene biosynthesis currently face significant bottlenecks, including low product titer, low substrate conversion rate, undesirable byproducts, and challenges in controlling the carbon length of these hydrocarbons (Wang and Zhu, 2018). Despite the innate ability of many plants, insects, bacteria, and fungi to biosynthesize alkanes, their natural production yields are often insufficient for scale-up applications. However, recent research studies have focused on applying metabolic engineering techniques to enhance product titer, improve substrate utilization rate, and achieve better control over the appropriate carbon chain length (Li et al., 2020; Lin et al., 2021; Liu et al., 2023). The progress in metabolic engineering over the past years holds promise for future commercial applications of microbial hydrocarbon biosynthesis.

The comparative analysis in **Table 1**, which presents key aspects covered in this review and recent publications (2020-2023) on microbial biosynthesis of alkanes and alkenes, is complemented by a bibliometric analysis of the biofuel biosynthesis research domain. This bibliometric analysis utilized co-occurrence network analysis and burst word detection analysis from the Web of Science Core Collection database, revealing dominant keywords such as alkane, biofuel, green synthesis, metabolic engineering, gene expression, *Escherichia coli*, and *Yarrowia lipolytica* over the last decade (2012-2023) (**Fig. 2**). Therefore, this comprehensive review further explores the opportunities and challenges in alkane and alkene biosynthesis, addresses limitations of metabolic pathways, investigates techniques for improving product titers, and provides valuable insights into economic and environmental considerations. By examining these aspects, this review aims to guide future research directions for achieving sustainable and efficient production of these valuable chemicals.

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Table 1.
Comparative analysis of key topics covered in this review and recent publications (2020-2023) on microbial biosynthesis of alkanes and alkenes.

Principles of Biosynthesis Pathways	Alkane and Alkene	Bio-Alkanes from CO_2	Economic and Environmental Analysis	Comparing *E. coli* and Yeast Chassis	Fatty Acids or Derivatives	Functions of the Key Enzymes	Biomass Pretreatment	Culture Condition Analysis	Ref.
×*	×	×	×	✓	×	✓	×	×	Fenibo et al. (2023)
✓	×	×	×	×	×	✓	✓	×	Bhushan et al. (2023)
✓	✓	×	×	✓	×	✓	×	×	Okoro et al. (2022)
✓	✓	×	×	✓	✓	✓	×	✓	Lu et al. (2022)
✓	✓	×	×	×	✓	✓	×	×	Monteiro et al. (2022)
×	✓	✓	×	×	×	✓	×	×	Lin et al. (2021)
×	✓	✓	×	×	×	✓	×	×	Panich et al. (2021)
×	✓	×	✓	×	×	×	×	✓	Abbasi et al. (2021)
✓	✓	×	✓	×	×	×	×	✓	Aamer Mehmood et al. (2021)
✓	✓	×	×	×	✓	✓	×	✓	Liu and Li (2020)
✓	✓	×	✓	×	×	✓	×	✓	Jaroensuk et al. (2020)
✓	✓	✓	✓	✓	✓	✓	×	✓	**This review**

*The"✓" symbol indicates that a particular topic is covered in a publication, while "×" indicates its absence.

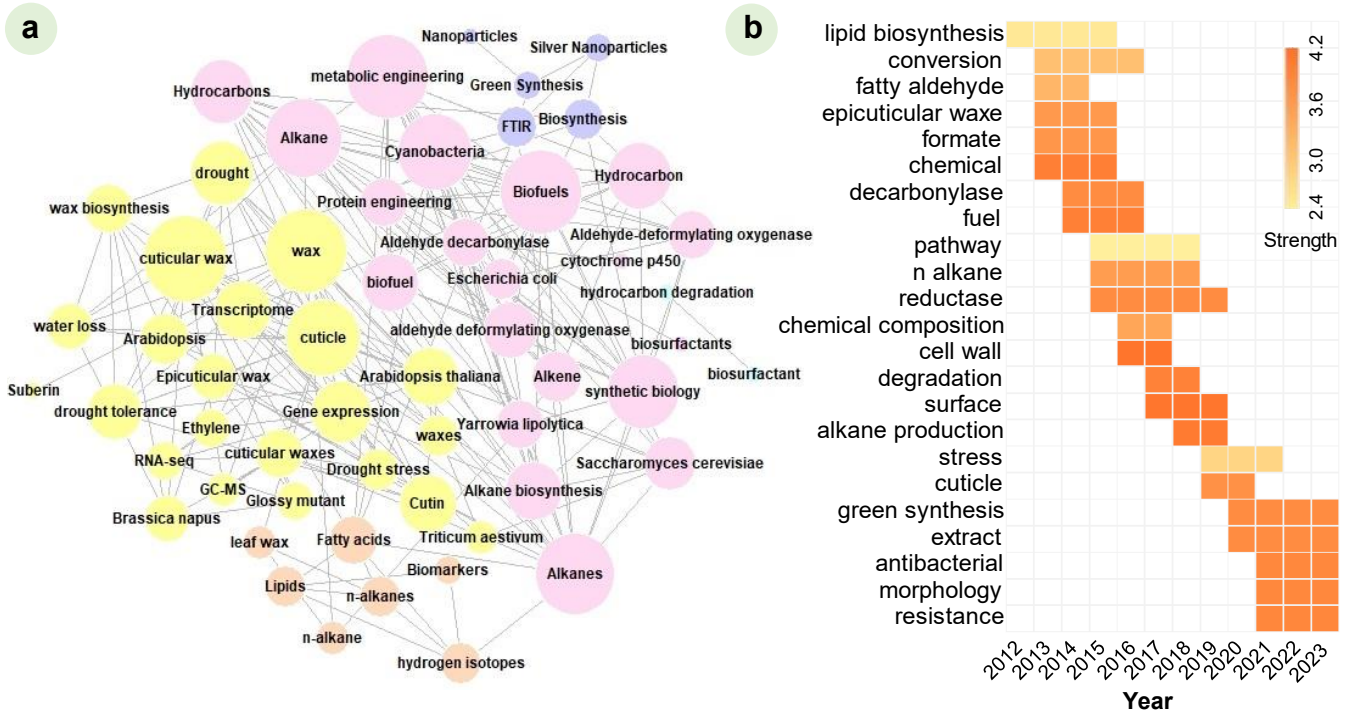

Fig. 2. Keyword analysis of research conducted based on the Web of Science Core Collection database from January 2012 to April 2023: **(a)** Keyword co-occurrence network analysis using BibExcel and Pajek, and **(b)** Burst keyword analysis using CiteSpace (Chen et al., 2010). The color saturation in the figure represents the burst strength of each keyword.

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2. Alkanes and alkenes biosynthesis – metabolic routes and challenges

Alkanes and alkenes are primarily synthesized through the fatty acid biosynthesis pathway in specific microorganisms. Enhancing these microbial hydrocarbon production titers and substrate utilization rates relies on a comprehensive understanding of the biosynthesis pathways. Precise modification of the biosynthetic pathway and key enzymes through metabolic engineering plays a crucial role in developing efficient microbial hydrocarbon biosynthesis systems. This section provides a detailed discussion of the five major fatty acid biosynthetic pathways (**Fig. 3**) and their implications for alkane and alkene biosynthesis.

2.1. Decarbonylation of fatty aldehydes pathway

The most common biosynthesis pathway for alkanes involves the conversion of long-chain fatty acids (FAs) to C_{n-1} alkanes through the action of decarbonylating enzymes, namely ADO/CYP4G/CER1. In this pathway, fatty aldehydes are processed by these enzymes, leading to the production of C_{n-1} alkanes and formate as a byproduct (Basri et al., 2020). Aldehyde deformylating oxygenase (ADO), initially discovered in cyanobacteria, is a metal-dependent enzyme with an alpha helix structure containing a diiron active site. It catalyzes the transformation of aldehydes, lacking a carbonyl group, into C_{n-1} alkanes (Basri et al., 2020). Aldehyde decarbonylases (ADCs), also known as cyanobacterial ADs or ADCs, have been identified in cyanobacteria, plants, and insects. These enzymes enable the conversion of fatty aldehydes into alkanes, with byproducts being carbon monoxide in plants, carbon dioxide in insects, and alkanes in different microorganisms (Feyereisen, 2020). Isotope tracer experiments have revealed the involvement of molecular O_2 in the formation of metal-bound peroxide nucleophiles, which attack aldehydes during the conversion process, with an oxygen atom from O_2 also becoming bound to formate (Li et al., 2012). The oxygenation of the reaction led to the renaming of the ADC enzyme as ADO, further confirming that the byproduct in cyanobacteria is formate rather than carbon monoxide.

There are two pathways for alkane production using the ADO/CYP4G/CER1 catalysis route (**Fig. 3a**). The first pathway involves the conversion of fatty acyl-ACP to alkanes after the formation of fatty aldehydes by Acyl-ACP reductase (AAR). The second pathway utilizes fatty acids as substrates, which are catalyzed by FAR/CAR/DOX enzymes, leading to the formation of fatty aldehydes that are subsequently converted to alkanes. During the catalysis process, ADO requires four electrons from an external reduction mechanism involving ferric oxide-reducing proteins Fd, $NADP^+$, NADPH, and FNR (Li et al., 2012; Basri et al., 2020). The fatty aldehydes produced by the two pathways are ultimately decarbonylated by the ADO/CYP4G/CER1 enzymes, resulting in alkane production. However, the poor catalytic activity of ADO often leads to the accumulation of intermediates and increased competition for endogenous substrates between aldehyde reductase and alcohol dehydrogenase during alkane production. This inefficiency hampers alkane production and leads to low titers (Lehtinen et al., 2018). Consequently, the enzymatic activity of ADO is considered a bottleneck for microbial alkane biosynthesis and serves as a focal point of the metabolic engineering technique.

Furthermore, CYP4G decarbonylase, a cytochrome P450 found in insects such as Drosophila, primarily utilizes fatty aldehydes with carbon chain lengths ranging from C_{24} to C_{28} as substrates, resulting in the production of alkanes with carbon chain lengths ranging from C_{23} to C_{27},

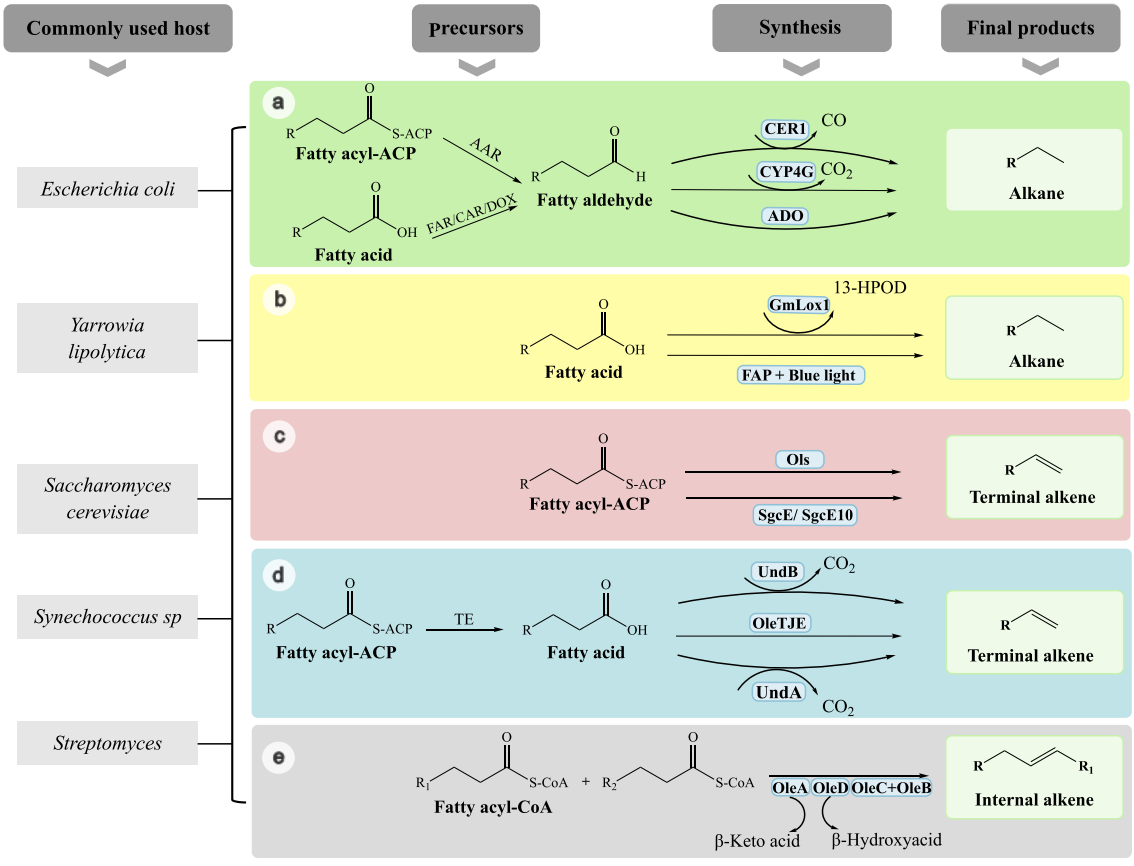

Fig. 3. The five different biosynthesis pathways **(a - e)** for producing alkanes or alkenes using different microbial chassis. TE: thioesterase; AAR: acyl–acyl carrier protein reductase; FAR: fatty acid reductase; CAR: carboxylic acid reductase; DOX: α-dioxygenase; CER1: aldehyde decarbonylase; CYP4G: cytochrome P450 aldehyde decarbonylase; ADO: Aldehyde deformylating oxygenases; GmLox1: soya lipoxygenase; FAP: fatty acid photodecarboxylase; Ols: olefin synthase; SgcE and SgcE10: a kind of thioesterase; UndA: non-heme iron oxidase; UndB: membrane-bound fatty acid decarboxylase; OleTJE: cytochrome P450 fatty acid decarboxylase; OleA: thiolytic enzyme; OleB: α/β hydrolase; OleC: AMP-dependent ligase/synthetase; OleD: reductase. These pathway diagrams are modified based on Kang and Nielsen (2017) and Liu and Li (2020).

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with the generation of carbon dioxide as a byproduct (Qiu et al., 2012). On the other hand, CER1, a membrane-bound diiron-containing aldehyde decarbonylase located in the plant endoplasmic reticulum, exhibits substrate preferences for fatty aldehydes mainly in the C_8 to C_{16} and C_{28} to C_{34} carbon chain length ranges and produces alkanes with carbon chain lengths ranging from C_7 to C_{15} and C_{27} to C_{33}, accompanied by the formation of carbon monoxide as a byproduct (Bernard et al., 2012). The distinct byproducts generated by CYP4G and CER1 suggest the presence of different catalytic mechanisms of action for these enzymes (Liu and Li, 2020).

Because ADO, CYP4G, and CER1 all exhibited significant catalytic activity towards aliphatic aldehydes, researchers compared the catalytic performance of these three enzymes in *Saccharomyces cerevisiae* (Kang et al., 2017). The results revealed that only ADO demonstrated the ability to produce medium-chain alkanes consistently across different hosts, leading many researchers to prefer using ADO as the key enzyme for alkane biosynthesis over CYP4G and CER1 (Kang et al., 2017; Shakeel et al., 2018). ADO enzymes obtained from various cyanobacteria strains differ significantly in their catalytic capacities, making it advantageous to reconstitute ADOs from different sources to optimize alkane production systems in terms of competitive inhibition, substrate selection, and yield (Kang et al., 2017).

For instance, the fusion protein ADO-catalase, known as CAT-ADO, combines the inhibitory byproduct hydrogen peroxide with its N- and C-termini, reducing substrate inhibition by converting hydrogen peroxide into the co-substrate O_2 (Andre et al., 2013); Mutations in specific sites within ADO can alter its affinity for substrates of different carbon chain lengths. Additionally, the fusion protein ADO-AAR enhances alkane yield in *E. coli* by 4.8-fold while modifying the spatial organization of ADO:AAR, particularly with a binding site ratio of 3:1, results in an 8.8-fold increase in alkane yield (Rahmana et al., 2014).

2.2. Decarboxylation of fatty acids pathway

Important industrial chemical compounds such as detergents, lubricants, and disinfectants can be derived from alkenes, also known as olefins. Within the chemical industry, 1-olefins are recognized as promising hydrocarbon biofuels (Xu et al., 2022). The production process involves the utilization of fatty acyl-ACP as a substrate, which is converted into olefins through the action of thioesterase (TE) enzymes. Subsequently, these olefins can be further transformed into fatty acids (**Fig. 3d**) (Xu et al., 2022). There are three distinct enzymatic processes that facilitate the conversion of fatty acids into olefins: OLeTJE, UndA, and UndB (Wang and Lu, 2013). OLeTJE, a newly identified member of the cytochrome P450 peroxidases within the CYP152 family, was named after its discovery in the genome sequence of *Jeotgalicoccus* sp. (Wang and Lu, 2013). OLeTJE utilizes H_2O_2 as the primary electron source to decarboxylate long-chain fatty acids (C_{16}-C_{20}) and generate α-olefins, with carbon dioxide produced as a byproduct (Rude et al., 2011).

The decarboxylases UndA and UndB are found in *Pseudomonas* sp. UndA is a non-heme iron oxidase that is ubiquitous in *Pseudomonas* sp. (Manley et al., 2019). The substrate range of the UndA enzyme is limited, as it can only utilize the C_{n-1} principle to produce terminal olefins from medium-chain fatty acids (C_{10}-C_{14}) (Lee et al., 2018). On the other hand, UndB, a membrane-bound fatty acid decarboxylase, is present in only a few *Pseudomonades*. Rui et al. discovered that UndB has a broader range of substrates (C_6-C_{16}) compared to UndA, but similar to UndA, it predominantly converts fatty acids within the C_{10}-C_{14} range (Rui et al., 2015). Both UndA and UndB produce olefins and carbon dioxide as byproducts (Lee et al., 2018). To date, OLeTJE, UndA, and UndB have been used to generate olefins in *E. coli* and *S. cerevisiae*. Among them, UndB has exhibited the highest yield, particularly for 1-olefins, which is three times higher than that of OLeTJE and UndA. This can be attributed to the unique catalytic function of UndB, which facilitates the export of the majority of the olefins it produces into the extracellular media (Liu and Li, 2020). Therefore, UndB shows greater potential for biosynthesizing 1-olefins compared to UndA and OLeTJE.

The production of 1-olefins from free fatty acids (FFA) requires only one step and does not produce intermediate fatty aldehydes. In contrast, the biosynthesis of alkanes involves two steps: the production of fatty aldehydes from FFA, followed by the conversion of aldehydes into alkanes. Yang et al. synthesized 39.6 mg/L of aliphatic olefins and 26.6 mg/L of aliphatic alkanes using the *Y. lipolytica* cytoplasm-designed lipase-based substrate production pathways TGL-OleTJE and TGL-CAR-ADC (Yang et al., 2019). Yang and co-workers demonstrated that the titer of olefins obtained through the single-step biosynthetic pathway exceeded the titer of the two-step pathway required for alkane production, even with equivalent strains and substrates. This indicates the superiority of the 1-olefin biosynthetic pathway compared to alkane biosynthesis. However, OleTJE has a narrow range of substrates and is highly specific for long-chain and linearly saturated FFAs, allowing for the selective production of long-chain α-olefins (Reinhard et al., 2020).

If OleTJE-produced hydrocarbons are intended for use as embedded hydrocarbon biofuels, they would need to be further processed into smaller chain hydrocarbons such as biogasoline (C_5-C_{10}) and green diesel (C_{10}-C_{25}). Some initiatives and modest successes have been made to expand OleTJE's substrate range, but further exploration in enzyme engineering is still required to enhance substrate options and increase conversion rates (Hsieh and Makris, 2016). Additionally, since alkane is the primary ingredient of biogasoline, bio-jet fuel, and green diesel, the saturation of α-olefins with alkanes may be necessary to produce standard biofuels.

2.2.1. Fatty acid photodecarboxylation pathway

Fatty acid photodecarboxylation is an alternative method for producing alkanes through fatty acid decarboxylation (**Fig. 3b**). This process primarily utilizes fatty acid photodecarboxylase (FAP) derived from the microalga *Chlorella variabilis* NC64A is mostly used in this process. FAP belongs to the glucose-methanol-choline oxidoreductase family, which is specific to algae, and it contains a light-capturing flavin adenine dinucleotide (FAD) cofactor. Under blue light, FAP catalyzes the decarboxylation of free fatty acids with carbon chain lengths ranging from C_{12} to C_{22}, resulting in the production of C_{n-1} alkanes or 1-olefins and notably, FAP exhibits a preference for C_{16} to C_{17} substrates (Sorigué et al., 2017). The decarboxylation process of FAP is triggered by the excitation of FAD with electrons obtained from free fatty acid substrates, utilizing quantum yield light with an efficiency greater than 80% and additionally, the process necessitates a continuous supply of blue photons (Sorigué et al., 2017). The requirement for a continuous input of blue photons also explains the naming of FAP as photodecarboxylase.

Subsequently, FAP can irreversibly decarboxylate triglycerides and fatty acids into alkanes using quantitative blue-light illumination, with a promising turnover number of up to 8000, indicating the potential of this biosynthesis pathway (Huijbers et al., 2018). However, the enzyme's activity was found to be significantly reduced in the presence of short-chain carboxylic acids, potentially due to FAP's preference for long-chain fatty acids, leading to less stability of shorter substrates within the long and narrow substrate channel of the decarboxylase (Sorigué et al., 2017). Based on this speculation, researchers explored a decoy method to enhance the production of short-chain alkanes. They utilized short-chain alkanes as co-catalysts to occupy the long-chain alkane substrate channels of FAP, thereby accelerating the FAP-catalyzed decarboxylation of short-chain carboxylic acids and promoting alkane production (Zhang et al., 2019). The results of this study not only demonstrate the practical application of a unique photoactivatable enzyme but also establish the foundation for the selective production of short-chain alkanes from waste carboxylic acids under mild reaction conditions.

2.3. Head-to-head hydrocarbon biosynthesis pathway

Fatty acids serve as substrates for the production of olefins through a head-to-head hydrocarbon biosynthesis process (**Fig. 3e**). This process involves the catalysis of acyl-CoA formation by TE enzymes and the subsequent non-decarboxylative thiolysis of two different acyl-CoAs by OleABCD enzymes to produce long-chain olefins (Song et al., 2016). The Ole genes, which can be expressed cooperatively or separately in the genome, consist of OleA, designated as a thiolytic enzyme; OleB, an α/β hydrolase; OleC, an AMP-dependent ligase/synthetase, and OleD, a reductase (Liu and Li, 2020). The entire head-to-head pathway, initiated by OleA, involves the non-decarboxylated Claisen condensation of two long-chain acyl-CoAs, resulting in the production of β-keto acids and the release of two CoAs (Liu and Li, 2020). The β-keto acids are then reduced to β-hydroxy acids by NADPH-dependent OleD, and finally, OleC utilizes ATP to transform β-hydroxy acids into olefins (Sukovich et al., 2010). The

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function and mechanism of OleB in the biosynthesis of olefins are still unknown. However, a copy of the OleA, OleB/OleC fusion, and OleD genes have been identified within the Ole gene family (MB46 09735- MB46 09745) in the A3 genome (Sun et al., 2016).

It is hypothesized that OleB may be associated with the expression activity of OleC and could potentially serve as a scaffolding or regulatory component of the Ole complex (Wackett and Wilmot, 2015). A recent study has identified an unprecedented β-lactone decarboxylation reaction carried out by OleB in the Gram-negative bacterium *Xanthomonas campestris*, which completes the biosynthesis of cis-olefins (Christenson et al., 2017b). However, the exact mode of action of OleB is still not fully understood and requires further investigation. The three-part head-to-head pathway converts two acyl-CoAs (C_{10-15} + C_{10-15}) into cis-olefins ranging from C_{19} to C_{29} (Christenson et al., 2017a). The structure of the final product is greatly influenced by OleA, the critical enzyme in the catalytic olefin biosynthesis process. Therefore, researchers have primarily focused on studying OleA to gain a better understanding of and manipulate the head-to-head biosynthesis pathway.

2.4. Lipoxygenase pathway

Several of the hydrocarbon biosynthesis processes mentioned above produce hydrocarbons without breaking the fatty acid carbon chain, which is more suitable for producing long-chain alkanes (carbon number >14) rather than short-chain olefins and alkanes. In contrast to the decarbonylase and decarboxylases mentioned earlier, soya lipoxygenase, GmLox1, exhibits a distinct catalytic activity (**Fig. 3b**) by converting linoleic acid ($C_{18:2}$) into pentane and 12-tridecanoic acid byproducts, resulting in the production of short-chain alkanes (Wang and Zhu, 2018). The biosynthesis of GmLox1 involves the catalysis of O_2 insertion into the linoleic acid ($C_{18:2}$) cis double bond, generating 13-hydroperoxylinoleic acid (13-HPOD) (Blazeck et al., 2013). Subsequently, 13-HPOD is converted to pentane and 13-oxo-cis-9-trans-11-tridecadienoic acid byproducts through homogenization-B-split.

Heterologous overexpression of GmLox1 in the oleaginous yeast *Y. lipolytica*, along with culture medium optimization and metabolic engineering, resulted in a pentane titer of 5 mg/L. However, the production of pentane was significantly hindered by the formation of tridecadienoic acid byproducts and the limited substrate range of GmLox1 (Blazeck et al., 2013). Although linoleic acid is abundant in nature, and the unique internal double bond breakage to produce pentane has potential, the presence of numerous accompanying byproducts increases waste disposal costs, which is environmentally unfriendly and may impede further commercial development. Therefore, the commercial potential of this approach relies on the ability to recycle the byproducts in large quantities.

2.5. Polyketide biosynthesis pathway

Both the fatty acid biosynthesis process and the polyketide biosynthesis system share similar mechanisms and are considered promising for biofuel production. Fatty acid synthases and polyketide synthases (PKSs) commonly utilize acyl-ACPs as substrates. The PKS consists of several components, including thioesterase (TE), acyltransferase (AT), acyl carrier protein (ACP), dehydratase (DH), enoyl reductase (ER), β-keto reductase (KR), and 3-β-keto-acyl synthase (KS) (Peirú et al., 2009). The biosynthesis process begins with acyl substrates and extends the carbon chain length using KS, AT, and ACP in conjunction with malonyl-CoA. The β-keto group is then reduced to a β-hydroxyl group by KR, DH, and ER, following carbon chain extension. Finally, the decarboxylation and dehydration reactions catalyzed by TE yield the olefin products (Kang and Nielsen, 2017).

In 2011, it was found that *Synechococcus* sp. PCC 7002 can produce two C_{19} olefins with terminal double bonds. To identify the enzymes involved in this process, the alkane biosynthesis pathway in PCC 7002 was examined for homologs using the BLAST search tool (Mendez-Perez et al., 2011). The search revealed that the last module encoded by CurM from the marine cyanobacterium *Lyngbya majuscule* plays a role in forming the terminal double bond. Based on sequence comparison, this enzyme was named olefin synthase (Ol) and shared 45% amino acid homology with CurM (Gu et al., 2009). Deletion mutants of Ol and strains overexpressing the *Ol* gene demonstrated the involvement of *Ol* in long-chain olefin biosynthesis. *Ol* deletion mutants were unable to produce olefins, while Ol overexpression resulted in an olefin yield of 4.2 mg/L (Mendez-Perez et al., 2011).

Another study discovered that PKS SgcE from Streptomyces and its homolog TE SgcE10 are essential for producing the central component of 1,3,5,7,9,11,13-pentadecaheptaene (PDH, 129.3 mg/L) and the enediyne antibiotic C-1027 (37.5 mg/L), which is present in numerous naturally occurring anticancer compounds (Chen et al., 2011). Heterologous expression of SgcE/SgcE10 led to a significant accumulation of the intermediate pentadecaheptaene (Zhang et al., 2008). In *E. coli*, PDH was produced by regulating the expression of SgcE/SgcE10, resulting in a yield of 140 mg/L, which could be chemically hydrogenated to generate pentadecane (Liu et al., 2015). The diverse nature of PKS and the controlled expression of these modular polymerases hold promise for efficient hydrocarbon production. Notably, TE enzymes can play a dominant role in decarboxylation functions, creating trimethyl double bonds that can be integrated into specially designed biocatalytic systems to generate suitable β-hydroxylated acyl–ACP precursors (Liu and Li, 2020).

3. Opportunities for advancing alkane and alkene synthesis

3.1. Metabolic engineering techniques

There is a wide variety of microorganisms in nature, offering numerous potential candidates as microbial platforms for hydrocarbon production (Pradhan et al., 2022; Selvasembian et al., 2022; Cronjé et al., 2023). From a theoretical standpoint, it is possible for microorganisms with efficient fatty acid metabolic pathways to serve as microbial platforms for alkane production, even if they do not naturally produce hydrocarbons. Among the microbial platforms, *E. coli* is currently the most popular choice for microbial metabolic engineering, followed by yeast (Wang and Zhu, 2018). In *E. coli*, one approach for microbial alkane production is to introduce genes encoding fatty acid biosynthesis pathways, such as type I fatty acid synthase (FASI) (Schweizer and Hofmann, 2004)) and/or type II fatty acid synthesis (FASII), into the bacterium (White et al., 2005). Type I FAS consists of large multifunctional enzymes that catalyze all reactions of fatty acid biosynthesis, while type II FAS employs separate enzymes for each reaction. Through this approach, the production of fatty acids can be increased, which can then be converted into alkanes using fatty acid decarboxylation pathways.

Another strategy involves the co-expression of multiple enzymes involved in alkane biosynthesis within *E. coli* (**Table 2**). This approach addresses existing limitations in the metabolic pathways of *E. coli*, such as low levels of alkane biosynthesis, and aims to enhance both the yield and efficiency of alkane production. In a notable study, a metabolic model for hydrocarbon production in *E. coli* was developed, combining recombinant DNA technology and the co-expression of multiple enzymes. The model was optimized using flux balance analysis (FBA) methods, resulting in a remarkable 36-fold improvement in overall alkane output (Fatma et al., 2018). This pioneering study applied a model-driven approach for hydrocarbon production, achieving the highest long-chain hydrocarbon titers in *E. coli*. In addition to genetic modifications, process optimizations, including the use of appropriate growth media and cultivation conditions, play a crucial role in improving alkane yield in *E. coli*.

While *E. coli* is commonly used as a host for alkane production, yeast may be more suitable for industrial-scale alkane production due to several advantages. Yeast exhibits higher stability in response to pH changes (Cao et al., 2016), possesses higher natural fatty acid fluxes, operates at lower optimal temperatures, can utilize inexpensive renewable substrates like sugars and oil wastes as carbon sources, and has resistance to phage infection (Chen et al., 2018). Two prominent yeast species in this context are *S. cerevisiae* and *Y. lipolytica* (**Table 3**). *S. cerevisiae* has been employed as a host for industrial biofuel production, including ethanol and isobutanol (Buijs et al., 2013). *Y. lipolytica*, on the other hand, has found application in the industrial production of fatty acid-derived chemicals thanks to its robust tricarboxylic acid (TCA) cycle, acetyl-CoA flux, and extensive lipid storage capacity (Wang et al., 2020).

Through meticulous metabolic engineering and fermentation optimization, researchers achieved a remarkable lipid titer of 99 g/L for direct hydrocarbon production using glucose as the carbon source in *Y. lipolytica* (Yang et al., 2019). Notably, *Y. lipolytica*'s oxidized pentose phosphate pathway supplies substantial amounts of NADPH, a crucial cofactor for fatty acid biosynthesis and hydrocarbon formation (Wasylenko

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Table 2.
Enzymatic profiles, primary products, and major byproducts in n-alkane and n-alkene biosynthesis using *Escherichia coli* as a chassis strain.

Host Microbial Chassis	Enzymes	Primary Products	Major Byproducts	n-Alkane and n-Alkene Titers	Ref.
Escherichia coli	CvFAP	alkane (C_{11}, C_{15}, C_{17})	-	Conversion rate of 13%, 88%, and 95%, respectively	Liu et al. (2023)
	Tes, Car, Adse	n-butane	-	6.74 mg/L	Liu et al. (2022)
	BudA, BudB, BudC (BDO dehydrogenase)	2,3-butanediol	Lactate, formate, acetate, and ethanol	14.2 g/L	Sathesh-Prabu et al. (2020)
	XaFDH, PrADO (MIT9313), AcACR1 (Fatty acyl-CoA reductase)	alkane (C_{13})	Fatty alcohols	Conversion yield of 50%	Jaroensuk et al. (2019)
	OleTMC, TesA	alkene (C_{11}, C_{13}, C_{15}, C_{17})	-	17.78 ± 0.63 mg/L	Lee et al. (2018)
	NoADO, SyADR	alkanes (C_{16})	Fatty alcohols	2.54 g/L	Fatma et al. (2018)
	UcTE	alkane (C_9, C_{11}, C_{13})	-	0.00062, 0.0052, 0.249 mg/g, respectively	Wang et al. (2017)
	NpADO, PmADO, SyADO	alkane (C_7, C_5, C_3)	-	11-143, 12-77, 7.2-23 μmol/L, respectively	Patrikainen et al. (2017)
	SeAAR, SeADO, fadR	alkene (C_{17})	-	255.6 mg/L	Song et al. (2016)
	SyADO, SyADR	alkanes (C_{15}, C_{16}, C_{17})	Fatty alcohols	1.31 g/L	Cao et al. (2016)
	PrAD, CarNi	alkanes (C_3 to C_9)	-	1.6 ± 0.3 mg/L	Sheppard et al. (2016)
	NoADC, NoACR, ucFatB	alkanes (C_{11}, C_{13}, C_{15})	Lauric acid	2.21 ± 0.18, 1.83 ± 0.12, 4.01 ± 0.43 mg/g, respectively	Yan et al. (2016)

Table 3.
Enzymatic profiles, primary products, and major byproducts in n-alkane and n-alkene biosynthesis using *Saccharomyces cerevisiae* and *Yarrowia lipolytica* as microbial chassis.

Host Microbial Chassis	Enzymes	Primary Products	Major Byproducts	n-Alkane and n-Alkene titers	Ref.
Saccharomyces cerevisiae	maFACR, SeADO	alkanes and alkenes (C_{13}, C_{15}, C_{17})	Fatty alcohols	1.54 mg/L	Foo et al. (2020)
	UndB	alkenes (C_{13}, C_{15}, C_{17})	-	35.3 mg/L	Zhou et al. (2018)
	UndA	alkenes (C_7, C_9, C_{11})	-	3.35 mg/L	Zhu et al. (2017)
	CwADO	alkane (C_8 to C_{14})	Fatty alcohols	0.13 mg/L	Kang et al. (2017)
	TeADO	alkane (C_7, C_9, C_{11}, C_{13})	-	30-36 μg/L	Zhu et al. (2017)
	NpADO	alkane (C_{14}, C_{16}, C_{18})	Formate	0.8 mg/L	Zhou et al. (2016)
	OleTJE	alkenes (C_{11}, C_{13}, C_{15}, C_{17}, C_{19})	-	3.7 mg/L	Chen et al. (2015)
Yarrowia lipolytica	CvFAP	alkanes and alkenes	-	1.47 g/L (highest reported so far in yeast)	Li et al. (2020)
	GmLox1	pentane (C_5)	13-oxo-cis-9-trans-11-tridecadienoic acid	4.98 mg/L	Li et al. (2020)
	MmCar-PmADO	alkanes (C_{15}, C_{17})	-	372.4 mg/L	Yang et al. (2019)
	OleTJE	alkenes (C_{15}, C_{17})	-	554.4 mg/L	Yang et al. (2019)
	CvFAP	alkanes and alkenes (C_{15}, C_{17})	-	58.69 mg/L	Bruder et al. (2019)
	MmCar-PmADO	alkanes and alkenes	-	81% alkanes from TAGs (triacylglycerides); 71% alkanes from microalgal oil	Yang et al. (2018)
	AbACR1-PmADO	alkane (C_{15}, C_{17})	-	16.8 mg/L	Xu et al. (2016)

et al., 2015). Building on these advantages, Li et al. achieved hydrocarbon titers of up to 1.47 g/L in an engineered *Y. lipolytica* strain in 2020 (Li et al., 2020), surpassing the highest hydrocarbon titer of 1.31 g/L obtained in *E. coli* (Cao et al., 2016). Recent experiments on microbial alkane production using both *E. coli* (**Table 2**) and yeast (**Table 3**) have demonstrated promising results, with improved yields and efficiencies compared to traditional methods. However, there is still ample scope for further improvement, and ongoing research focuses on developing more efficient and scalable approaches for microbial alkane production in both *E. coli* and yeast.

3.2. Mitigating competition pathways in alkane production

The presence of competitive pathways significantly limits the yield of alkane biosynthesis in microorganisms. One such competitive pathway occurs at the initial step of alkane biosynthesis, where the carbon source is primarily converted into fatty aldehydes, fatty acids, acyl-CoA, and acyl-ACP (**Fig. 4a**). Fatty aldehydes can be reduced to fatty alcohols by natural aldehyde reductases (AHR) in *E. coli* (Rodriguez and Atsumi, 2014), resulting in the generation of a large number of aliphatic alcohol byproducts

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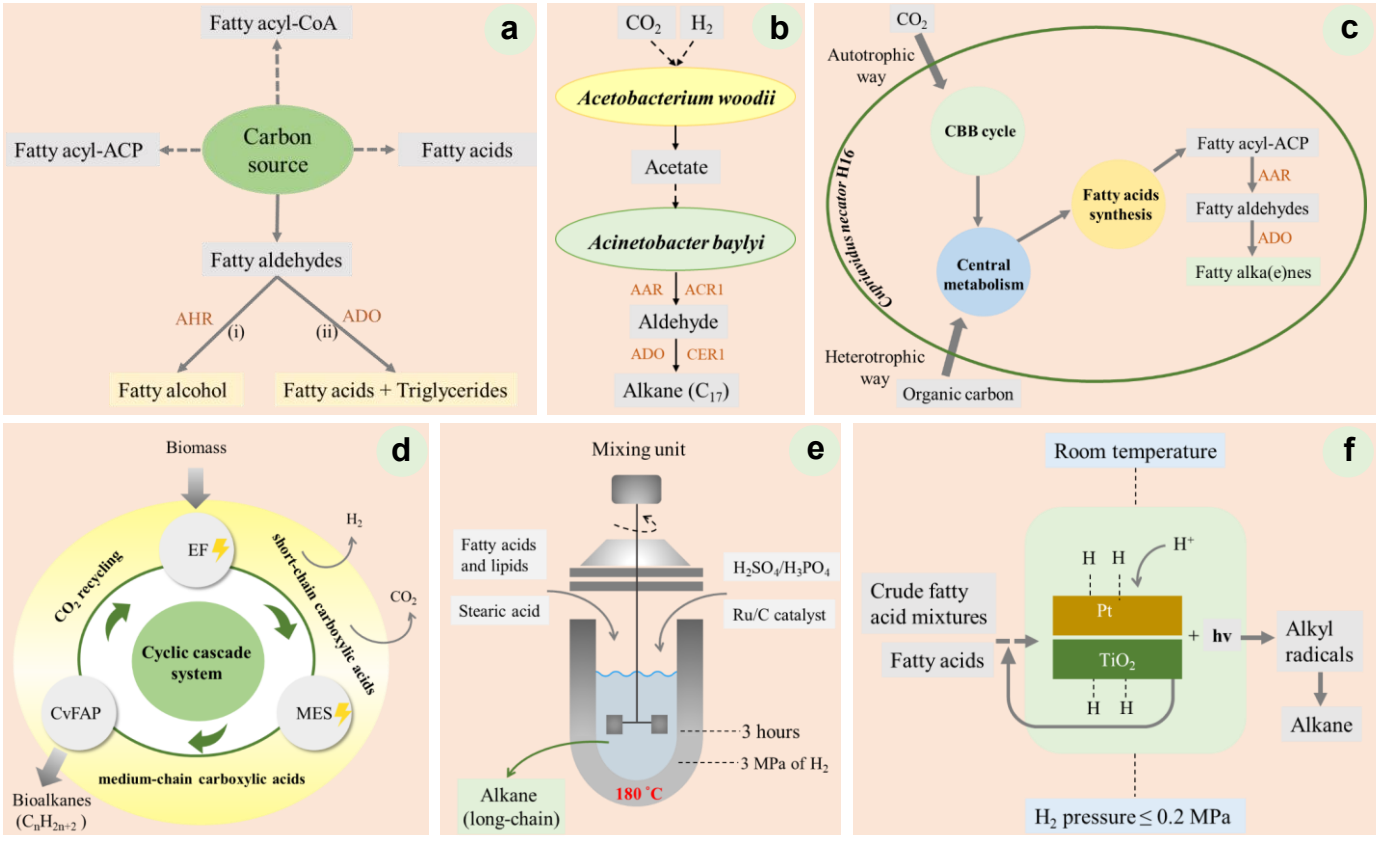

Fig. 4. Different strategies and new ways to improve the synthesis of alkane/alkene. **(a)** Two competitive pathways (i and ii) occur during alkane biosynthesis. **(b)** Alkane production pathway module with upstream acetic acid bacteria *Acetobacterium woodii* to produce acetate from CO_2 and H_2 and then converted to alkane by *Acinetobacter baylyi*. **(c)** Two genes (encoding acyl-ACP reductase AAR and aldehyde-deformylating oxygenase ADO) from *Synechococcus elongatus* are overexpressed in *Cupriavidus necator* to biosynthesize alkane/alkene by autotrophic (Calvin-Benson-Bassham (CBB) cycle or heterotrophic (i.e., via central metabolism) means (Panich et al., 2021). **(d)** Electro-fermentation (EF) and microbial electrosynthesis (MES) technology to produce alkanes. EF can convert biomass to short-chain carboxylic acids (SCCA), which are subsequently transformed to medium-chain carboxylic acids (MCCA) by MES. Finally, carboxylic acids are successfully converted to C_nH_{2n+2} alkanes by photoactivated decarboxylase (CvFAP) (Lin et al., 2021). **(e)** Hydrothermal deoxygenation method for the addition of common inorganic acids (H_2SO_4, H_3PO_4) using inexpensive raw materials (fatty acids and lipids), stearic acid, and commercial Ru/C catalysts under mild hydrothermal conditions (180 °C) to produce long-chain alkanes (Cai et al., 2022). **(f)** Photocatalytic decarboxylation pathway to produce alkane. The fatty acids or industrial fatty acids mixture is oxidized to alkyl radicals by adding Pt/TiO_2 catalysts combined with photogenerated holes generated by light at room temperature and H_2 pressure ≤ 0.2 MPa (Zhou and Zhang, 2020).

that can even surpass the yield of alkanes (Zhou et al., 2016). Another competitive pathway occurs during the dehydrogenation of fatty aldehydes, where fatty aldehyde dehydrogenase converts them into specific fatty acids. Co-expression of AAR and ADO in *Aspergillus niger* has shown an increase in internal fatty acids, triglycerides, and alkane production (Zhang et al., 2017).

Initially, scientists aimed to enhance alkane production by blocking or eliminating the competing pathways and making appropriate metabolic engineering modifications to boost the accumulation of fatty aldehyde precursors. However, it was discovered that complete elimination of AHR and a significant reduction in fatty alcohol byproducts resulted in lower alkane titers (Cao et al., 2016). AHR plays a crucial role in maintaining a balance between fatty aldehyde production and consumption by AAR and ADO. Maximizing alkane production requires maintaining appropriate endogenous AHR activity, keeping the alkane:alcohol titer within the range of 1.5-3.3 (Warui et al., 2015). By enhancing the inhibition of the first competitive pathway, alkane biosynthesis in *E. coli* was increased from trace amounts to 58.8 mg/L by promoting the right amount of fatty alcohol biosynthesis.

Through the regulation of fatty acid biosynthesis, lipid breakdown, and electron transfer systems, alkane yields of 1.31 g/L (C_{15}, C_{17}) were achieved (Cao et al., 2016), marking a significant advancement in the field of microbial alkane biosynthesis. In 2019, Jaroensuk et al. demonstrated the highest alkane yield on record by achieving a 50% bioconversion yield of alkanes (C_{13}) in *E. coli*. They introduced the formate dehydrogenase gene (*fdh*) and ADO, encoded by *Xanthobacter* sp. 91 (XaFDH), into *E. coli*, using ferredoxin (Fd) as a co-substrate (Jaroensuk et al., 2019).

3.3. Optimizing culture conditions for alkane and alkene biosynthesis

In the process of alkane production using microorganisms, culture conditions are often overlooked despite being crucial factors. Factors such as temperature, pH, and carbon source play significant roles in the overall production process and outcomes. Optimizing the temperature is important as it can influence enzyme activity and increase alkane yield. For instance, when introducing different aldehyde decarbonylases from Arabidopsis and cyanobacteria into *E. coli*, it was found that CER1 exhibited the highest enzyme activity at 30 °C, resulting in a titration of alkanes up to 580.8 mg/L (Rodriguez and Atsumi, 2014). Similarly, ADO from cyanobacteria achieved the highest alkane titer of 26 mg/L at 24 °C, 5.3 mg/L at 18 °C, and 4.2 mg/L at 37 °C (Song et al., 2016). These findings highlight the importance of adjusting the culture temperature according to the optimal temperature for each enzyme to maximize alkane production.

pH regulation is another crucial factor, with microorganisms involved in alkane production generally preferring a neutral to slightly acidic pH range of 6 to 7. Within this range, the activity of enzymes involved in alkane biosynthesis is optimal, leading to higher alkane yields (Fitzgerald et al., 2010). Extreme pH values can inhibit enzyme activity, resulting in reduced yield and quality of produced alkanes. For example, raising the pH of the growth medium to 7 using phosphate significantly increased the titer of aldehyde precursors and, subsequently, the titer of alkanes from 3 to 337 mg/L (Foo et al., 2017).

Glucose is commonly used as the primary carbon source in many studies for alkane production. Typically, fatty acid biosynthesis methods are

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employed to convert glucose into alkanes through microbial metabolic modification. However, this metabolic pathway is prone to various feedback inhibitions, resulting in the accumulation of fatty acid precursors within the host organism. Consequently, this accumulation inhibits alkane biosynthesis. For instance, an initial production of 28.5 g/L of crude heavy oils (composed of 87.12% alkanes) was achieved using *Aureobasidium melanogenum* 9-1, isolated from the mangrove ecosystem of Dongzai Harbor, Hainan Province, China, in an environment containing 120.0 g/L of glucose. However, when the carbon source was switched from glucose to inulin, the production increased to 30.2 g/L of crude heavy oils (Xin et al., 2017). To overcome these limitations, researchers have explored alternative carbon sources, including non-food and non-glucose feedstocks, such as carbon dioxide and waste grease. These carbon sources not only reduce feedstock costs but also bypass the feedback mechanism of the fatty acid biosynthetic pathway, leading to improved yield and selectivity of the final product (Sinha et al., 2015).

3.4. Alkane production from CO_2

Currently, there are two main realities to the continued consumption of petroleum fuels. One is the anthropogenic CO_2 emissions from petroleum-derived fuels that contribute to global warming, a problem that can be mitigated or solved by capturing CO_2 from production processes or directly using fuels with near-zero net CO_2 balances (d'Ippolito et al., 2020; Abbasi et al., 2021; Pradhan et al., 2021). Second, petroleum itself is a highly polluting, non-renewable energy source, and scientists predict that it will be significantly depleted in the future (Ragauskas et al., 2006); therefore, it is necessary to diversify renewable fuels, especially biofuels, that can replace oil and other fossil hydrocarbons. CO_2 is a cheap and non-toxic starting material; its emissions remain at 31.5 Gt, and in 2020, the annual average concentration of CO_2 in the atmosphere reached a maximum level of 412.5 ppm (Bhatt et al., 2023). More importantly, CO_2 is not in competition with the global food supply chain and can be considered an ideal feedstock to produce alkanes. Most metabolic engineering studies on microbial production of alkanes have focused on sugars as a carbon source. The use of CO_2 to produce alkanes, which can bypass the biomass step (convert biomass waste into a mixture of liquids, gases, and carbon chars) and serve as a sustainable carbon source for large-scale production of alkanes, is a relatively novel approach that has attracted the attention of scientists in recent years. Currently, there are two main bottlenecks in the production of alkanes from CO_2: (i) the reduction of CO_2 to organic compounds and (ii) the conversion of the reduced organic compounds to long-chain alkanes (Aamer Mehmood et al., 2021).

In 2018, Lehtinen and team used a non-natural alkane producer, *Acinetobacter baylyi*, combined with acetogen *Acetobacterium woodii* to produce acetate from CO_2 and H_2 (**Fig. 4b**). The alkanes were biosynthesized by introducing the key enzymes AAR, ADO, Acr1, and CER1 through the modified heterologous microorganism *A. baylyi*. Finally, 540 μg/L of heptadecane (C_{17}) was obtained (Lehtinen et al., 2018). This is the first study to combine the alkane production pathway module with upstream acetic acid bacteria to produce acetate from CO_2 and H_2; it also means that the first bottleneck of alkane production from CO_2 has been circumvented. Although the alkane titers produced are still very low, this study provides a foundation for future scholars to investigate the field of alkane production using CO_2 heterologous organisms and can encourage scientists to explore further.

In metabolic engineering, *Cupriavidus necator* H16 was discovered by scientists to be the most attractive and promising chassis (Fedorov and Linke, 2022) capable of bioconverting CO_2 into biological hydrocarbons and, so far, is one of the most advanced genetic systems. *C. necator* produces alkanes autotrophically by assimilating CO_2 through its native Calvin-Benson-Bassham (CBB) cycle (Crepin et al., 2016). The authors have shown that two genes (encoding acyl-ACP reductase AAR and aldehyde-deformylating oxygenase ADO) from *Synechococcus elongatus* are overexpressed in *C. necator* to biosynthesize olefins and alkanes by autotrophic or heterotrophic means (**Fig. 4c**). The results showed that pentadecane, hexadecane, and heptadecane produced 435 mg/L of heterotrophic alkanes or alkenes and 4.4 mg/L of autotrophic alkanes or alkenes (i.e., pentadecane, hexadecane, and heptadecane) (Panich et al., 2021). Currently, the yield of this method is still low, mainly due to the low conversion efficiency of the CBB cycle. However, the flexible metabolic capacity of *C. necator* and its genetic ease of handling are more direct indications that *C. necator* is an ideal chassis for biofuel production using CO_2 in a way that not only has the potential to mitigate anthropogenic CO_2 emissions but also produces sustainable biofuels. With the expansion of scholarly research on *C. necator* and the implementation of scalable electrolysis, it is likely that in the next few years, a technically and economically feasible bioconversion of CO_2 into commercial chemicals and fuels will be achieved.

Electro-fermentation (EF) and microbial electrosynthesis (MES) are novel interdisciplinary biotechnologies (**Fig. 4d**) through which renewable fuels and carboxylic acids can be produced (Selvasembian et al., 2022; Sriram et al., 2022). As scientists gradually study EF and MES to produce carboxylic acids, the latest research shows that porous composites with high electrical conductivity and biocompatibility are the most promising for future applications in MES. Higher yields (3.1-9.3 g/L) of short-chain carboxylic acids (SCCA) have been successfully produced from MES, but yields of medium-chain carboxylic acids (MCCA) are still low. More research can be invested into the study of variables such as applied potential, regulation of enzyme activity, and functionality for biotope aspects to improve carboxylic acid yield (Lin et al., 2021).

Production of carboxylic acids combined with photoactivated decarboxylase from *Chlorella variabilis* NC64A (CvFAP), which is fully compatible with EF and MES and can successfully convert carboxylic acids to C_nH_{2n+2} alkanes (n = 2-5) (Sorigué et al., 2017). This innovative cyclic cascade system combines photo/biological/electrochemical catalysis to convert biomass and CO_2 to microbial alkanes; the method enables carbon cycling and simultaneous product separation and conversion (**Fig. 4d**). On this basis, a two-step enzymatic cascade reaction, lipase-catalyzed hydrolysis, and photodecarboxylation have been proposed. This method resulted in a conversion efficiency of 83% and a turnover number of over 8,000 (Huijbers et al., 2018). Subsequently, the recombinant engineered strain was able to convert carboxylic acids (C_4, C_5) to alkanes (C_3, C_4) by encoding the CvFAP gene into *E. coli* and *Halomonas*. The use of *Halomonas* for large-scale production of alkanes on existing infrastructure has been evaluated and proven to be economically viable (Amer et al., 2020).

3.5. Novel approaches for alkane and alkene production

The production of renewable diesel fuel from waste grease has garnered significant attention. However, the traditional preparation process is limited by strict reaction conditions or complex catalytic systems (Yao et al., 2021). Instead of relying on these conventional approaches, which involve designing key enzymes, inhibiting competing pathways, and increasing fatty acid supply, Cai et al. explored a simple method for the addition of common inorganic acids using inexpensive raw materials and co-catalysts under mild hydrothermal conditions. They combined this approach with commercial Ru/C catalysts to improve the conversion of fatty acids and lipids with a higher carbon number into long-chain alkanes (**Fig. 4e**). This hydrothermal deoxygenation (HTDO) method enables the complete conversion of stearic acid at 180°C within 3 h, with a selectivity of 97.85% for long-chain alkanes (C_{15}, C_{16}, C_{17}, C_{18}) (Cai et al., 2022). Efficiently producing alkanes while controlling the conversion of hydrogen radical intermediates under mild conditions is challenging, but it is a favored route compared to other methods.

A photocatalytic decarboxylation pathway has been developed to achieve early termination of hydrogen radicals, enabling efficient production of alkanes from bio-derived fatty acids (Zhou and Zhang, 2020). In this process, Pt/TiO_2 catalysts are added to oxidize fatty acids to alkyl radicals, combined with photogenerated holes generated by light at room temperature and H_2 partial pressure of 0.2 MPa (**Fig. 4f**). The electron-induced proton reduction and the interaction between H_2 and Pt/TiO_2 catalyst create a catalytic surface with abundant hydrogen elements, facilitating rapid hydrogenation of alkyl radicals with a 90% conversion rate. Moreover, crude fatty acid mixtures from industrial processes can be converted with up to 95% conversion (Zhou and Zhang, 2020). Although the alkanes produced by this method are not as abundant as those derived from fossil fuels, the ability to efficiently produce alkanes under mild conditions (room temperature, H_2 partial pressure of 0.2 MPa), in contrast to the traditional energy-intensive conditions (i.e., temperature of 250 °C and H_2 partial pressure of 2 MPa) required for deoxygenating fatty acids to alkanes, highlights the potential of this novel production method (Betts et al., 2018).

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4. Alkane and alkene biosynthesis: An economic and environmental perspective

4.1. Economic aspects of alkane and alkene biosynthesis

Unlike mature biofuel technologies such as ethanol and butanol production, the establishment of microbial hydrocarbon (i.e., alkane and alkene) based production facilities faces significant uncertainties due to the nascent stage of this hydrocarbon biosynthesis technology and limited commercial adoption. Furthermore, estimating the yield of intermediates and final products in the microbial hydrocarbon production process relies heavily on mathematical modeling and theoretical data, resulting in a lack of comprehensive and detailed production cost information for microbial alkanes. However, by dissecting the various stages of microbial hydrocarbon production and analyzing the factors influencing production costs at each step, researchers can gain valuable insights into the cost aspects associated with microbial hydrocarbon production. Thus, a comprehensive economic evaluation is crucial when transitioning from laboratory-scale to commercial-scale microbial hydrocarbon production.

For instance, the cost of biosynthesizing biofuels includes raw material prices, operational costs, capital costs, transportation, and time costs (Abbasi et al., 2021; Gheewala et al., 2022; Gheewala, 2023). Thus, the choice of microorganisms used for microbial alkanes and alkenes biosynthesis will significantly impact production costs. For example, using poplar as a feedstock for wood fiber ethanol incurs a cost of goods sold of approximately 4.50 USD/gallon (Phulara et al., 2018). Algae, on the other hand, can be used as a feedstock for ethanol production, with costs ranging from 2.95 to 3.2 USD/gallon depending on the production size and technology selected (Aamer Mehmood et al., 2021). While commercial-scale alkane and alkene biosynthesis is not yet widely available, it is possible to estimate the operational and capital costs based on similar biofuel production processes.

4.2. Environmental implications of alkane and alkene biosynthesis

To address the environmental issues associated with conventional chemical manufacturing methods and reduce dependence on non-renewable fossil fuels, the future adoption of the microbial biosynthesis of hydrocarbons holds promise (Aghbashlo et al., 2022; Gea et al., 2022; Selvasembian et al., 2022). Environmental analyses have demonstrated that biofuels and bio-based chemicals play a crucial role in ensuring environmental safety, thereby reducing emissions of harmful gases such as nitrogen oxides (NO and NO_2), carbon monoxide (CO), and sulfur oxides (SO_2 and SO), compared to traditional industrial fuels. Consequently, they help mitigate respiratory diseases and even cancer caused by these gases. Biologically synthesized alkanes and alkenes offer an eco-friendly alternative that can help mitigate the emission of greenhouse gases, thereby reducing the formation of ground-level ozone, which can have detrimental impacts on living organisms (Rao and Vizuete, 2021). Although CO_2 is a common byproduct in microbial alkane production, it can be recycled through the corresponding alkane production process due to the carbon cycling process in the ecosystem. For instance, scientists have optimized methanol production by CO_2 hydrogenation, resulting in an annual reduction of 7526.35-ton CO_{2eq} in greenhouse gas emissions and 19.43 tons in air pollutants (GhasemiKafrudi et al., 2022). It is estimated that microbial biosynthesis of biofuels can potentially reduce greenhouse gas emissions by 50% (Tarafdar et al., 2021).

Microbial alkanes, however, present environmental challenges by potentially causing soil and water contamination and negatively impacting the health of plants, animals, and humans. Alkanes and olefins can be toxic to microorganisms and can disrupt cell membrane function and integrity, inhibiting cell growth and potentially causing cell death when present in large quantities (Sikkema et al., 1995). Additionally, as the most common intermediate in microbial alkane production, fatty aldehydes are toxic, and the emission of substantial amounts of intermediate aldehydes can be detrimental to both the environment and human health. Scientists have explored reconstitution methods for common non-native biofuel-producing strains such as *E. coli*, *Lactobacillus*, and photosynthetic cyanobacteria to mitigate the impacts of chemical toxicity on microbial hosts (Jin et al., 2014). However, these methods only offer partial solutions, necessitating further research to overcome the toxicity of alkanes and olefins.

5. Conclusions and prospects

Microbial alkane and alkene biosynthesis show great promise as sustainable alternatives for high-value platform chemical production, but overcoming challenges is crucial for their large-scale implementation. The limitations of current metabolic pathways, including low product titers and undesirable byproducts, highlight the need for further research and optimization. The application of metabolic engineering techniques, such as enzyme modification and suppression of competing pathways, has shown promise in enhancing productivity. Additionally, the exploration of autotrophic metabolic pathways that utilize CO_2 as a carbon source, along with the development of non-biosynthetic routes, presents exciting avenues for improving alkane and alkene biosynthesis. Furthermore, the economic and environmental aspects of these processes must be carefully considered to ensure sustainable and efficient production.

Future research should focus on systematically addressing the challenges in microbial alkane and alkene biosynthesis to enable their widespread implementation. One crucial area of investigation is improving product titers and substrate conversion rates, which can be achieved through continued efforts in metabolic engineering, including the optimization of enzyme activities and the exploration of novel genetic modifications. Additionally, developing a robust microbial chassis capable of efficient alkane and alkene production is essential for scaling up the bioprocess. The integration of systems biology approaches, computational modeling, and the utilization of artificial intelligence and machine learning models can provide valuable insights into the complex metabolic networks involved, facilitating targeted improvements.

Furthermore, research should strive to enhance control over carbon chain length and minimize the formation of undesired byproducts. This requires a deeper understanding of the underlying enzymatic reactions and regulatory mechanisms involved in alkane and alkene biosynthesis. Novel strategies for enzyme engineering and pathway optimization can be employed to achieve precise control over product characteristics. Moreover, exploring the extensive utilization of renewable and sustainable feedstocks, such as lignocellulosic biomass, agricultural residues, and carbon dioxide, is crucial to ensure both environmental friendliness and cost-effectiveness of the process.

Acknowledgements

This work was supported by the Hong Kong Baptist University with grant numbers RC-OFSGT2/20-21/SCI/010. Further, it was supported by the Start-up fund of Hong Kong Baptist University (BIOL-22-23-01) and the Innovation Technology Fund of Innovation Technology Commission: Funding Support to State Key Laboratory of Agrobiotechnology. Any opinions, findings, conclusions, or recommendations expressed in this publication do not reflect the views of the Government of the Hong Kong Special Administrative Region or the Innovation and Technology Commission.

References

[1] Aamer Mehmood, M., Shahid, A., Malik, S., Wang, N., Rizwan Javed, M., Nabeel Haider, M., Verma, P., Umer Farooq Ashraf, M., Habib, N., Syafiuddin, A., Boopathy, R., 2021. Advances in developing metabolically engineered microbial platforms to produce fourth-generation biofuels and high-value biochemicals. Bioresour. Technol. 337, 125510.

[2] Abbasi, M., Pishvaee, M.S., Mohseni, S., 2021. Third-generation biofuel supply chain: a comprehensive review and future research directions. J. Clean. Prod. 323, 129100.

[3] Aghbashlo, M., Hosseinzadeh-Bandbafha, H., Shahbeik, H., Tabatabaei, M., 2022. The role of sustainability assessment tools in realizing bioenergy and bioproduct systems. Biofuel Res. J. 9(3), 1697-1706.

[4] Amer, M., Wojcik, E.Z., Sun, C., Hoeven, R., Hughes, J.M.X., Faulkner, M., Yunus, I.S., Tait, S., Johannissen, L.O., Hardman, S.J.O., Heyes, D.J., Chen, G.Q., Smith, M.H., Jones, P.R., Toogood, H.S., Scrutton, N.S., 2020. Low carbon strategies for sustainable bio-

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alkane gas production and renewable energy. Energy Environ. Sci. 13(6), 1818-1831.

[5] Andre, C., Kim, S.W., Yu, X.H., Shanklin, J., 2013. Fusing catalase to an alkane-producing enzyme maintains enzymatic activity by converting the inhibitory byproduct H_2O_2 to the cosubstrate O_2. Proc. Natl. Acad. Sci. U S A. 110(8), 3191-3196.

[6] Basri, R.S., Rahman, R., Kamarudin, N.H.A., Ali, M.S.M., 2020. Cyanobacterial aldehyde deformylating oxygenase: structure, function, and potential in biofuels production. Int. J. Biol. Macromol. 164, 3155-3162.

[7] Bernard, A., Domergue, F., Pascal, S., Jetter, R., Renne, C., Faure, J.D., Haslam, R.P., Napier, J.A., Lessire, R., Joubes, J., 2012. Reconstitution of plant alkane biosynthesis in yeast demonstrates that Arabidopsis ECERIFERUM1 and ECERIFERUM3 are core components of a very-long-chain alkane synthesis complex. Plant Cell. 24(7), 3106-3118.

[8] Betts, L.M., Dappozze, F., Guillard, C., 2018. Understanding the photocatalytic degradation by P25 TiO_2 of acetic acid and propionic acid in the pursuit of alkane production. Applied Catal., A. 554, 35-43.

[9] Bhatt, H., Davawala, M., Joshi, T., Shah, M., Unnarkat, A., 2023. Forecasting and mitigation of global environmental carbon dioxide emission using machine learning techniques. Clean. Chem. Eng. 5, 100095.

[10] Bhushan, S., Jayakrishnan, U., Shree, B., Bhatt, P., Eshkabilov, S., Simsek, H., 2023. Biological pretreatment for algal biomass feedstock for biofuel production. J. Environ. Chem. Eng. 11(3), 109870.

[11] Blazeck, J., Liu, L., Knight, R., Alper, H.S., 2013. Heterologous production of pentane in the oleaginous yeast Yarrowia lipolytica. J. Biotechnol. 165(3-4), 184-194.

[12] Bruder, S., Moldenhauer, E.J., Lemke, R.D., Ledesma-Amaro, R., Kabisch, J., 2019. Drop-in biofuel production using fatty acid photodecarboxylase from Chlorella variabilis in the oleaginous yeast Yarrowia lipolytica. Biotechnol. Biofuels. 12, 202.

[13] Buijs, N.A., Siewers, V., Nielsen, J., 2013. Advanced biofuel production by the yeast Saccharomyces cerevisiae. Curr. Opin. Chem. Biol. 17(3), 480-488.

[14] Cai, X., Zhang, Z., Ye, Y., Wang, D., Li, S., Wang, D., Zheng, Z., 2022. Conversion of higher fatty acids or higher fatty acid esters to long-chain alkanes by acid added metal catalyst under mild hydrothermal conditions. Biomass Bioenergy. 156, 106328.

[15] Cao, Y.X., Xiao, W.H., Zhang, J.L., Xie, Z.X., Ding, M.Z., Yuan, Y.J., 2016. Heterologous biosynthesis and manipulation of alkanes in Escherichia coli. Metab. Eng. 38, 19-28.

[16] Chen, B., Lee, D.Y., Chang, M.W., 2015. Combinatorial metabolic engineering of Saccharomyces cerevisiae for terminal alkene production. Metab. Eng. 31, 53-61.

[17] Chen, B., Lee, H.L., Heng, Y.C., Chua, N., Teo, W.S., Choi, W.J., Leong, S.S.J., Foo, J.L., Chang, M.W., 2018. Synthetic biology toolkits and applications in Saccharomyces cerevisiae. Biotechnol. Adv. 36(7), 1870-1881.

[18] Chen, C., Ibekwe-SanJuan, F., Hou, J., 2010. The structure and dynamics of co-citation clusters: a multiple-perspective co-citation analysis. J. Am. Soc. Inf. Sci. Technol. 61(7), 1386-1409.

[19] Chen, Y., Yin, M., Horsman, G.P., Shen, B., 2011. Improvement of the enediyne antitumor antibiotic C-1027 production by manipulating its biosynthetic pathway regulation in Streptomyces globisporus. J. Nat. Prod. 74(3), 420-424.

[20] Christenson, J.K., Richman, J.E., Jensen, M.R., Neufeld, J.Y., Wilmot, C.M., Wackett, L.P., 2017a. β-Lactone synthetase found in the olefin biosynthesis pathway. Biochemistry. 56(2), 348-351.

[21] Christenson, J.K., Robinson, S.L., Engel, T.A., Richman, J.E., Kim, A.N., Wackett, L.P., 2017b. OleB from bacterial hydrocarbon biosynthesis is a β-lactone decarboxylase that shares key features with haloalkane dehalogenases. Biochemistry. 56(40), 5278-5287.

[22] Crepin, L., Lombard, E., Guillouet, S.E., 2016. Metabolic engineering of Cupriavidus necator for heterotrophic and autotrophic alka(e)ne production. Metab. Eng. 37, 92-101.

[23] Cronjé, Y., Farzad, S., Mandegari, M., Görgens, J.F., 2023. A critical review of multiple alternative pathways for the production of a high-value bioproduct from sugarcane mill byproducts: the case of adipic acid. Biofuel Res. J. 10(03), 1933-1947.

[24] d'Ippolito, G., Landi, S., Esercizio, N., Lanzilli, M., Vastano, M., Dipasquale, L., Pradhan, N., Fontana, A., 2020. CO_2-induced transcriptional reorganization: molecular basis of capnophillic lactic fermentation in Thermotoga neapolitana. Front. Microbiol. 11, 171.

[25] Fatma, Z., Hartman, H., Poolman, M.G., Fell, D.A., Srivastava, S., Shakeel, T., Yazdani, S.S., 2018. Model-assisted metabolic engineering of Escherichia coli for long chain alkane and alcohol production. Metab. Eng. 46, 1-12.

[26] Fedorov, A., Linke, D., 2022. Data analysis of CO_2 hydrogenation catalysts for hydrocarbon production. J. CO_2 Util. 61.

[27] Fenibo, E.O., Selvarajan, R., Abia, A.L.K., Matambo, T., 2023. Medium-chain alkane biodegradation and its link to some unifying attributes of alkB genes diversity. Sci. Total Environ. 877, 162951.

[28] Feyereisen, R., 2020. Origin and evolution of the CYP4G subfamily in insects, cytochrome P450 enzymes involved in cuticular hydrocarbon synthesis. Mol Phylogenet. Evol. 143, 106695.

[29] Fitzgerald, L.A., Ziemer, C., Lizewski, S.E., Ringeisen, B.R., Henry, K., Biffinger, J.C., 2010. Microbial treatment of swine fecal waste to generate long-chain linear alkanes after fast pyrolysis. J. Biotechnol. 150, 151.

[30] Foo, J.L., Rasouliha, B.H., Susanto, A.V., Leong, S.S.J., Chang, M.W., 2020. Engineering an alcohol-forming fatty Acyl-CoA reductase for aldehyde and hydrocarbon biosynthesis in Saccharomyces cerevisiae. Front. Bioeng. Biotechnol. 8, 585935.

[31] Foo, J.L., Susanto, A.V., Keasling, J.D., Leong, S.S., Chang, M.W., 2017. Whole-cell biocatalytic and de novo production of alkanes from free fatty acids in Saccharomyces cerevisiae. Biotechnol. Bioeng. 114(1), 232-237.

[32] Gea, S., Irvan, I., Wijaya, K., Nadia, A., Pulungan, A.N., Sihombing, J.L., Rahayu, R., 2022. Bio-oil hydrodeoxygenation over acid activated-zeolite with different Si/Al ratio. Biofuel Res. J. 9(2), 1630-1639.

[33] GhasemiKafrudi, E., Samiee, L., Mansourpour, Z., Rostami, T., 2022. Optimization of methanol production process from carbon dioxide hydrogenation in order to reduce recycle flow and energy consumption. J. Clean. Prod. 376.

[34] Gheewala, S.H., 2023. Life cycle assessment for sustainability assessment of biofuels and bioproducts. Biofuel Res. J. 10(1), 1810-1815.

[35] Gheewala, S.H., Jaroenkietkajorn, U., Nilsalab, P., Silalertruksa, T., Somkerd, T., Laosiripojana, N., 2022. Sustainability assessment of palm oil-based refinery systems for food, fuel, and chemicals. Biofuel Research Journal 9(4), 1750-1763.

[36] Gu, L., Wang, B., Kulkarni, A., Gehret, J.J., Lloyd, K.R., Gerwick, L., Gerwick, W.H., Wipf, P., Håkansson, K., Smith, J.L., Sherman, D.H., 2009. Polyketide decarboxylative chain termination preceded by O-sulfonation in curacin a biosynthesis. J. Am. Chem. Soc. 131(44), 16033-16035.

[37] Hsieh, C.H., Makris, T.M., 2016. Expanding the substrate scope and reactivity of cytochrome P450 OleT. Biochem. Biophys. Res. Commun. 476(4), 462-466.

[38] Huijbers, M.M.E., Zhang, W., Tonin, F., Hollmann, F., 2018. Light-driven enzymatic decarboxylation of fatty acids. Angew. Chem. Int. Ed. Engl. 57(41), 13648-13651.

[39] Jaroensuk, J., Intasian, P., Kiattisewee, C., Munkajohnpon, P., Chunthaboon, P., Buttranon, S., Trisrivirat, D., Wongnate, T., Maenpuen, S., Tinikul, R., Chaiyen, P., 2019. Addition of formate dehydrogenase increases the production of renewable alkane from an engineered metabolic pathway. J. Biol. Chem. 294(30), 11536-11548.

[40] Jaroensuk, J., Intasian, P., Wattanasuepsin, W., Akeratchatapan, N., Kesornpun, C., Kittipanukul, N., Chaiyen, P., 2020. Enzymatic reactions and pathway engineering for the production of renewable hydrocarbons. J. Biotechnol. 309, 1-19.

[41] Jin, H., Chen, L., Wang, J., Zhang, W., 2014. Engineering biofuel tolerance in non-native producing microorganisms. Biotechnol. Adv. 32(2), 541-548.

[42] Kang, M.K., Nielsen, J., 2017. Biobased production of alkanes and alkenes through metabolic engineering of microorganisms. J. Ind. Microbiol. Biotechnol. 44(4-5), 613-622.

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[43] Kang, M.K., Zhou, Y.J., Buijs, N.A., Nielsen, J., 2017. Functional screening of aldehyde decarbonylases for long-chain alkane production by Saccharomyces cerevisiae. Microb. Cell Fact. 16(1), 74.
[44] Knoot, C.J., Pakrasi, H.B., 2019. Diverse hydrocarbon biosynthetic enzymes can substitute for olefin synthase in the cyanobacterium Synechococcus sp. PCC 7002. Sci. Rep 9(1), 1360.
[45] Lee, J.W., Niraula, N.P., Trinh, C.T., 2018. Harnessing a P450 fatty acid decarboxylase from Macrococcus caseolyticus for microbial biosynthesis of odd chain terminal alkenes. Metab. Eng. Commun. 7, e00076.
[46] Lehtinen, T., Virtanen, H., Santala, S., Santala, V., 2018. Production of alkanes from CO_2 by engineered bacteria. Biotechnol. Biofuels. 11, 228.
[47] Li, J., Ma, Y., Liu, N., Eser, B.E., Guo, Z., Jensen, P.R., Stephanopoulos, G., 2020. Synthesis of high-titer alka(e)nes in Yarrowia lipolytica is enabled by a discovered mechanism. Nat. Commun. 11(1), 6198.
[48] Li, N., Chang, W.C., Warui, D.M., Booker, S.J., Krebs, C., Bollinger, J.M., Jr., 2012. Evidence for only oxygenative cleavage of aldehydes to alk(a/e)nes and formate by cyanobacterial aldehyde decarbonylases. Biochemistry. 51(40), 7908-7916.
[49] Lin, R., Deng, C., Zhang, W., Hollmann, F., Murphy, J.D., 2021. Production of bio-alkanes from biomass and CO_2. Trends Biotechnol. 39(4), 370-380.
[50] Liu, K., Li, S., 2020. Biosynthesis of fatty acid-derived hydrocarbons: perspectives on enzymology and enzyme engineering. Curr. Opin. Biotechnol. 62, 7-14.
[51] Liu, Q., Wu, K., Cheng, Y., Lu, L., Xiao, E., Zhang, Y., Deng, Z., Liu, T., 2015. Engineering an iterative polyketide pathway in Escherichia coli results in single-form alkene and alkane overproduction. Metab. Eng. 28, 82-90.
[52] Liu, Y., Khusnutdinova, A., Chen, J., Crisante, D., Batyrova, K., Raj, K., Feigis, M., Shirzadi, E., Wang, X., Dorakhan, R., Wang, X., Stogios, P.J., Yakunin, A.F., Sargent, E.H., Mahadevan, R., 2022. Systems engineering of Escherichia coli for n-butane production. Metab. Eng. 74, 98-107.
[53] Liu, Y., Liu, W.Q., Huang, S., Xu, H., Lu, H., Wu, C., Li, J., 2023. Cell-free metabolic engineering enables selective biotransformation of fatty acids to value-added chemicals. Metab. Eng. Commun. 16, e00217.
[54] Lu, R., Shi, T.Q., Lin, L., Ledesma-Amaro, R., Ji, X.J., Huang, H., 2022. Advances in metabolic engineering of yeasts for the production of fatty acid-derived hydrocarbon fuels. Green Chem. Eng. 3(4), 298-303.
[55] Manley, O.M., Fan, R., Guo, Y., Makris, T.M., 2019. Oxidative decarboxylase UndA utilizes a dinuclear iron cofactor. J. Am. Chem. Soc. 141(22), 8684-8688.
[56] Mendez-Perez, D., Begemann, M.B., Pfleger, B.F., 2011. Modular synthase-encoding gene involved in alpha-olefin biosynthesis in Synechococcus sp. strain PCC 7002. Appl. Environ. Microbiol. 77(12), 4264-4267.
[57] Mokhtar, M., Sukmono, A., Setiapraja, H., Ma'ruf, M., Yubaidah, S., Haryono, I., Rochmanto, B., Soewono, R.T., Adhi Sukra, K.F., Thahar, A., Manurung, E., Wibowo, C.S., Widodo, S., Supriyadi, F., Abriyant, R.Y., Abriyant, R.Y., Suntoro, D., Faridha, F., Reksowardojo, I.K., 2023. Towards nationwide implementation of 40% biodiesel blend fuel in Indonesia: a comprehensive road test and laboratory evaluation. Biofuel Res. J. 10(03), 1876-1889.
[58] Monteiro, R.R.C., da Silva, S.S.O., Cavalcante, C.L., de Luna, F.M.T., Bolivar, J.M., Vieira, R.S., Fernandez-Lafuente, R., 2022. Biosynthesis of alkanes/alkenes from fatty acids or derivatives (triacylglycerols or fatty aldehydes). Biotechnol. Adv. 61, 108045.
[59] Okoro, V., Azimov, U., Munoz, J., 2022. Recent advances in production of bioenergy carrying molecules, microbial fuels, and fuel design-a review. Fuel. 316, 123330.
[60] Panich, J., Fong, B., Singer, S.W., 2021. Metabolic engineering of Cupriavidus necator H16 for sustainable biofuels from CO_2. Trends Biotechnol. 39(4), 412-424.
[61] Patrikainen, P., Carbonell, V., Thiel, K., Aro, E.M., Kallio, P., 2017. Comparison of orthologous cyanobacterial aldehyde deformylating oxygenases in the production of volatile C3-C7 alkanes in engineered E. coli. Metab. Eng. Commun. 5, 9-18.
[62] Peirú, S., Gramajo, H., Menzella, H.G., 2009. Chapter 14 Design and synthesis of pathway genes for polyketide biosynthesis, Methods in Enzymology. Academic Press, pp. 319-337.
[63] Phulara, S.C., Chaurasia, D., Diwan, B., Chaturvedi, P., Gupta, P., 2018. In-situ isopentenol production from Bacillus subtilis through genetic and culture condition modulation. Process Biochem. 72, 47-54.
[64] Pradhan, N., d'Ippolito, G., Dipasquale, L., Esposito, G., Panico, A., Lens, P.N.L., Fontana, A., 2021. Kinetic modeling of hydrogen and L-lactic acid production by Thermotoga neapolitana via capnophilic lactic fermentation of starch. Bioresour. Technol. 332, 125127.
[65] Pradhan, N., Dipasquale, L., d'Ippolito, G., Panico, A., Lens, P.N., Esposito, G., Fontana, A., 2015. Hydrogen production by the thermophilic bacterium Thermotoga neapolitana. Int. J. Mol. Sci. 16(6), 12578-12600.
[66] Pradhan, N., Kumar, S., Selvasembian, R., Rawat, S., Gangwar, A., R, S., Kit Yuen, Y., Luo, L., Ayothiraman, S., Dattatraya Saratale, G., Mal, J., 2022. Emerging trends in the pretreatment of microalgal biomass and recovery of value-added products: a review. Bioresour. Technol. 369, 128395.
[67] Qiu, Y., Tittiger, C., Wicker-Thomas, C., Le Goff, G., Young, S., Wajnberg, E., Fricaux, T., Taquet, N., Blomquist, G.J., Feyereisen, R., 2012. An insect-specific P450 oxidative decarbonylase for cuticular hydrocarbon biosynthesis. Proc. Natl .Acad.Sci. 109(37), 14858-14863.
[68] Ragauskas, A.J., Williams, C.K., Davison, B.H., Britovsek, G., Cairney, J., Eckert, C.A., Frederick, W.J., Hallett, J.P., Leak, D.J., Liotta, C.L., Mielenz, J.R., Murphy, R., Templer, R., Tschaplinski, T., 2006. The path forward for biofuels and biomaterials. Science. 311(5760), 484-489.
[69] Rahmana, Z., Sung, B.H., Yi, J.Y., Bui le, M., Lee, J.H., Kim, S.C., 2014. Enhanced production of n-alkanes in Escherichia coli by spatial organization of biosynthetic pathway enzymes. J. Biotechnol. 192(Part A), 187-191.
[70] Rao, V., Vizuete, W., 2021. Chapter Three - Ozone: Good high, bad nigh, in: Rao, V., Vizuete, W. (Eds.), Particulates Matter. Elsevier, pp. 39-55.
[71] Reinhard, F.G.C., Lin, Y.T., Stanczak, A., de Visser, S.P., 2020. Bioengineering of cytochrome P450 OleTJE: how does substrate positioning affect the product distributions?. Molecules. 25(11), 2675.
[72] Rodriguez, G.M., Atsumi, S., 2014. Toward aldehyde and alkane production by removing aldehyde reductase activity in Escherichia coli. Metab. Eng. 25, 227-237.
[73] Rude, M.A., Baron, T.S., Brubaker, S., Alibhai, M., Del Cardayre, S.B., Schirmer, A., 2011. Terminal olefin (1-alkene) biosynthesis by a novel p450 fatty acid decarboxylase from Jeotgalicoccus species. Appl. Environ. Microbiol. 77(5), 1718-1727.
[74] Rui, Z., Harris, N.C., Zhu, X., Huang, W., Zhang, W., 2015. Discovery of a family of desaturase-like enzymes for 1-alkene biosynthesis. ACS Catal. 5(12), 7091-7094.
[75] Sathesh-Prabu, C., Kim, D., Lee, S.K., 2020. Metabolic engineering of Escherichia coli for 2,3-butanediol production from cellulosic biomass by using glucose-inducible gene expression system. Bioresour. Technol. 309, 123361.
[76] Schweizer, E., Hofmann, J., 2004. Microbial type I fatty acid synthases (FAS): major players in a network of cellular FAS systems. Microbiol. Mol. Biol. Rev. 68(3), 501-517.
[77] Selvasembian, R., Mal, J., Rani, R., Sinha, R., Agrahari, R., Joshua, I., Santhiagu, A., Pradhan, N., 2022. Recent progress in microbial fuel cells for industrial effluent treatment and energy generation: fundamentals to scale-up application and challenges. Bioresour. Technol. 346, 126462.
[78] Shakeel, T., Gupta, M., Fatma, Z., Kumar, R., Kumar, R., Singh, R., Sharma, M., Jade, D., Gupta, D., Fatma, T., Yazdani, S.S., 2018. A consensus-guided approach yields a heat-stable alkane-producing enzyme and identifies residues promoting thermostability. J. Biol. Chem. 293(24), 9148-9161.
[79] Sheppard, M.J., Kunjapur, A.M., Prather, K.L.J., 2016. Modular and selective biosynthesis of gasoline-range alkanes. Metab. Eng. 33, 28-40.
[80] Sikkema, J., Bont, J.A.D., Poolman, B., 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev. 59(2), 201-222.

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[81] Sinha, M., Sørensen, A., Ahamed, A., Ahring, B.K., 2015. Production of hydrocarbons by Aspergillus carbonarius ITEM 5010. Fungal Biology. 119(4), 274-282.
[82] Song, X., Yu, H., Zhu, K., 2016. Improving alkane synthesis in Escherichia coli via metabolic engineering. Appl. Microbiol. Biotechnol. 100(2), 757-767.
[83] Sorigué, D., Légeret, B., Cuiné, S., Blangy, S., Moulin, S., Billon, E., Richaud, P., Brugière, S., Couté, Y., Nurizzo, D., Müller, P., Brettel, K., Pignol, D., Arnoux, P., Li-Beisson, Y., Peltier, G., Beisson, F., 2017. An algal photoenzyme converts fatty acids to hydrocarbons. Science. 357(6354), 903-907.
[84] Sriram, S., Wong, J.W.C., Pradhan, N., 2022. Recent advances in electro-fermentation technology: a novel approach towards balanced fermentation. Bioresour. Technol. 360, 127637.
[85] Sukovich, D.J., Seffernick, J.L., Richman, J.E., Gralnick, J.A., Wackett, L.P., 2010. Widespread head-to-head hydrocarbon biosynthesis in bacteria and role of OleA. Appl. Environ. Microbiol. 76(12), 3850-3862.
[86] Sun, H., Gao, T., Chen, X., Hitchings, M.D., Li, S., Chen, T., Zhang, H., An, L., Dyson, P., 2016. Complete genome sequence of a psychotrophic Arthrobacter strain A3 (CGMCC 1.8987), a novel long-chain hydrocarbons producer. J. Biotechnol. 222, 23-24.
[87] Tarafdar, A., Sirohi, R., Gaur, V.K., Kumar, S., Sharma, P., Varjani, S., Pandey, H.O., Sindhu, R., Madhavan, A., Rajasekharan, R., Sim, S.J., 2021. Engineering interventions in enzyme production: lab to industrial scale. Bioresour. Technol. 326, 124771.
[88] Wackett, L.P., Wilmot, C.M., 2015. Chapter 2-Hydrocarbon Biosynthesis in Microorganisms, in: Himmel, M.E. (Ed.) Direct microbial conversion of biomass to advanced biofuels. Elsevier, Amsterdam, pp. 13-31.
[89] Wang, J., Ledesma-Amaro, R., Wei, Y., Ji, B., Ji, X.J., 2020. Metabolic engineering for increased lipid accumulation in Yarrowia lipolytica-a review. Bioresour. Technol. 313, 123707.
[90] Wang, J., Xia, A., Deng, Z., Huang, Y., Zhu, X., Zhu, X., Liao, Q., 2022. Intensifying biofuel production using a novel bionic flow-induced peristaltic reactor: biodiesel production as a case study. Biofuel Res. J. 9(4), 1721-1735.
[91] Wang, J., Zhu, K., 2018. Microbial production of alka(e)ne biofuels. Curr. Opin. Biotechnol. 50, 11-18.
[92] Wang, M., Nie, K., Cao, H., Xu, H., Fang, Y., Tan, T., Baeyens, J., Liu, L., 2017. Biosynthesis of medium chain length alkanes for bio-aviation fuel by metabolic engineered Escherichia coli. Bioresour. Technol. 239, 542-545.
[93] Wang, W., Lu, X., 2013. Microbial synthesis of alka(e)nes. Front. Bioeng. Biotechnol. 1, 10.
[94] Warui, D.M., Pandelia, M.E., Rajakovich, L.J., Krebs, C., Bollinger, J.M., Jr., Booker, S.J., 2015. Efficient delivery of long-chain fatty aldehydes from the Nostoc punctiforme Acyl-Acyl carrier protein reductase to its cognate aldehyde-deformylating oxygenase. Biochemistry. 54(4), 1006-1015.
[95] Wasylenko, T.M., Ahn, W.S., Stephanopoulos, G., 2015. The oxidative pentose phosphate pathway is the primary source of NADPH for lipid overproduction from glucose in Yarrowia lipolytica. Metab. Eng. 30, 27-39.
[96] White, S.W., Zheng, J., Zhang, Y.M., Rock, 2005. The structural biology of type II fatty acid biosynthesis. Annu. Rev. Biochem. 74, 791-831.
[97] Xin, F.H., Zhang, Y., Xue, S.J., Chi, Z., Liu, G.L., Hu, Z., Chi, Z.M., 2017. Heavy oils (mainly alkanes) over-production from inulin by Aureobasidium melanogenum 9-1 and its transformant 88 carrying an inulinase gene. Renewable Energy. 105, 561-568.
[98] Xu, P., Qiao, K., Ahn, W.S., Stephanopoulos, G., 2016. Engineering Yarrowia lipolytica as a platform for synthesis of drop-in transportation fuels and oleochemicals. Proc. Natl. Acad. Sci. U.S.A. 113(39), 10848-10853.
[99] Xu, W., Chen, Y., Li, D., Wang, Z., Xu, J., Wu, Q., 2022. Rational design of fatty acid photodecarboxylase enables the efficient decarboxylation of medium- and short-chain fatty acids for the production of gasoline bio-alkanes. Mol. Catalysis. 524, 112261.
[100] Yan, H., Wang, Z., Wang, F., Tan, T., Liu, L., 2016. Biosynthesis of chain-specific alkanes by metabolic engineering in Escherichia coli. Eng. Life Sci. 16(1), 53-59.
[101] Yang, K., Li, F., Qiao, Y., Zhou, Q., Hu, Z., He, Y., Yan, Y., Xu, L., Madzak, C., Yan, J., 2018. Design of a New multienzyme complex synthesis system based on Yarrowia lipolytica simultaneously secreted and surface displayed fusion proteins for sustainable production of fatty acid-derived hydrocarbons. ACS Sustainable Chem. Eng. 6(12), 17035-17043.
[102] Yang, K., Qiao, Y., Li, F., Xu, Y., Yan, Y., Madzak, C., Yan, J., 2019. Subcellular engineering of lipase dependent pathways directed towards lipid related organelles for highly effectively compartmentalized biosynthesis of triacylglycerol derived products in Yarrowia lipolytica. Metab. Eng. 55, 231-238.
[103] Yao, X., Strathmann, T.J., Li, Y., Cronmiller, L.E., Ma, H., Zhang, J., 2021. Catalytic hydrothermal deoxygenation of lipids and fatty acids to diesel-like hydrocarbons: a review. Green Chem. 23(3), 1114-1129.
[104] Zhang, J., Van Lanen, S.G., Ju, J., Liu, W., Dorrestein, P.C., Li, W., Kelleher, N.L., Shen, B., 2008. A phosphopantetheinylating polyketide synthase producing a linear polyene to initiate enediyne antitumor antibiotic biosynthesis. Proc. Natl. Acad. Sci. U.S.A. 105(5), 1460-1465.
[105] Zhang, W., Ma, M., Huijbers, M.M.E., Filonenko, G.A., Pidko, E.A., van Schie, M., de Boer, S., Burek, B.O., Bloh, J.Z., van Berkel, W.J.H., Smith, W.A., Hollmann, F., 2019. Hydrocarbon synthesis via photoenzymatic decarboxylation of carboxylic acids. J. Am. Chem. Soc. 141(7), 3116-3120.
[106] Zhang, X., Cheng, Z., Ma, L., Li, J., 2017. A study on accumulation of volatile organic compounds during ochratoxin a biosynthesis and characterization of the correlation in Aspergillus carbonarius isolated from grape and dried vine fruit. Food Chem. 227, 55-63.
[107] Zhou, C., Zhang, T., 2020. Photocatalytic alkane production from fatty acid decarboxylation over hydrogenated catalyst. Sci. Bull. 65(11), 870-871.
[108] Zhou, Y.J., Buijs, N.A., Zhu, Z., Qin, J., Siewers, V., Nielsen, J., 2016. Production of fatty acid-derived oleochemicals and biofuels by synthetic yeast cell factories. Nat. Commun. 7, 11709.
[109] Zhou, Y.J., Hu, Y., Zhu, Z., Siewers, V., Nielsen, J., 2018. Engineering 1-alkene biosynthesis and secretion by dynamic regulation in yeast. ACS Synth. Biol. 7(2), 584-590.
[110] Zhu, Z., Zhou, Y.J., Kang, M.K., Krivoruchko, A., Buijs, N.A., Nielsen, J., 2017. Enabling the synthesis of medium chain alkanes and 1-alkenes in yeast. Metab. Eng. 44, 81-88.

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Please cite this article as: Geng J., Liao P., Tan G.Y.A., Zhu F.-Y., Pradhan N. Opportunities and challenges for n-alkane and n-alkene biosynthesis: A sustainable microbial biorefinery. Biofuel Research Journal 40 (2023) 1974-1988. DOI: 10.18331/BRJ2023.10.4.4

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