Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Microbial production of alka(e)ne biofuels

Juli Wang[1,2] and Kun Zhu[1]

Addresses
[1] CAS Key Laboratory of Microbial Physiological and Metabolic Engineering, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
[2] University of Chinese Academy of Sciences, Beijing, 100049, China

Corresponding author: Zhu, Kun (zhuk@im.ac.cn)

Current Opinion in Biotechnology 2018, **50**:11–18

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

Edited by **Akihiko Kondo** and **Hal Alper**

http://dx.doi.org/10.1016/j.copbio.2017.08.009

Introduction

Due to depleting petroleum supply and environmental issue presented by fractional distillation of crude oil, many researchers seek to exploit the use of biofuels as a clean energy source [1,2]. Many governments have invested greatly in the application of biofuels for years [3]. Although various types of biofuels have already been put in everyday use, many of them have several drawbacks due to their chemical properties. Bioethanol, for example, has considerably lower energy density than petroleum based fuels; its high hygroscopicity and volatility make it harder to preserve [4]. Alka(e)ne, however, as one of the hydrocarbon biofuels and chemical feedstock derived from microbial fatty acid metabolic pathway, has no such disadvantages. Key features of product alka(e)ne (chain length; degree of saturation; straight or branched) are determining factors for their use. According to its chain length, alka(e)ne constitutes most of the petroleum based fuels (gasoline, aviation fuel, *etc.*). The presence of unsaturated and branched alka(e)ne ensures fuels low freezing-point during high altitude [5]. Alkenes, on the other hand, have also been in use for the production of chemical products such as detergents and lubricants.

The potential of alka(e)ne generating system is determined by three essential factors: metabolic pathway engineering, microbial platform and culture condition. In this review, we discuss these three factors' impact on alka(e)ne production individually. With Table 1 and Figure 1 summarizing detailed strategies for alka(e)ne biosynthesis in recent years, we elaborate several unique findings in main text. Among these factors, pathway engineering attract most of the attention. However, to some extent, we deem it is equally important to investigate suitable host and culture method for alka(e)ne production.

Metabolic pathway engineering for alka(e)ne biosynthesis

Since the confirmation of two main steps responsible for the synthesis of alka(e)nes in cyanobacteria [6], many researches attempt to construct this alka(e)ne biosynthesis pathway heterologously with the expectation of elevated yield and altered alkane properties. As shown in Table 1 and Figure 1, core alka(e)ne generating pathway often involves a simple binary structure with one enzyme (Table 2) tapping into the host fatty acid metabolism to provide precursor, while second enzyme (Table 3) converting this precursor into hydrocarbons [7,8••,9,10,11•,12,13].

The first step of alka(e)ne biosynthesis usually directs carbon source into three precursors (Figure 1 and Table 2): fatty aldehydes (in most cases), fatty acids and acyl-CoA/ACP. This step is highly susceptible to native competing pathways. For instance, when using fatty aldehyde as precursor, many studies detect fatty alcohol byproduct (Table 1). In some extreme cases, the titer of fatty alcohols even exceeds the target alka(e)nes' [7,14•,15••]. This is mainly because the presence of multiple fatty aldehyde reductase homologues in *Escherichia coli* (more than 13) as well as in *Saccharomyces cerevisiae* [16,17]. Another pathway competing for aldehyde precursor is fatty aldehyde dehydrogenation pathway [7,14•,15••,16,18]. Study shows that, when expressing AAR and ADO in *Aspergillus carbonarius*, the level of internal fatty acids and triacylglycerol rise as soon as alkane emerges. This is attributed to the presence of fatty aldehyde dehydrogenase which allows aldehydes to be redirected into fatty acids [19•]. Many experiments start with the deletion of these competing pathways in order to raise the accumulation of fatty aldehyde precursor thus improve the overall performance of alka(e)ne generating system. However, considering the intensity of competing pathway elimination, Cao *et al.* show that the total removal of aldehyde reductase activity might not be the best scenario for alkane production. Only if the alkane/alcohol titer is within the range of 1.5–3.3, alkane titer is able to reach its peak. The intensive reduction of fatty alcohol byproduct will somehow lower the titer of alkane [20•]. It is worth mentioning that, in need of supplying the system with sufficient precursor, researchers often introduce different types of thioesterase into the system. These thioesterase successfully improve the concentration of internal free fatty acids by more than

Table 1

Metabolic strategies and engineered microorganisms for alka(e)ne production

Strain	Precursor + alka(e)ne generating enzyme	Other metabolic alterations[a]	Carbon source	Product chain length	Titer	Byproduct	Year	Reference
Escherichia coli	CAR + ADO	ΔyjgB; ΔyqhD; +sfp; +fdx; +fpr	Fatty acids	C3/C5/C7	40–134 μmol/L/OD	Fatty alcohols	2017	[45]
	AAR + ADO	ΔyqhD; +fadR	Glucose	C15/C17	255.6 mg/L	–	2016	[29]
	ACR + ADO	+UcFatB; +FadD	Glucose	C11/C13/C15	8.05 mg/g	Fatty aldehydes	2016	[27]
	CAR + ADO	FAS modules or reverse β-oxidation modules	Glucose or glycerol	C3/C4/C5/C7/C9	2.8–4.3 mg/L	–	2016	[25••]
	AAR + ADO	+Fd/FNR (among various metabolic alterations)	Glycerol	C15/C17	101.7 mg/L	Fatty alcohols/ aldehydes	2016	[20•]
	2-Keto-acid decarboxylase + mutated ADO	+alsS; +ilvCD; +Kivd; ΔALRs	Glucose	C3	267 μg/L	Isobutanol/ isobutyraldehyde	2016	[34]
	ACR + mutated ADO	+fadD; +thioesterase; ΔfadE	–	C11/C15	0.094 mg/L/OD	–	2016	[21]
	UcFatB2 + UndB	–	Glucose	C11	55 mg/L	–	2015	[39••]
	Mutated ADO	+atoB-TPC7 route; +ferredoxin PetF; ΔyqhD	Glucose	C3	3.40 mg/L	Butanol	2015	[35]
	AAR + ADO	+FAS	Glucose	C15	57 mg/L	Fatty alcohols	2015	[9]
	Spatial organized fusion ADO-AAR	–	Glucose	C13/C15/C17	44 mg/L	–	2014	[37]
	$oleT_{JE}$	pMSD8; pMSD15(fatty acid overproducing strain)	Glucose	C13/C15/C17	97.6 mg/L	–	2014	[55]
	CAR + ADO	+Sfp; +C_4 thioesterase; +PetF; Fpr; +catalase (KatE); ΔYqhD; Δahr	Glucose	C3	32 mg/L	Butanol	2014	[28]
	AAR + ADO	+fabH2	Glucose + 6.5 mM propanoate	C13/C14/C15/C16/C17	98.3 mg/L	–	2013	[10]
	FAR + ADO	+thioesterase; +FabH2; +BCKD	Fatty acids (branched and linear) or Glucose	C13/C15/C16/C17 (branched and linear)	2–5 mg/L	Fatty alcohols	2013	[22]
	ACR + CER1	ΔfadE; ΔfadR; +fadD; +TesA'	Glucose	C9/C12/C13/C14	580.8 mg/L	Fatty alcohols	2013	[23]
	CAR + ADO	+TesA′; +Sfp	Glucose	C11/C13/C15/C17	2 mg/L	Fatty alcohols	2013	[24]
	AAR + ADO	–	Glucose	C13/C15/C17	300 mg/L	Fatty alcohols/ aldehydes	2010	[6]
S. cerevisiae	CAR + ADO	ΔPOX1; ΔHFD1; ΔADH5; +FNR/Fd	Glucose	C11/C13/C15/C17	1.14 mg/L	Fatty alcohols (∼6.5 mg/L)	2017	[14•]
	DOX + ADO	–	Fatty acids	C12/C14/C16	337.8 μg/L	Fatty alcohols/ aldehydes	2017	[11•]
	CAR + ADO	+Chimeric citrate lyase (ACL/ME/Ctp1/'Mdh3); +FAS; +ACC; ΔAdh5; Δfaa1; Δfaa4; ΔPOX1; ΔHFD1	Glucose	C13/C15/C17	0.82 mg/L	Fatty alcohols (5 mg/L)	2016	[7]
	CAR + ADO	+NpgA; ΔHFD1; ΔPOX1; ΔPEX31; ΔPEX32; ΔPEX34	Glucose	C15/C17	3.55 mg/L	Fatty alcohols (∼7 mg/L)	2016	[15••]
	AAR + ADO	+Fdx/FNR; Δ HFD1	Glucose	C13/C15/C17	22 μg/g	–	2015	[16]
	OleT	Δfaa1; Δfaa4; Δctt1; Δcta1; Δccp1; PTEF1-HEM3; PTEF1-$oleT_{JE}$-CO (pRS41K)	Glucose	C17/C19	3.7 mg/L	–	2015	[56]
	CYP4G1	+CPR	–	C23/C25/C27	Low and erratic	–	2012	[30]