

## Types of liquid fuels.

Fuel type	Major components	Important property	Biosynthetic alternatives
Gasoline	C <sub>4</sub> –C <sub>12</sub> hydrocarbons Linear, branched, cyclic, aromatics Anti-knock additives	Octane number <sup>a</sup> Energy content <sup>b</sup> Transportability	Ethanol, <i>n</i> -butanol and <i>iso</i> -butanol Short chain alcohols Short chain alkanes
Diesel	C <sub>9</sub> –C <sub>23</sub> (average C <sub>16</sub> ) Linear, branched, cyclic, aromatic Anti-freeze additives	Cetane number <sup>c</sup> Low freezing temperature Low vapor pressure	Biodiesel (FAMEs) Fatty alcohols, alkanes Linear or cyclic isoprenoids
Jet fuel	C <sub>8</sub> –C <sub>16</sub> hydrocarbons Linear, branched, cyclic, aromatic Anti-freeze additives	Very low freezing temperature Net heat of combustion Density	Alkanes Biodiesel Linear or cyclic isoprenoids

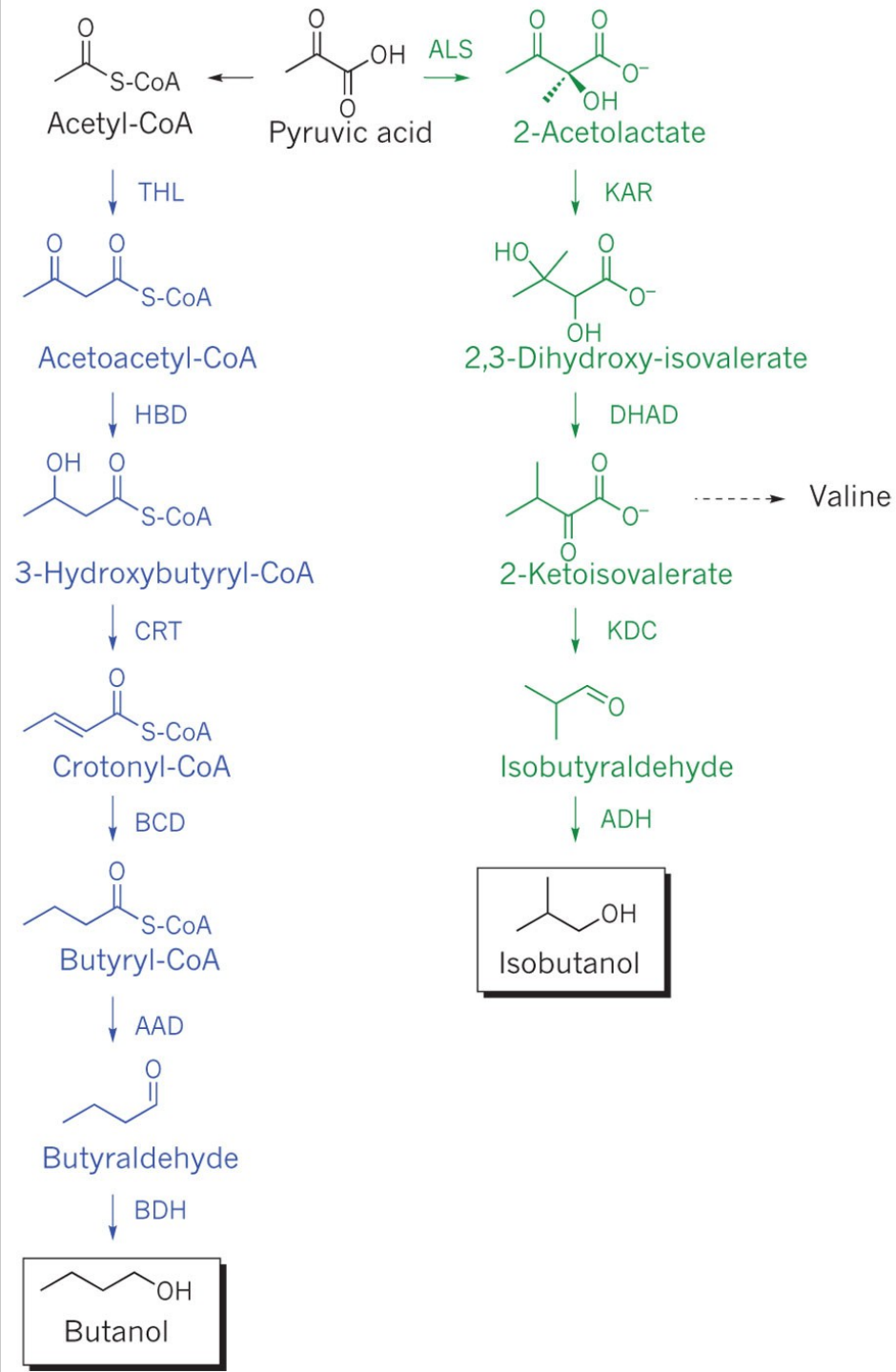
<sup>a</sup> A measurement of its resistance to knocking. Knocking occurs when the fuel/air mixture spontaneously ignites before it reaches the optimum pressure and temperature for spark ignition.

<sup>b</sup> The amount of energy produced during combustion. The number of C–H and C–C bonds in a molecule is a good indication of how much energy a particular fuel will produce.

<sup>c</sup> A measurement of the combustion quality of diesel fuel during compression ignition. A shorter ignition delay, the time period between the start of injection and start of combustion of the fuel is preferred, and the ignition delay is indexed by the cetane number.

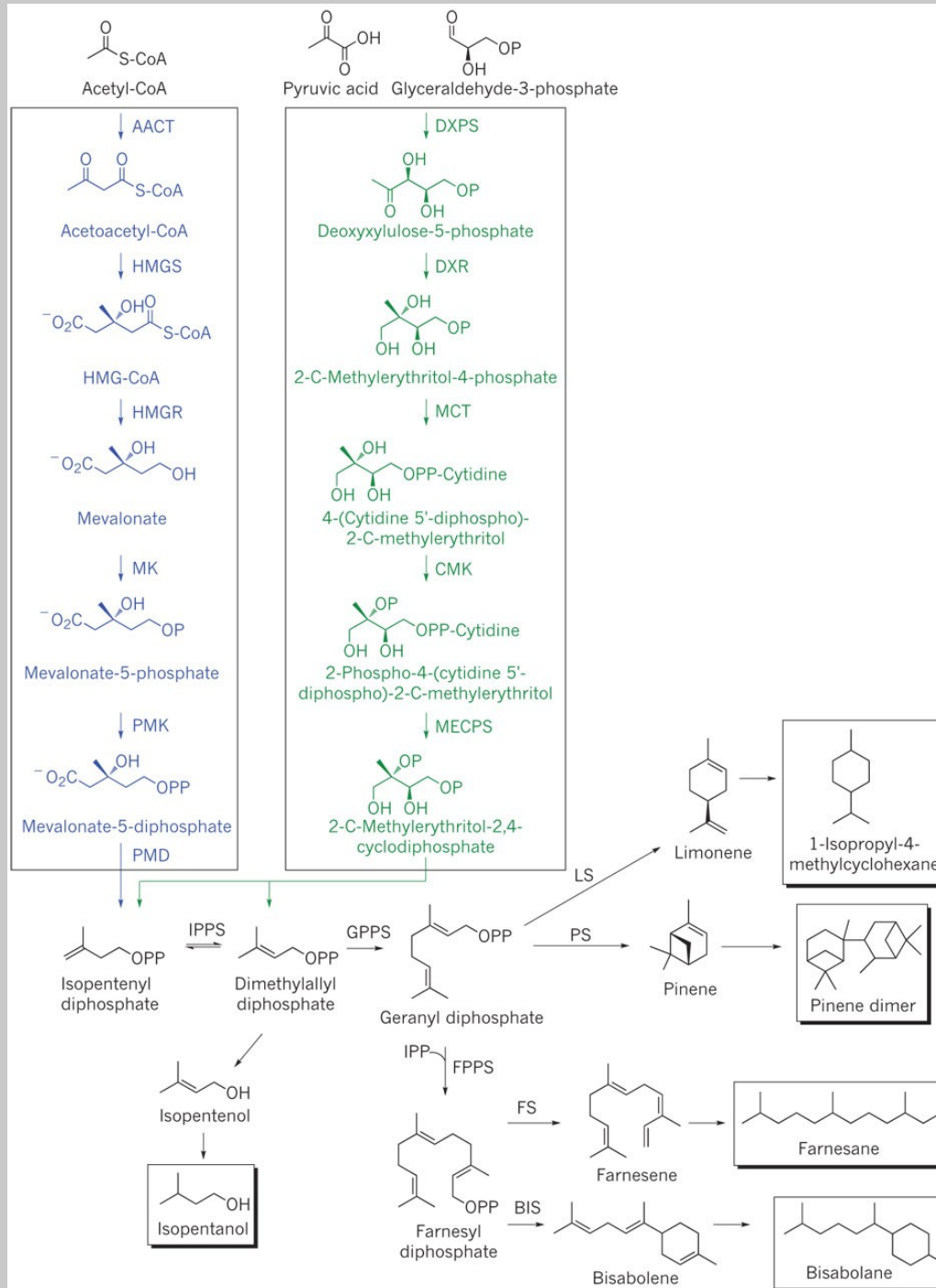


## Butanolna pot pri klostridijih

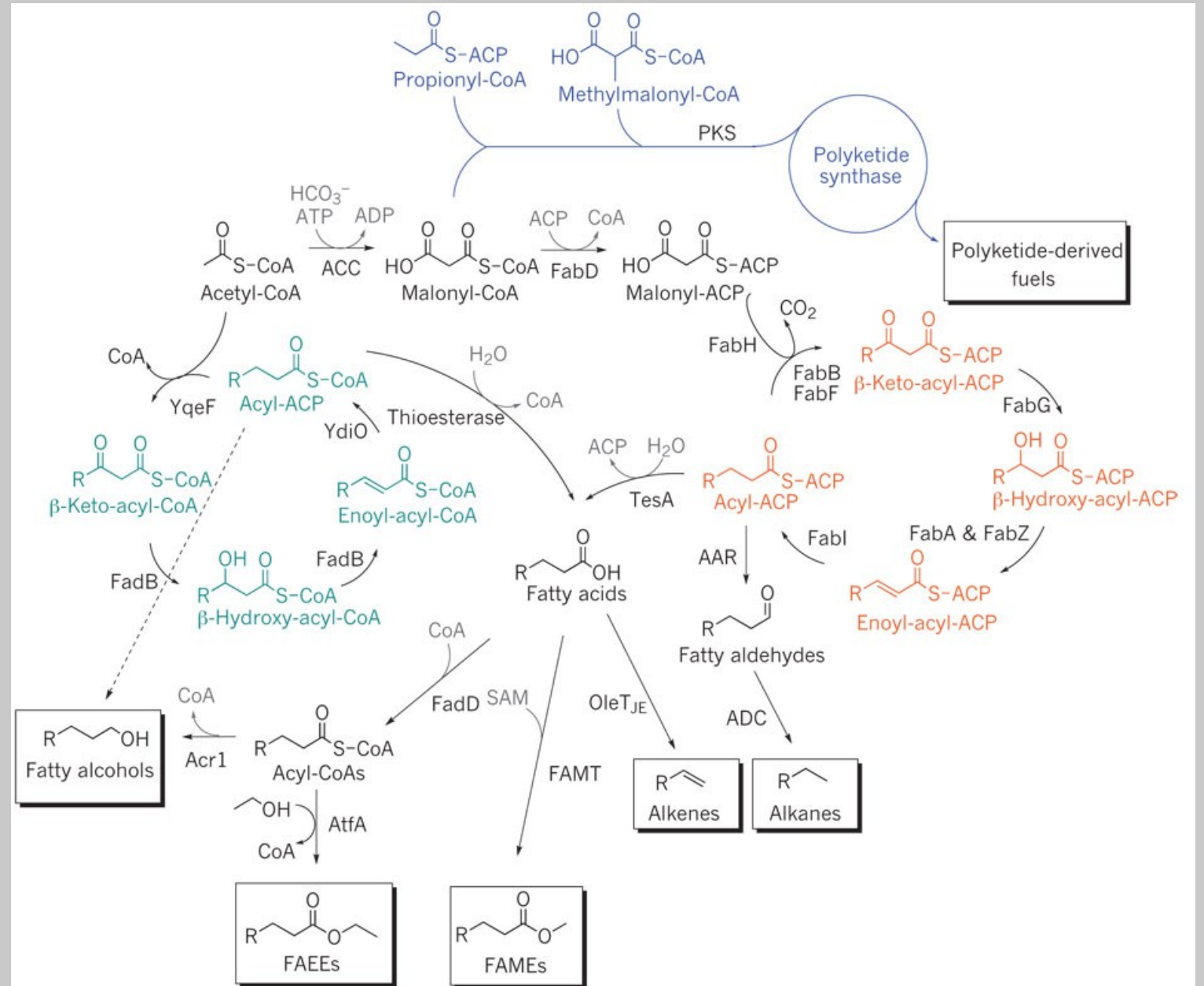


## Pot 2-keto kislin

# Mevalonatna pot (modro) in pot deoksiksiluloza-5-fosfata: načini priprave izoprenoidnih goriv



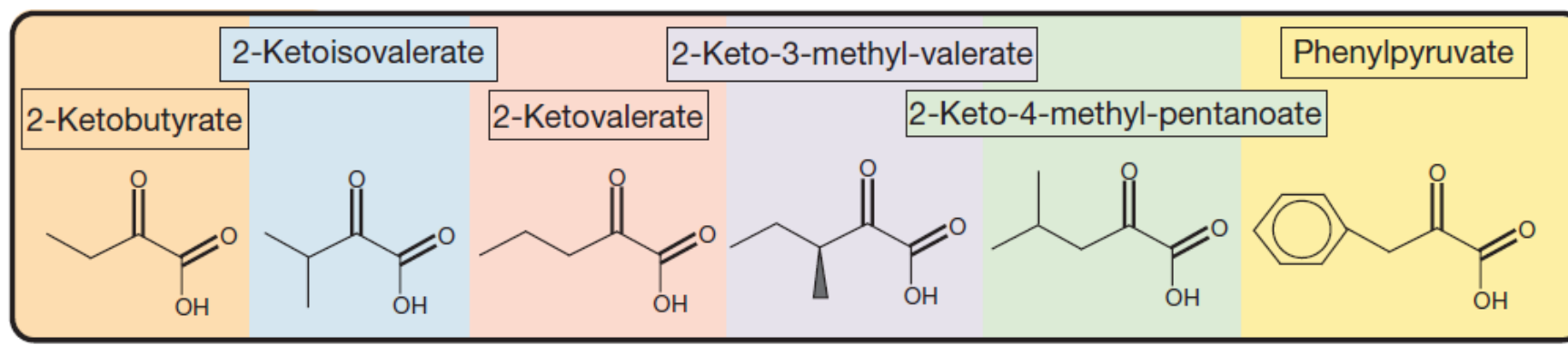
# Metabolne poti za pripravo biogoriv na osnovi maščobnih kislin in poliketidov



**Table 2****Examples of different metabolic engineering strategies for increasing yields of various biofuels.**

Biofuel	Strategy	Yield <sup>a</sup>	Reference
Ethanol	Engineering of phosphoketolase pathway to increase the availability of NAD <sup>+</sup> during xylose metabolism in <i>S. cerevisiae</i>	0.42	[29]
	Modulation of redox metabolism by modifying ammonium assimilation in order to increase xylose utilization by deleting GDH1 and overexpressing GDH2 in <i>S. cerevisiae</i>	0.34	[30]
	<i>In silico</i> gene insertion predicted that heterologous expression of <i>gapN</i> would increase ethanol yield and eliminate glycerol production in <i>S. cerevisiae</i> during growth on glucose and xylose	0.36	[48*]
	EM analysis directed knockout strategy optimized ethanol production from pentose and hexose and removed extraneous pathways in <i>E. coli</i>	0.36	[42]
Butanol	Expression of different gene combinations for butanol production in <i>E. coli</i> modeled after the <i>C. acetobutylicum</i> pathway; deletion of competing pathways; increased NADH availability	.0056	[18]
	Expression of different gene combinations for isobutanol production in <i>E. coli</i> modeled after the amino acid catabolic pathway; deletion of competing pathways; overexpression of valine biosynthetic genes	0.35	[4**]
Pentanol	Expression of different gene combinations for isopentanol production in <i>E. coli</i> modeled after the amino acid catabolic pathway; deletion of competing pathways; overexpression of leucine biosynthetic genes	0.11	[22]
Propanol	Expression of different gene combinations for propanol production in <i>E. coli</i> modeled after the <i>C. beijerinckii</i> pathway	0.14	[17]

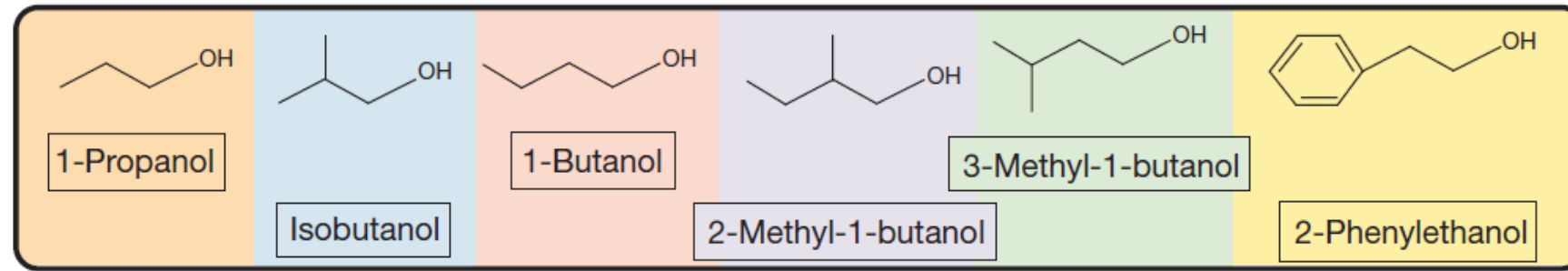
<sup>a</sup> Reported yield [g biofuel/g carbon source].



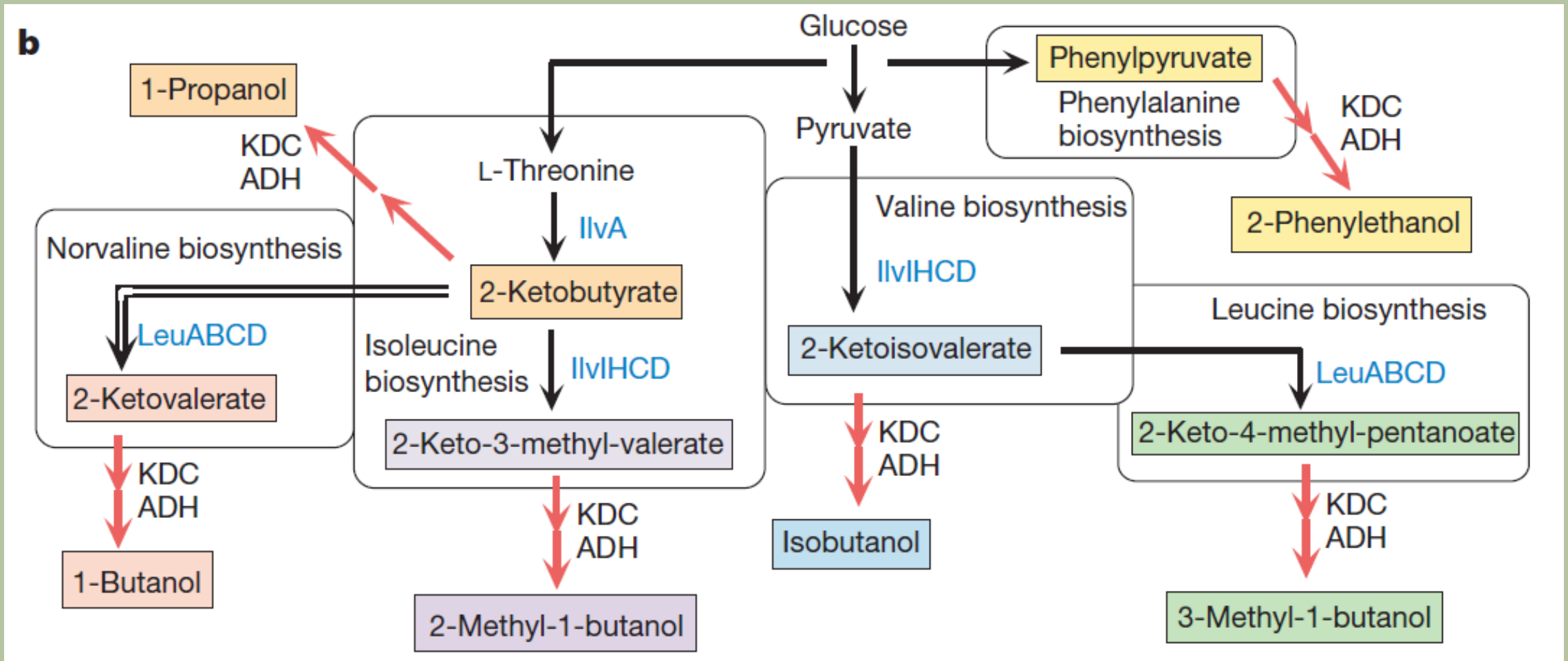
2-Keto-acid decarboxylase



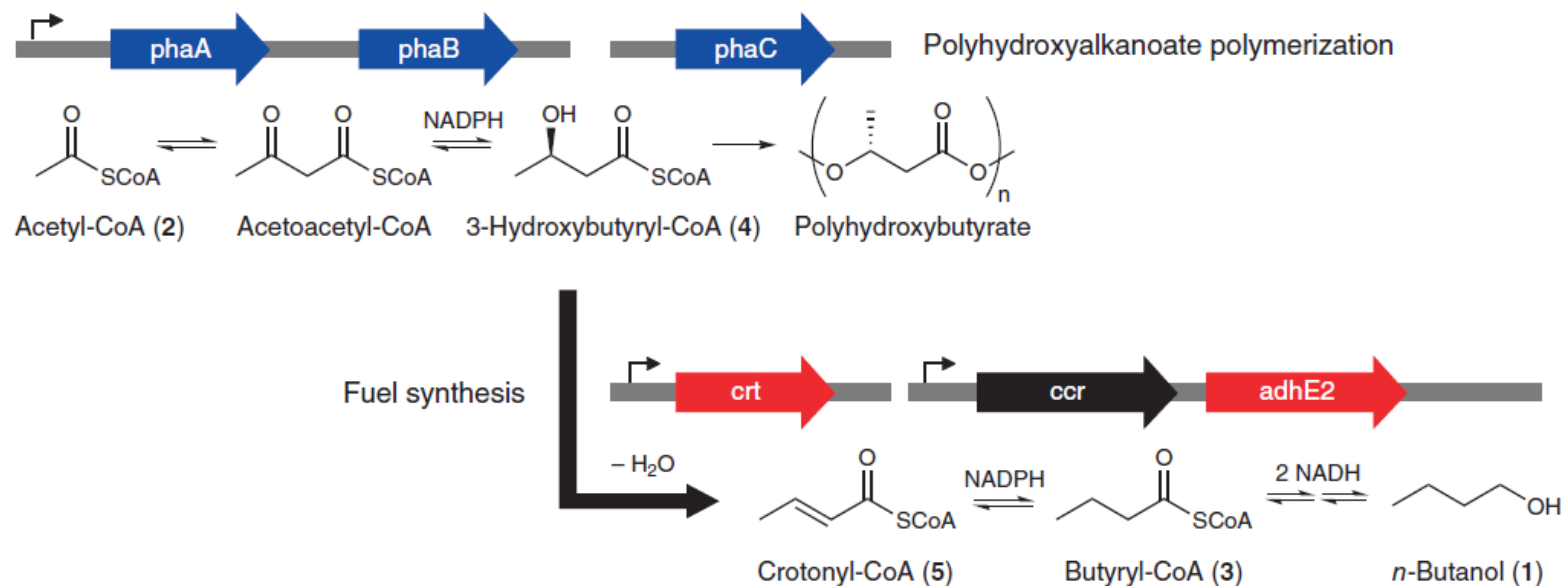
Alcohol dehydrogenase



# Preureditev biosintezne poti aminokislin v *E. coli* za prekomerno proizvodnjo višjih alkoholov

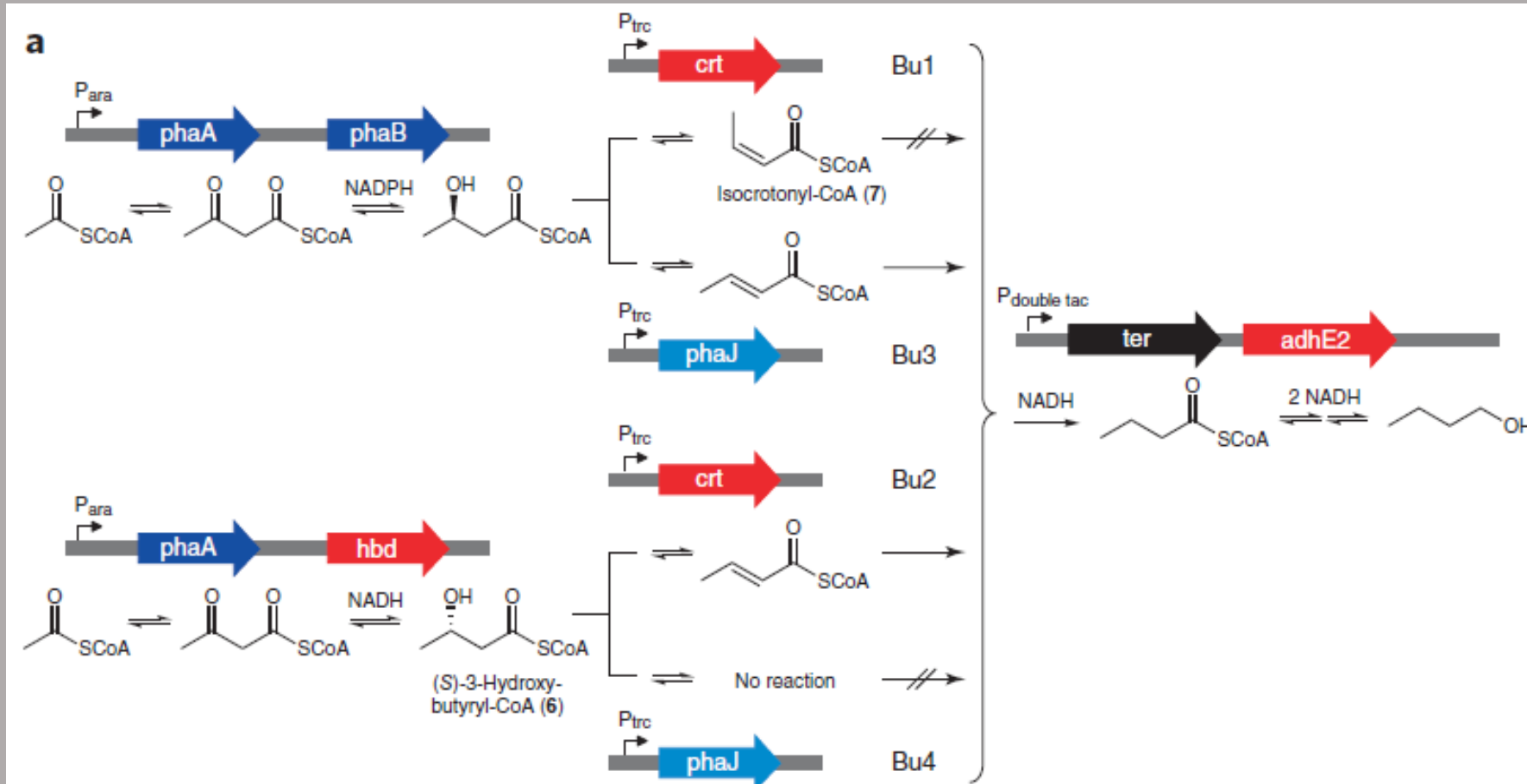




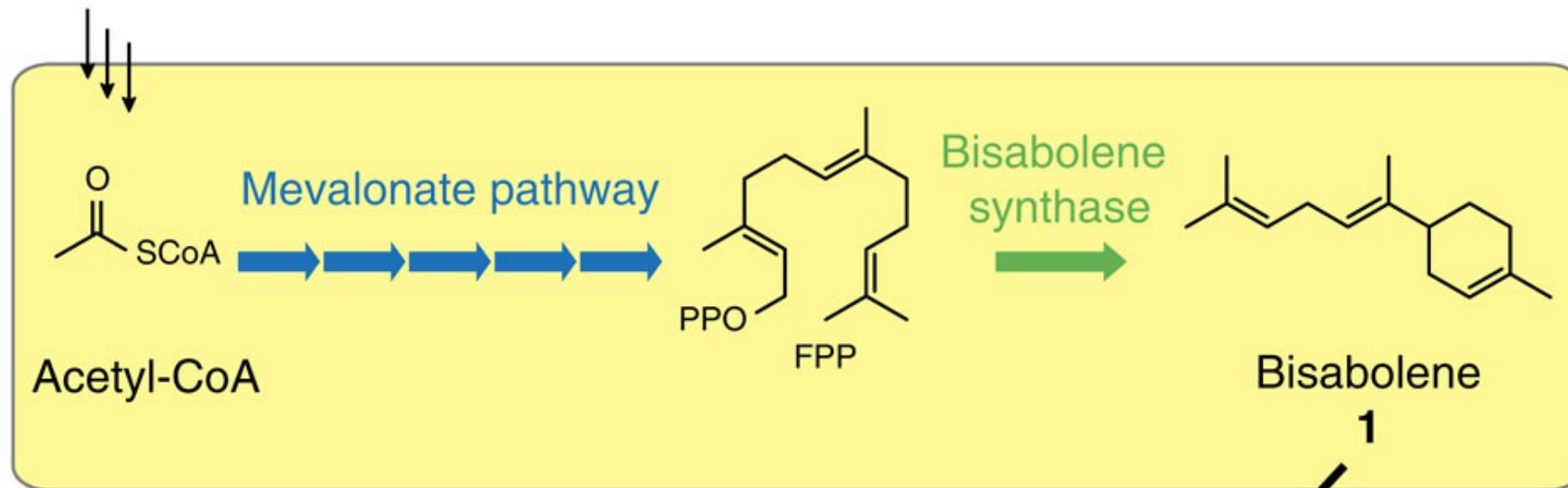


**Scheme 1 | Comparison of a high-yielding pathway for production of polyhydroxyalkanoates (PHAs) to the design of a chimeric pathway for fuel synthesis derived from three different organisms.** Blue, *R. eutrophus*; red, *C. acetobutylicum*; black, *S. collinus*; *phaA*, acetoacetyl-CoA thiolase/synthase; *phaB*, 3-hydroxybutyryl-CoA dehydrogenase; *phaC*, PHA synthase; *crt*, crotonase; *ccr*, crotonyl-CoA reductase; *adhE2*, bifunctional butyraldehyde and butanol dehydrogenase.

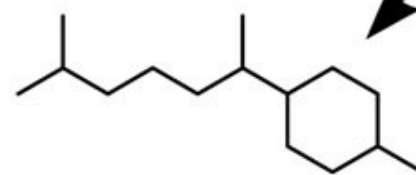
Optimizacija biosintezne poti n-butanola s posredovanjem trans-enoil-CoA reduktaze (ter):  
identifikacija ozkih grl



Simple sugars



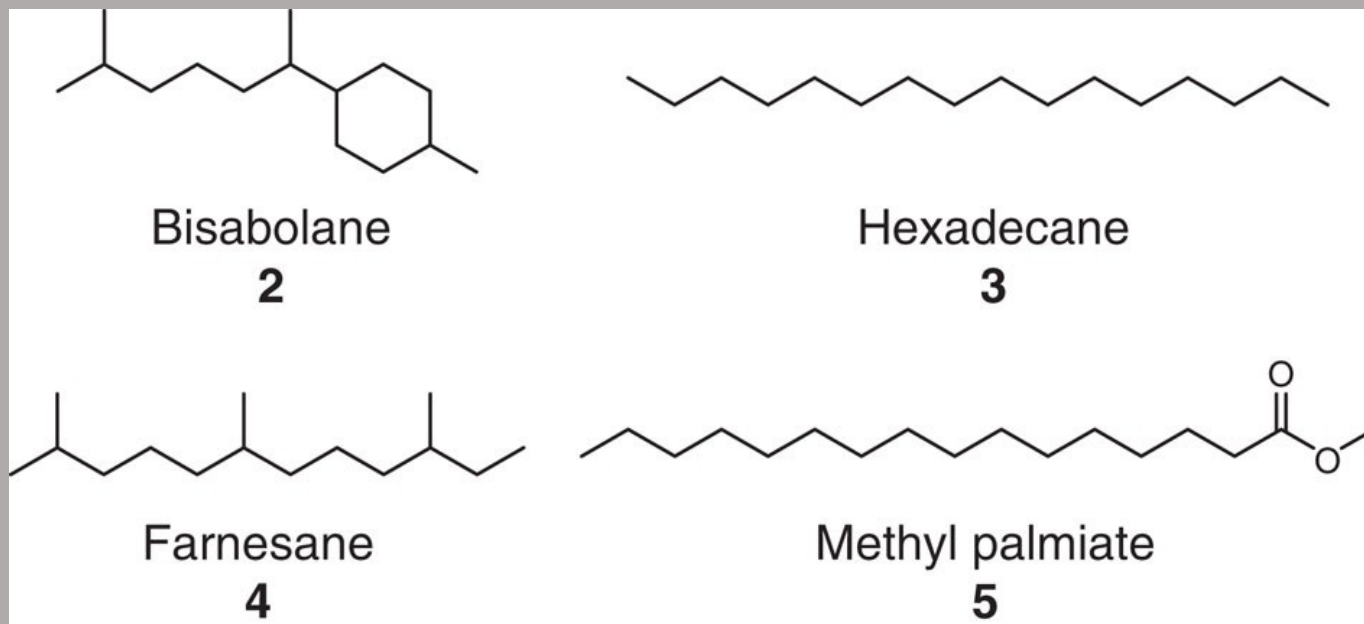
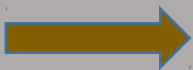
Biosynthetic alternative to D2 diesel fuel



Bisabolane  
2

Chemical hydrogenation

dizel



metilna estra  
mašč. kislin



**Table 1 | Fuel properties of D2 diesel fuel and biosynthetic alternatives.**

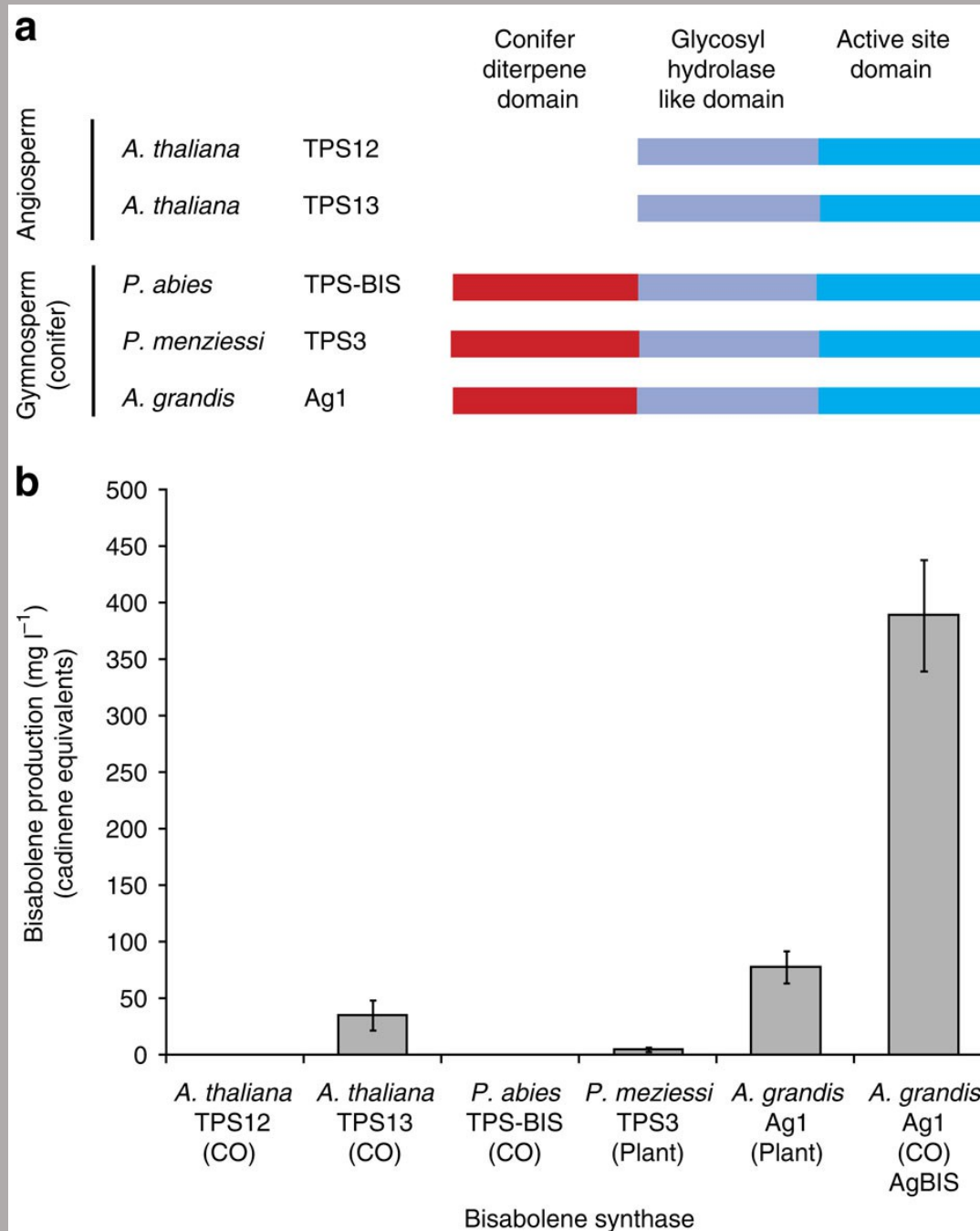
Properties	D2 Diesel fuel*	Biodiesel†	Hydrogenated commercial bisabolene‡
Density (g ml <sup>-1</sup> )	0.85	0.88	0.82
API Gravity	35.0	29.3	41.1
Flash point (°C)	60–80	100–170	111
Kinetic viscosity (mm <sup>2</sup> s <sup>-1</sup> )	1.3–4.1	4.0–6.0	2.91
Boiling point (°C)	180–340	315–350	267
Cloud point (°C)	–35 to 5	–3 to 15	< –78
Cetane number	40–55	48–65	41.9

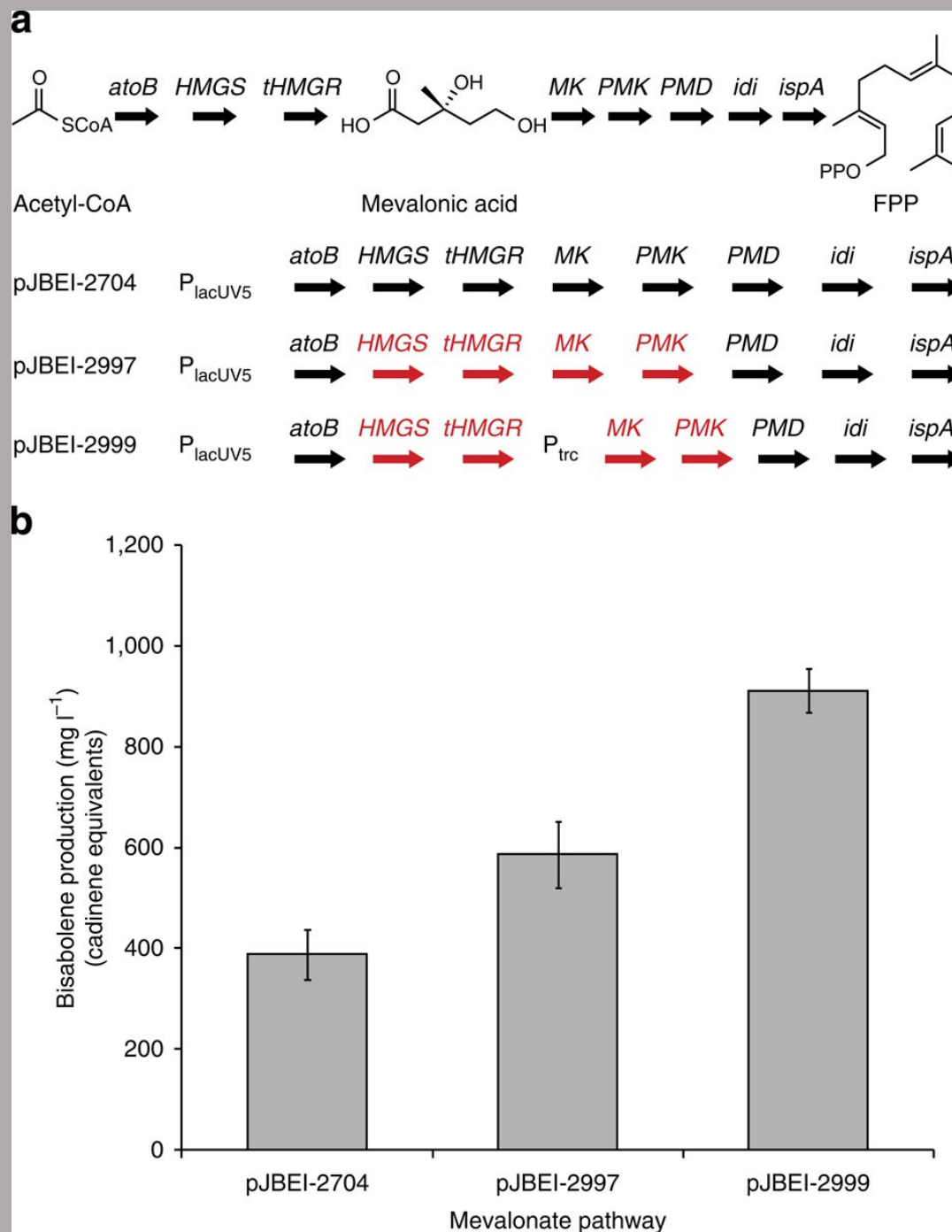
\*Biodiesel Handling and Use Guide, National Renewable Energy Laboratories<sup>15</sup>.

†Biodiesel: Fatty acid methyl esters. Biodiesel Handling and Use Guide, National Renewable Energy Laboratories<sup>15</sup>.

‡Hydrogenated commercial bisabolene: bisabolane: ~50%, farnesane: ~20%, partially hydrogenated bisabolene: ~20%, and aromatized bisabolene: ~7% (Fig 3b).

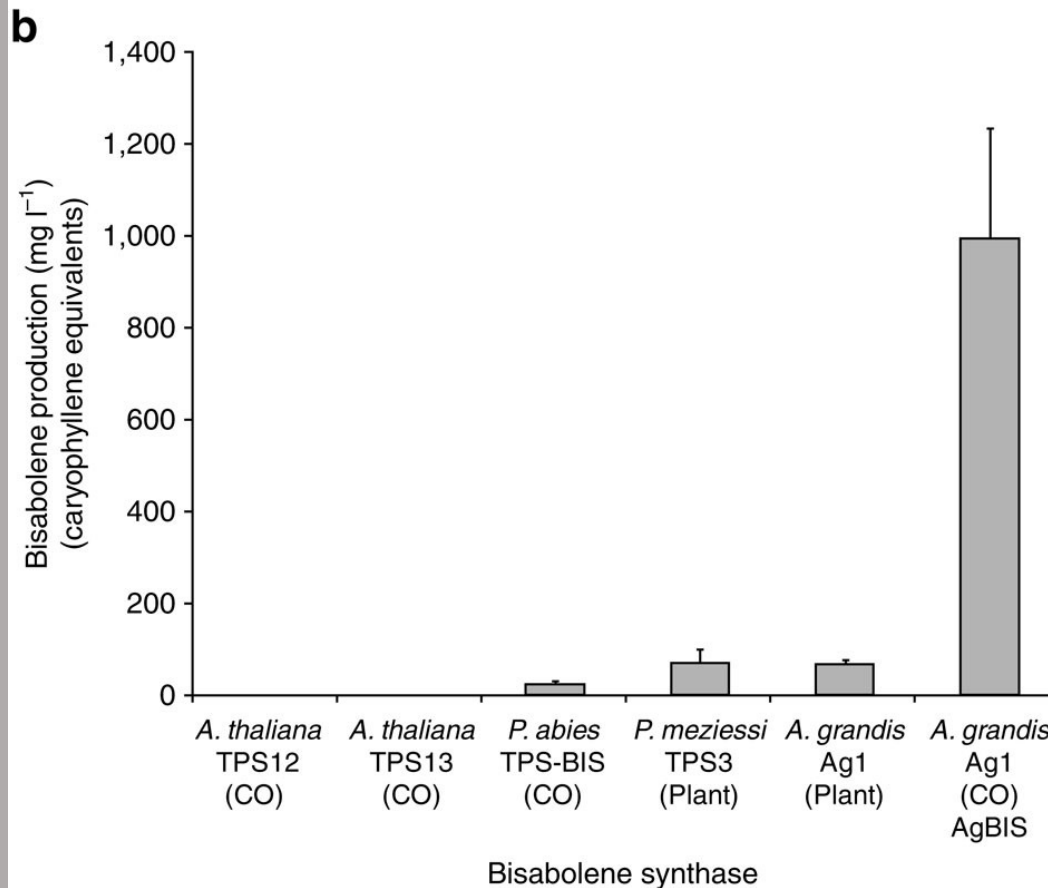
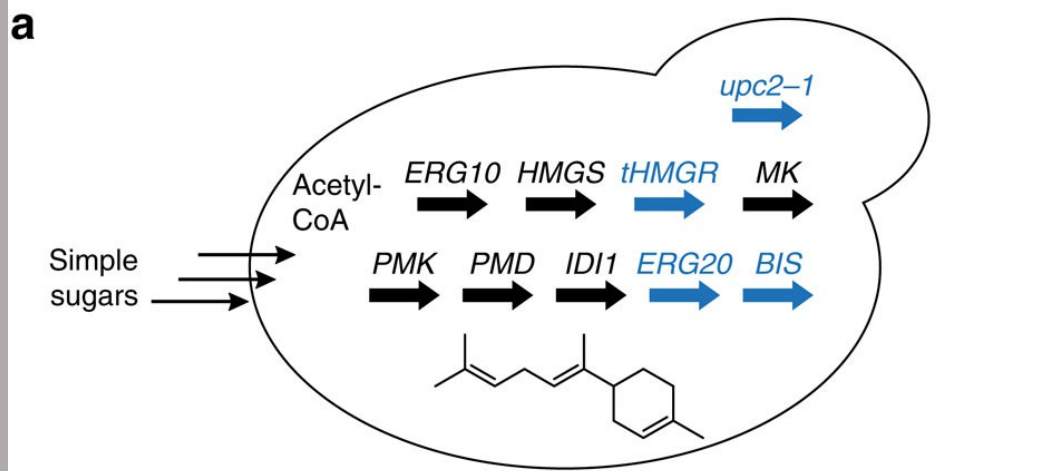
# Genska struktura in aktivnost bisabolen sintaz





Izboljšave mevalonatne poti za pretvorbo Ac-CoA v farnezildifosfat (FPP) geni iz *S.c.* (velike črke) in *E. coli* (male črke), sintezni geni s prilagojeno rabo kodona za *E. coli* (rdeče)

Biosinteza bisabolena ob uporabi sintaze AgBIS v *E. coli*



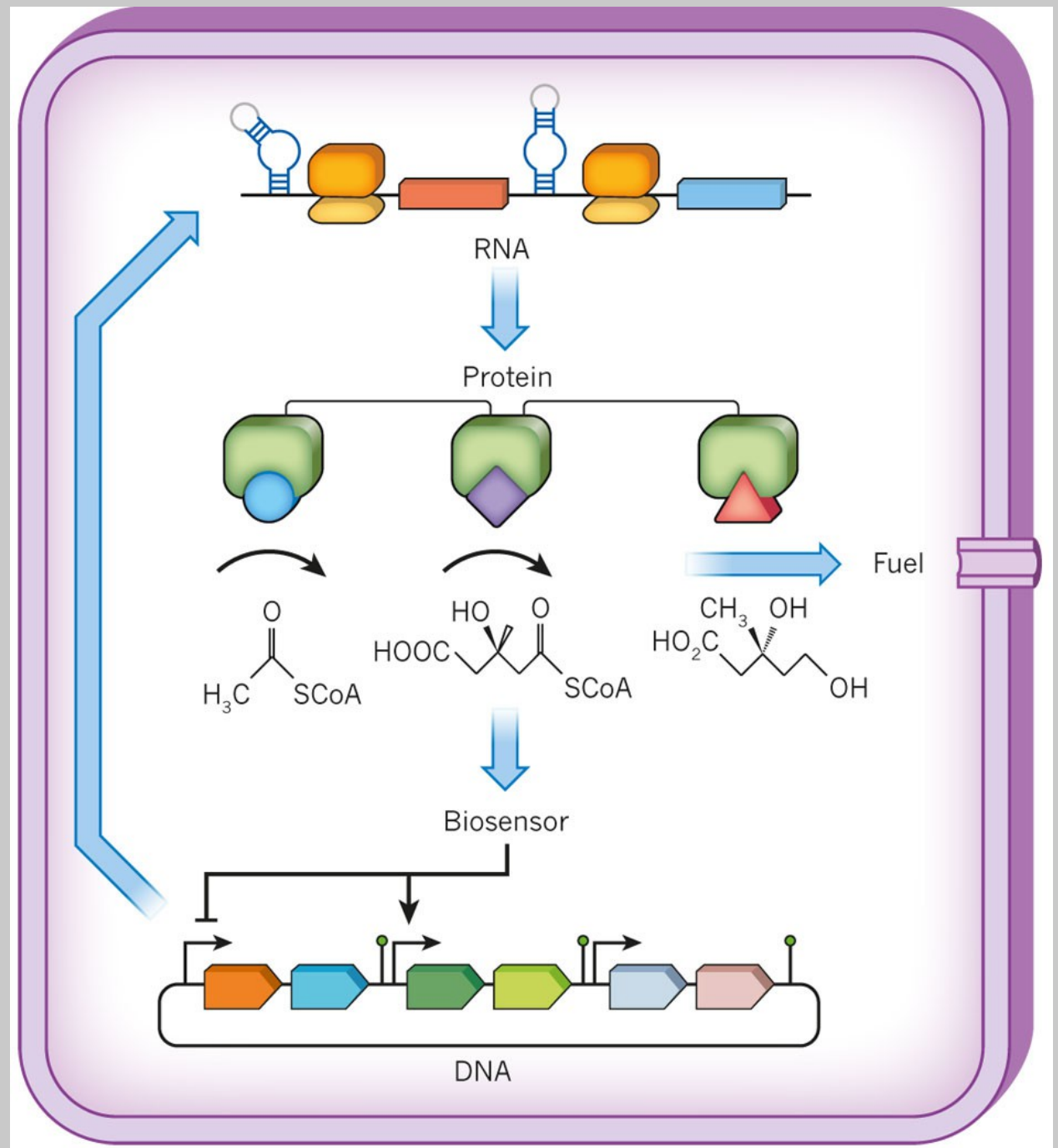
## Prilagoditev kvasovke za proizvodnjo bisabolena

celice so predhodno optimizirali za proizvodnjo amorfadiena (→ artemizinska kislina)

encimi označeni z modro so bili prekomerno izraženi

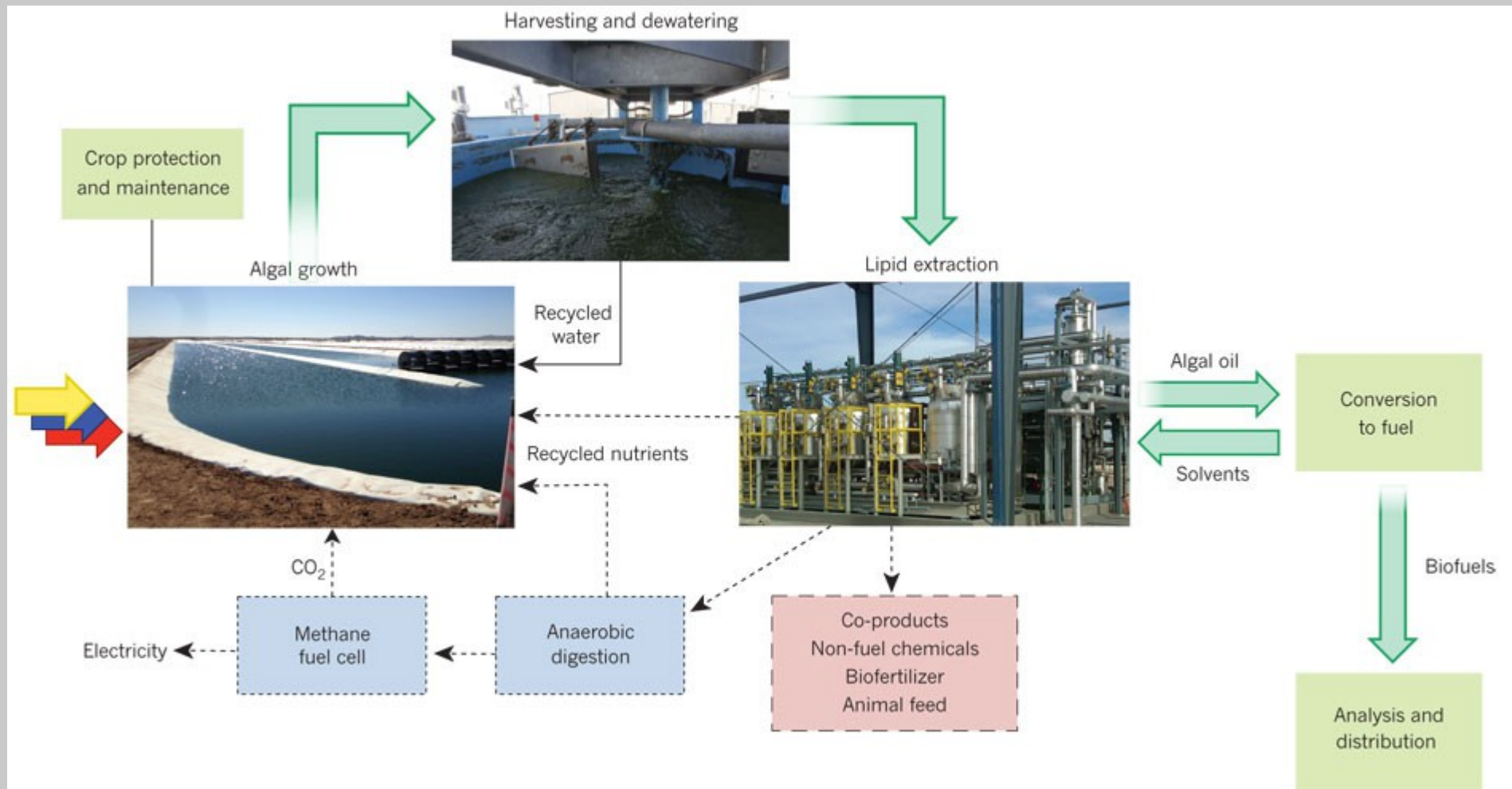
Biosinteza bisabolena ob uporabi različnih sintaz v *S. c.*

# Sinteznobiološki pristop k proizvodnji biogoriv

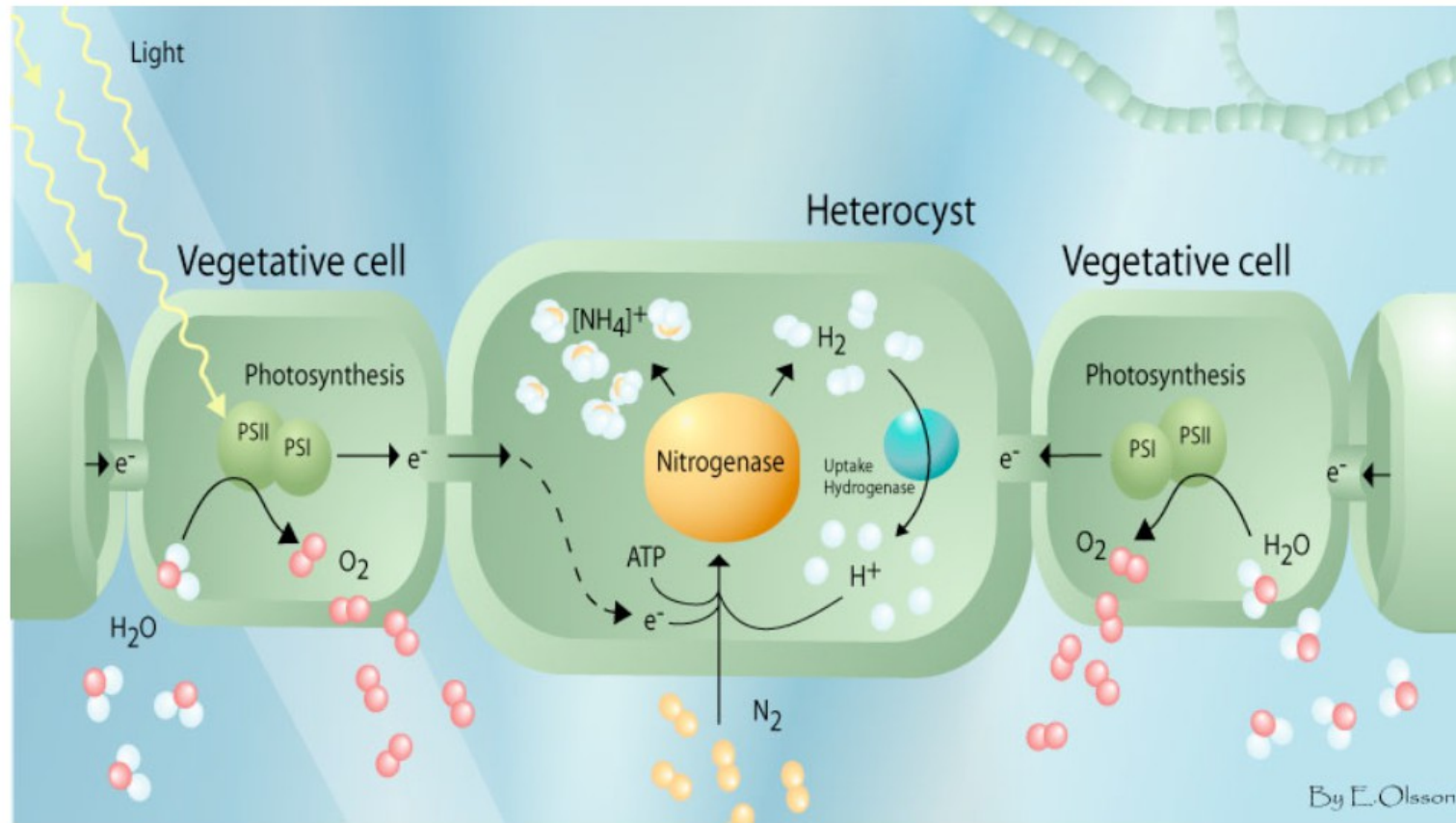








## Hydrogen production by heterocystous cyanobacteria



By **nitrogenase** catalysed reaction:

