Advanced School on the Present and Future of Bioenergy
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School of Chemical Engineering (FEQ) / University of Campinas (Unicamp)
Campinas - Sp - Brazil

Biofuels beyond ethanol

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Lecture overview

- Introduction

Why do we need alternatives to ethanol (and biodiesel)?

Physicochemical parameters of biofuels

- Metabolic engineering for biofuel production

Example - isobutanol

- Summary/Outlook

Requirements for advanced biofuels – the drop-in concept

- high energy density (energy per mass [MJoule/kg] or volume [MJoule/L])
- low hygroscopicity (to prevent corrosion; compatibility with current infrastructure and engines)
- miscibility with conventional fuels
- acceptable energy per CO_2 ratio

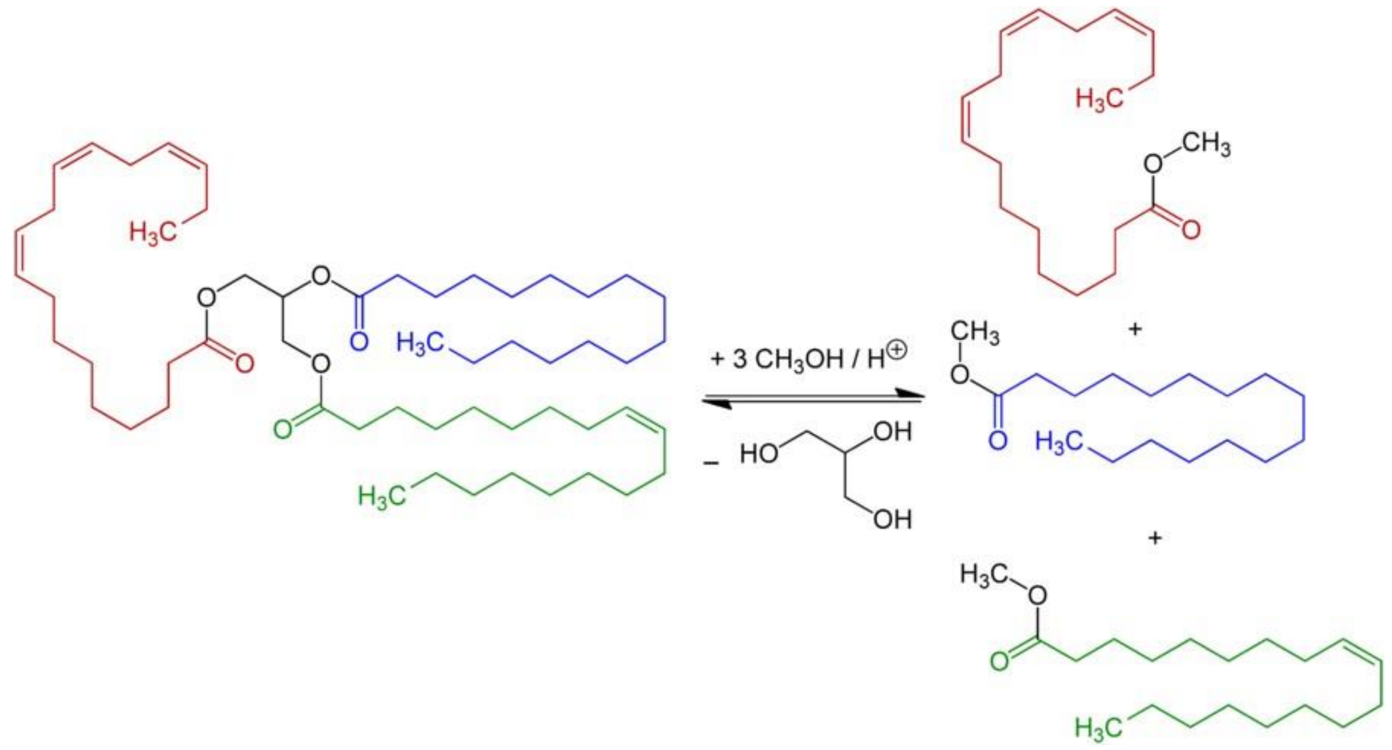
Physicochemical properties of fuels

Biofuel	Specific energy [MJ/kg]	Energy density [MJ/L]	Hygroscopicity	Energy/CO2 [MJ/kg]
Gasoline	45 – 48.3	32 – 34.8	no	13.64-14.64
Diesel	48.1	40.3	no	14.15
Jet fuel	46	37.4	no	n.i.
Ethanol	23.4 –26.8	18.4 - 21.2	yes	12.25-14.03
Butanol	36.6	29.2	no	15.16
Biodiesel	37.8	33.3 – 35.7	no	13.26

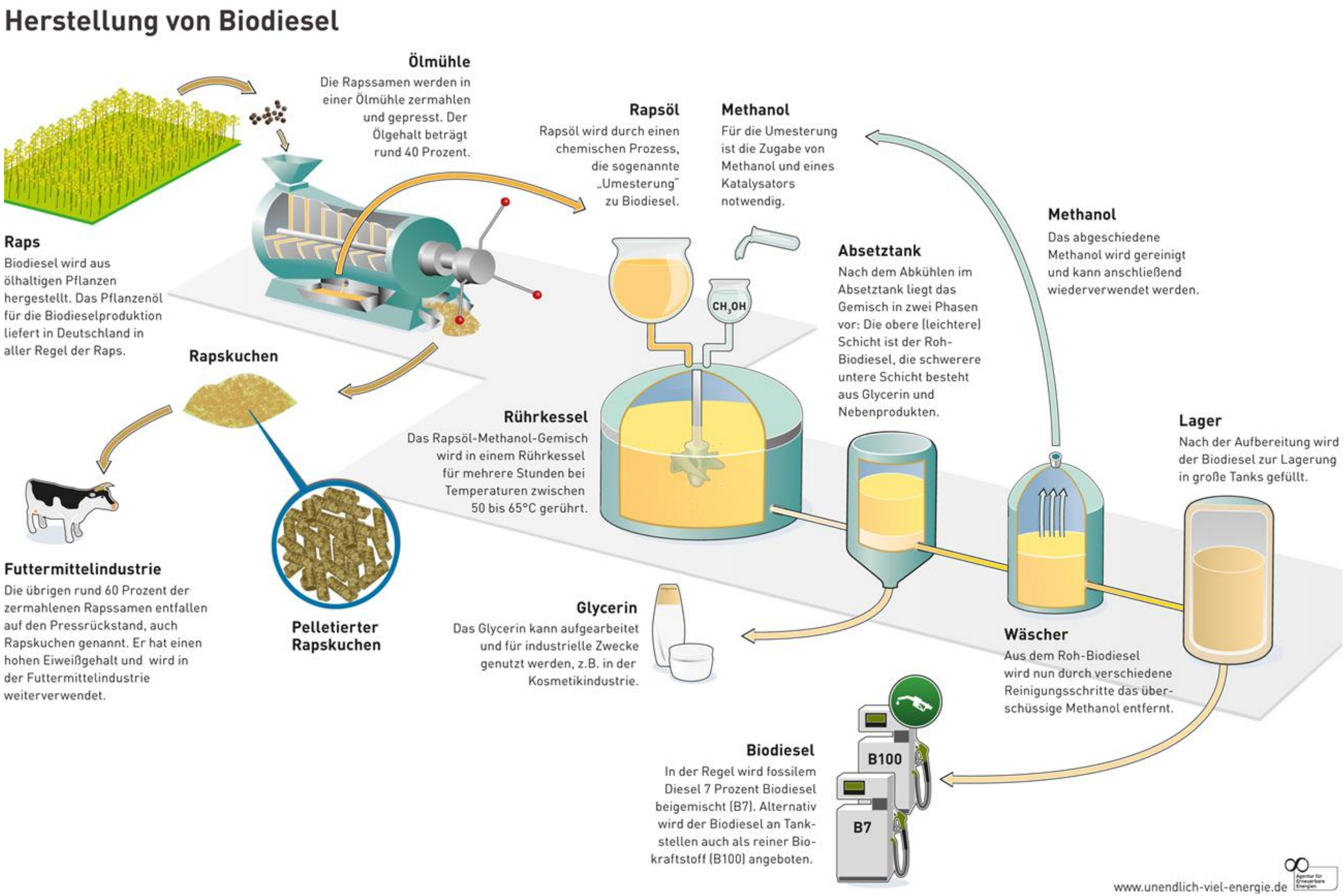
http://en.wikipedia.org/wiki/Energy_content_of_biofuel

A current alternative to Diesel: 1st generation Biodiesel

- fatty acid-ethanol or –methanol esters (FAEE or FAME)



Production of 1st generation Biodiesel



www.ufop.de

Biodiesel has a low energy yield per hectare

Table 2.1 The annual energy output of various biofuels

	Liters per hectare* (1)	MJ per hectare** (2)	KWh per hectare (3)	KWh per sq m (4)
Ethanol from				
Corn[1]	3,730	89,517	24,886	2.489
Corn[2]	3,003	72,063	20,033	2.003
Corn stover[1]	1,544	37,051	10,300	1.030
Miscanthus[1]	6,945	166,676	46,336	4.634
Switchgrass[1]	2,009	48,208	13,402	1.340
Sugar cane[2]	6,744	161,861	44,997	4.500
Biodiesel[3]				
Oil palm	4,752	156,810	43,593	4.359
Coconut	2,151	70,997	19,737	1.974
Rapeseed	954	31,485	8,753	0.875
Peanut	842	27,781	7,723	0.772
Sunflower	767	25,312	7,037	0.704
Soybean	524	17,286	4,806	0.481

Nelson in „Handbook of Bioenergy, Economics and Policy", Springer (2010)

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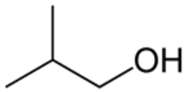
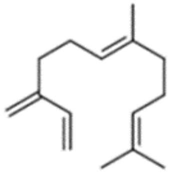
Alternatives for both ethanol and biodiesel are needed

Nelson in „Handbook of Bioenergy, Economics and Policy“, Springer (2010)

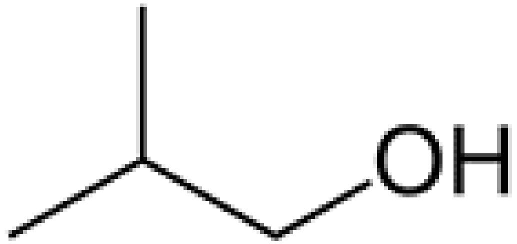
Advanced biofuels that can be obtained by metabolic engineering of microbes



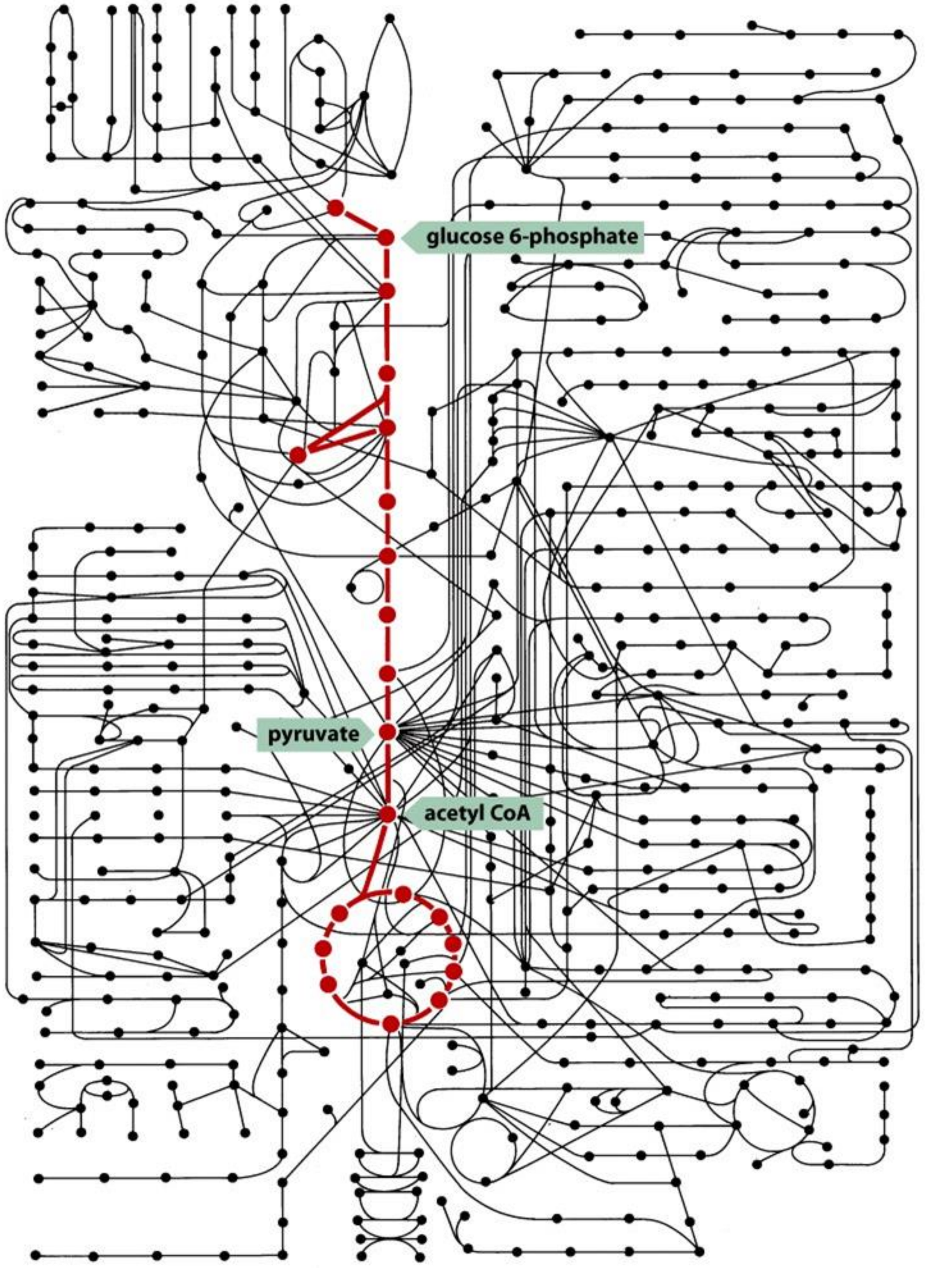


Compound (Examples)	Chemical classification	Subsitutes for
Isobutanol	Higher alcohols	Gasoline
Nonane, Tridecane	Alkanes/Alkenes	Gasoline
Farnesene, Amorphadiene	Isoprenoids	Jet Fuel, Diesel

Properties of isobutanol



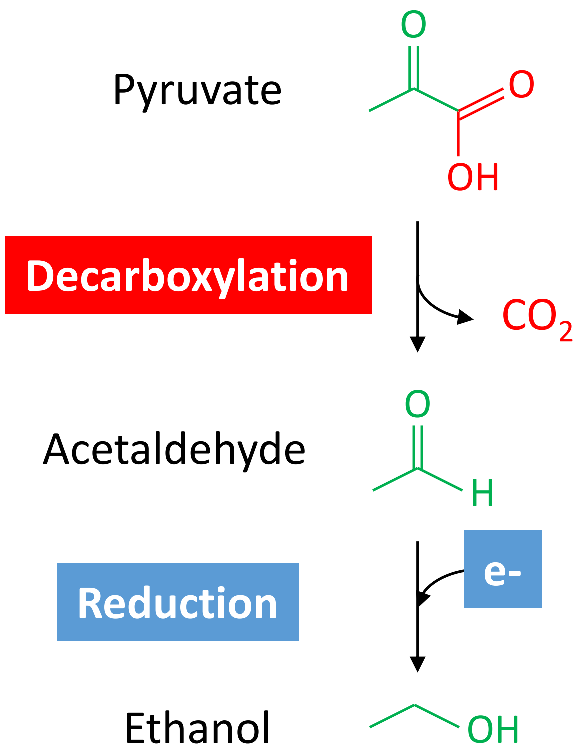
- high energy density (98% of gasoline)
- less miscible with water
- far less corrosive
- can be distributed through existing infrastructure
- can replace fossil fuels up to 100%
- can be blended with diesel
- can be used as a building block for other compounds, e.g. PTA >plastics

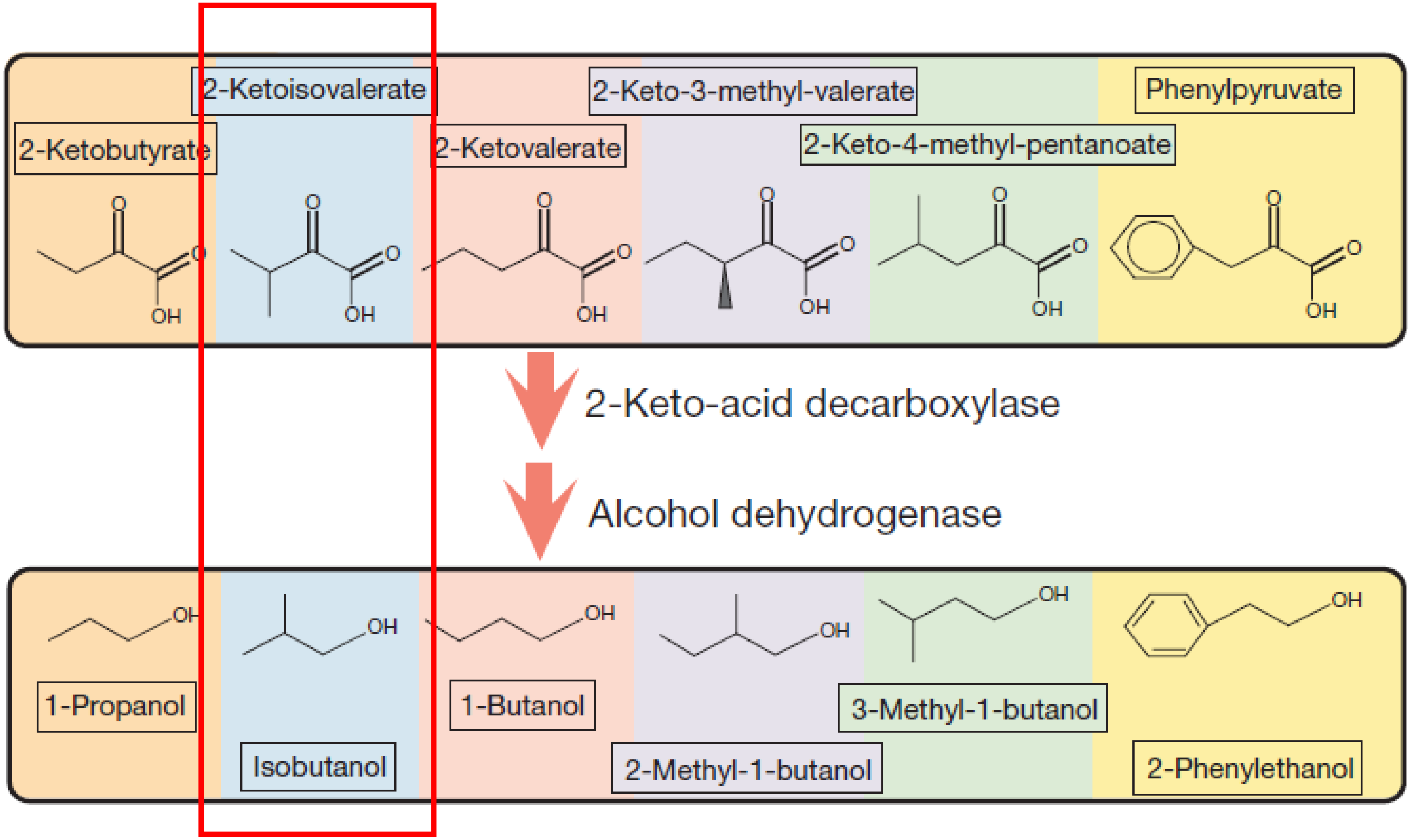


Principles of metabolic engineering

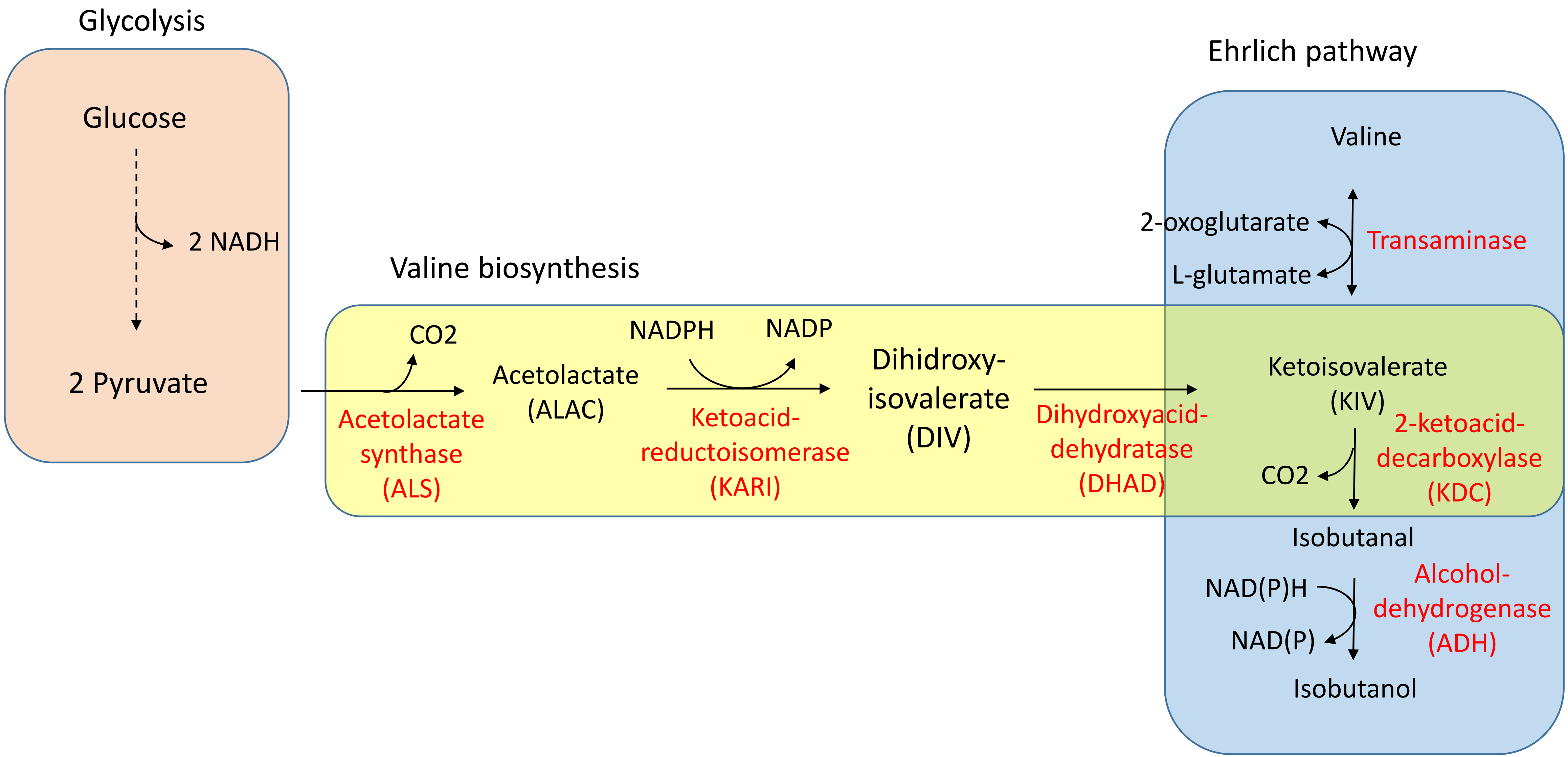
- exploring nature´s emzyme repertoire
- thermodynamic constraints/driving forces
- redox cofactor balance
- rational enzyme engineering
- cellular compartmentalization (eukaryotes)

2-ketoacid derivatives

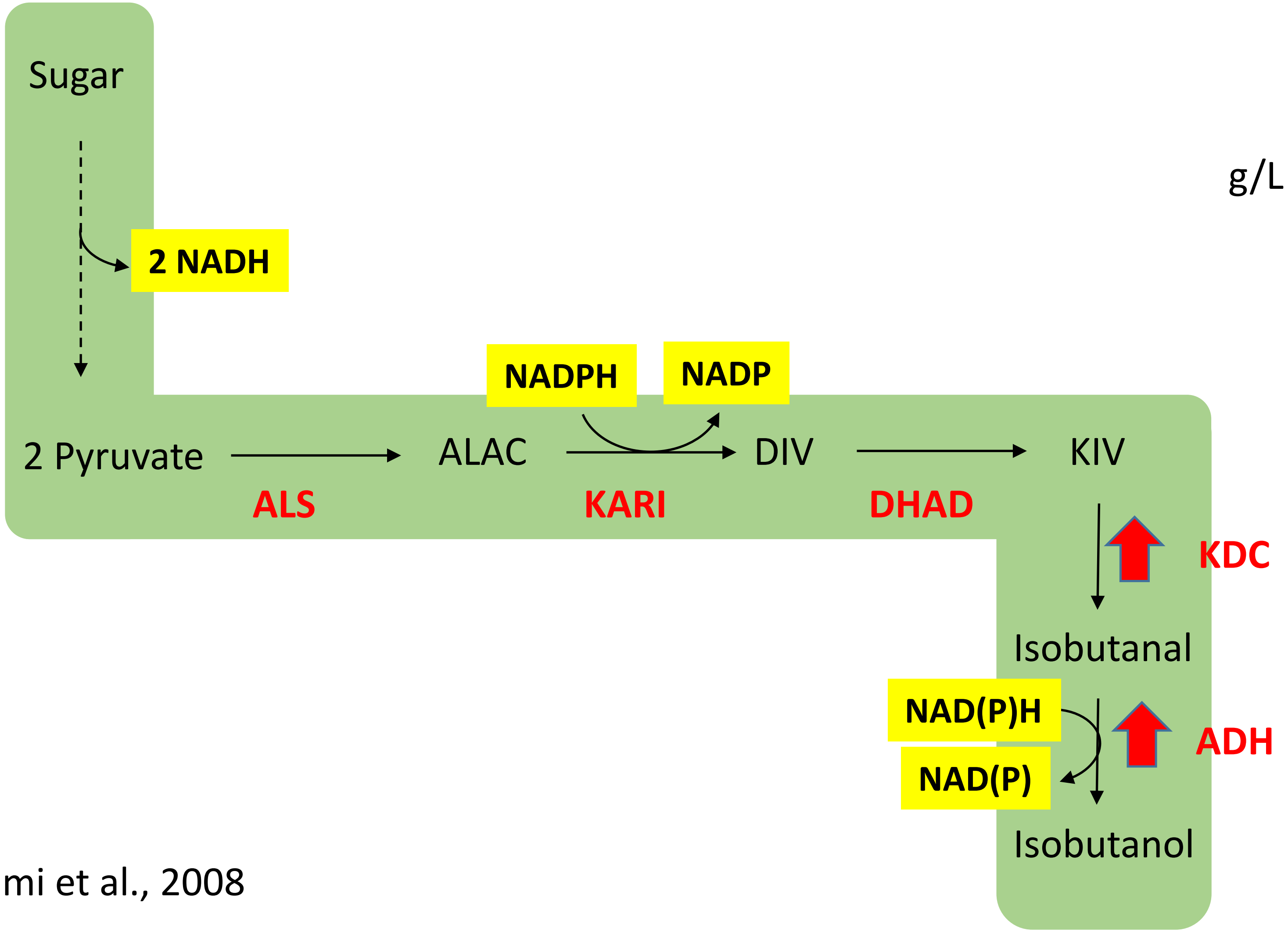


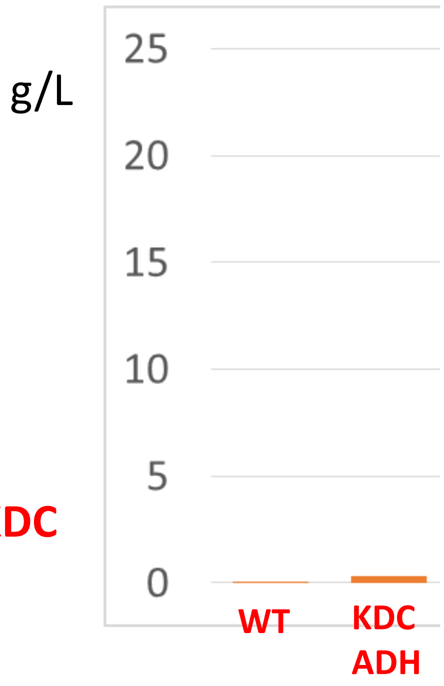


Isobutanol as a product of valine metabolism



Metabolic engineering of *E. coli* for isobutanol production





Atsumi et al., 2008

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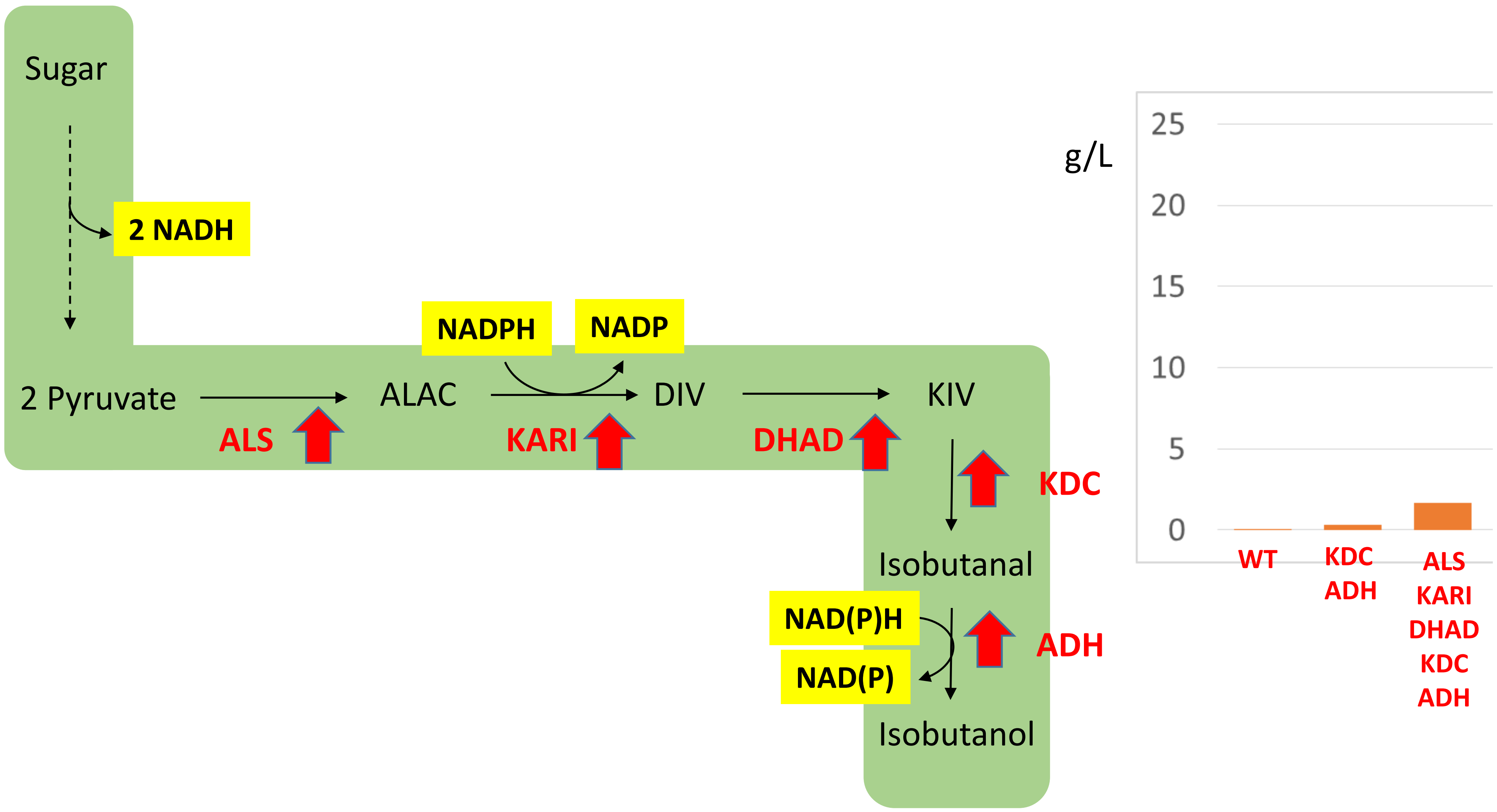

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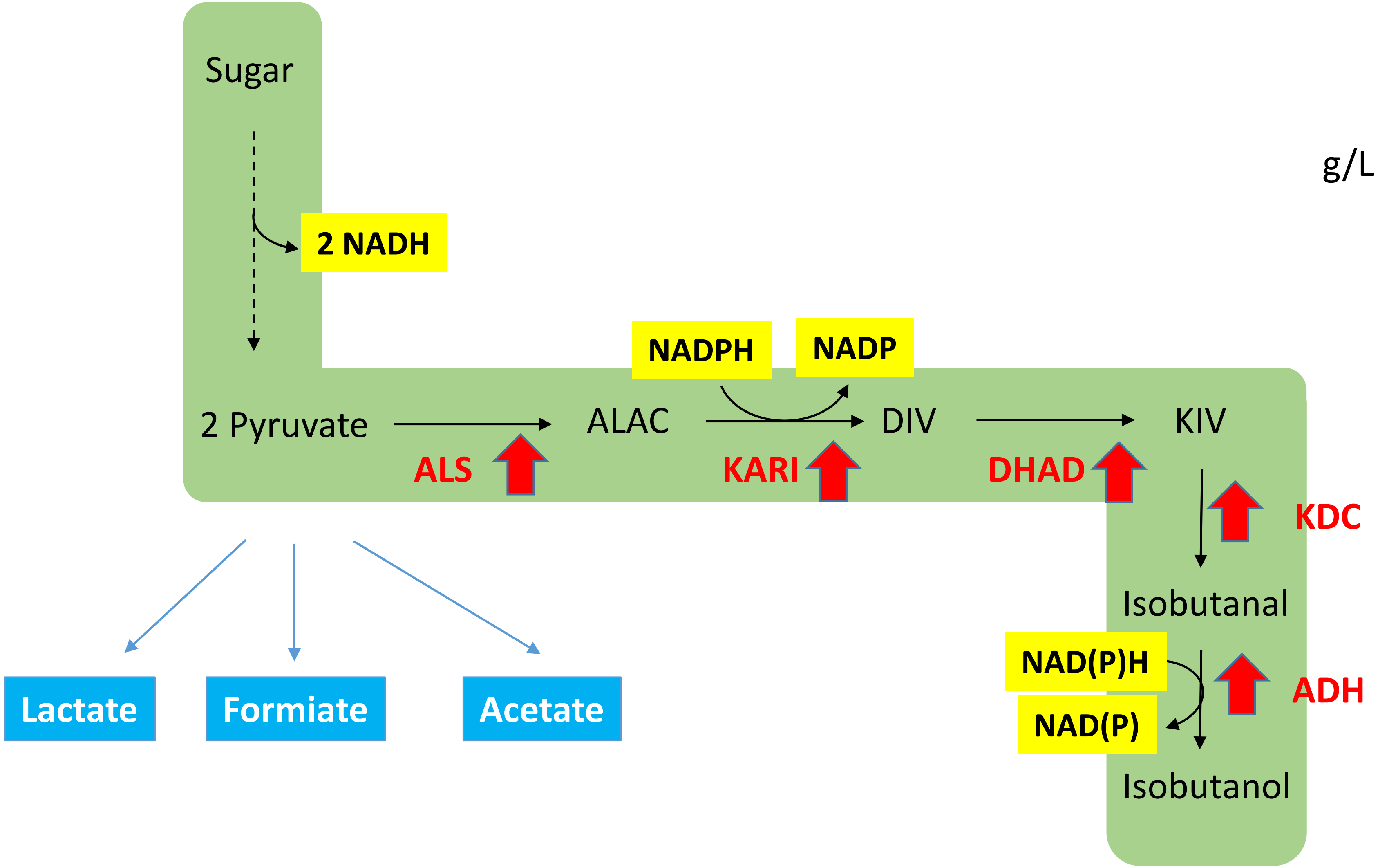

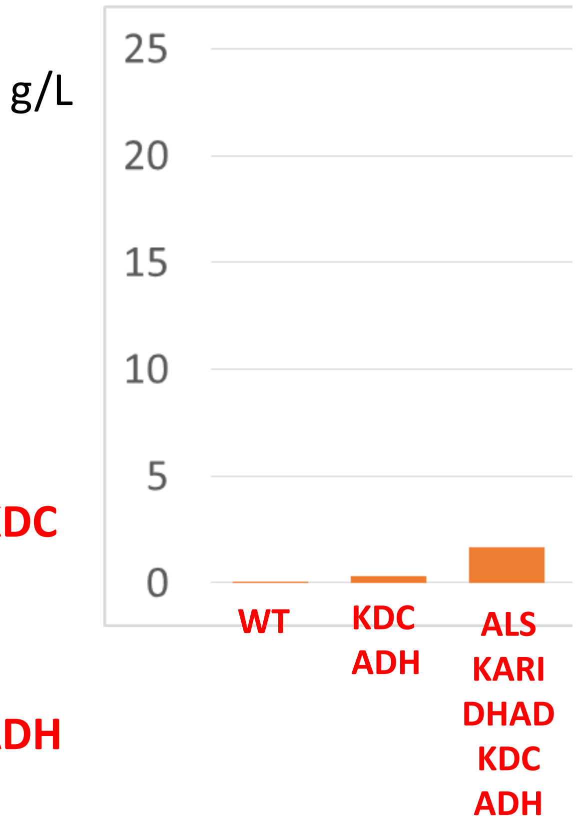

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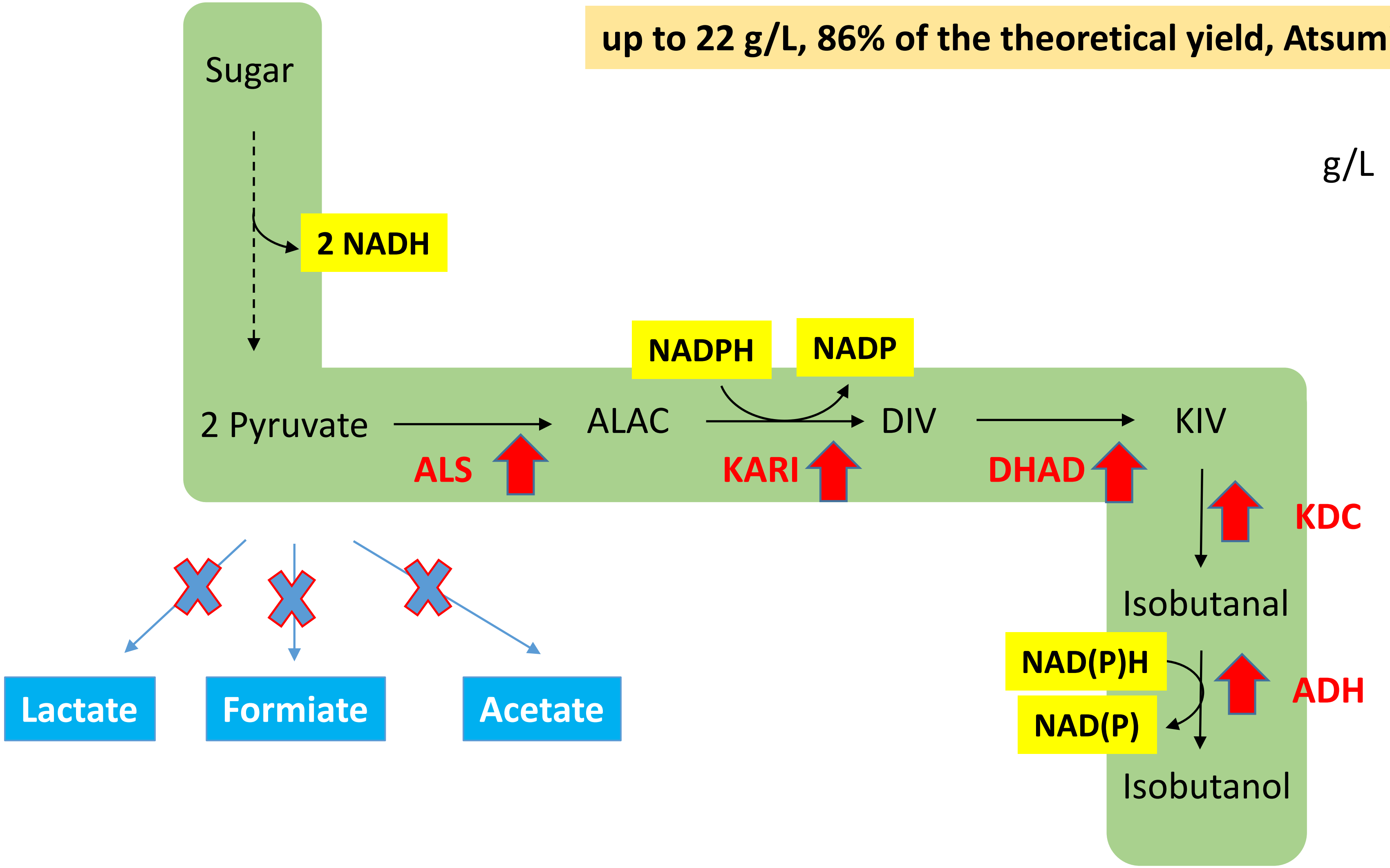
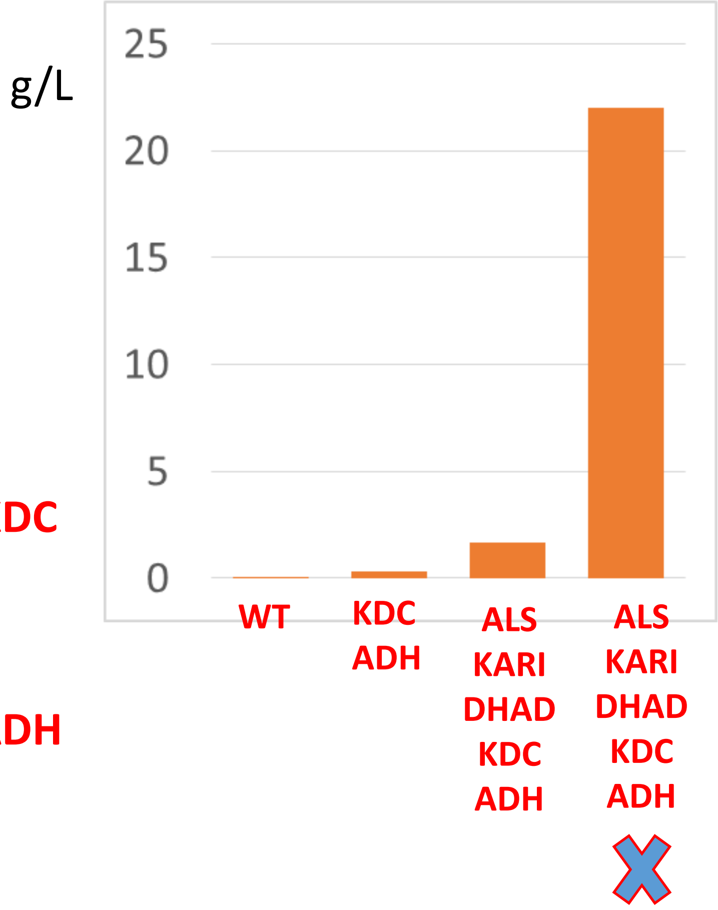
up to 22 g/L, 86% of the theoretical yield, Atsumi et al., 2008





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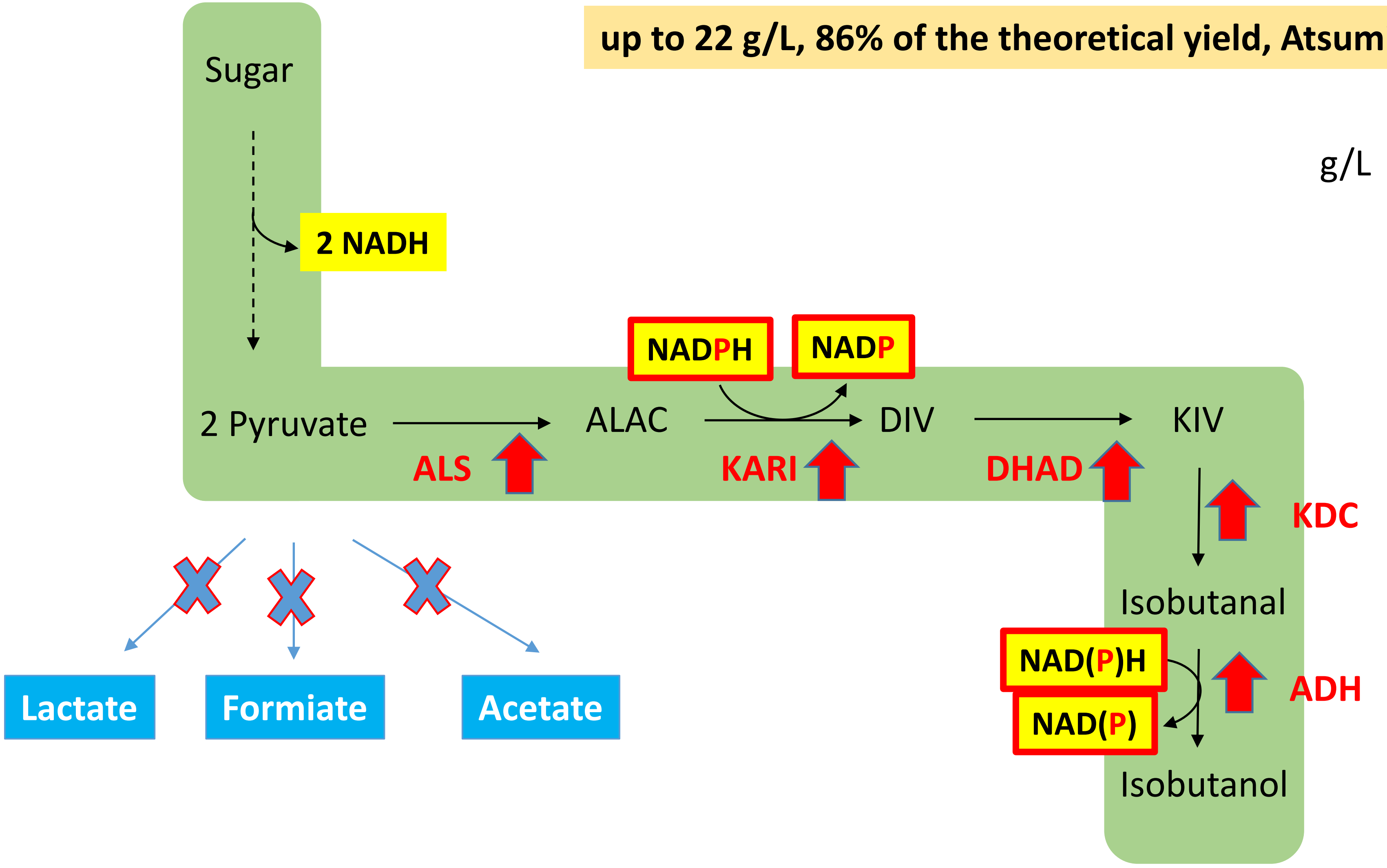
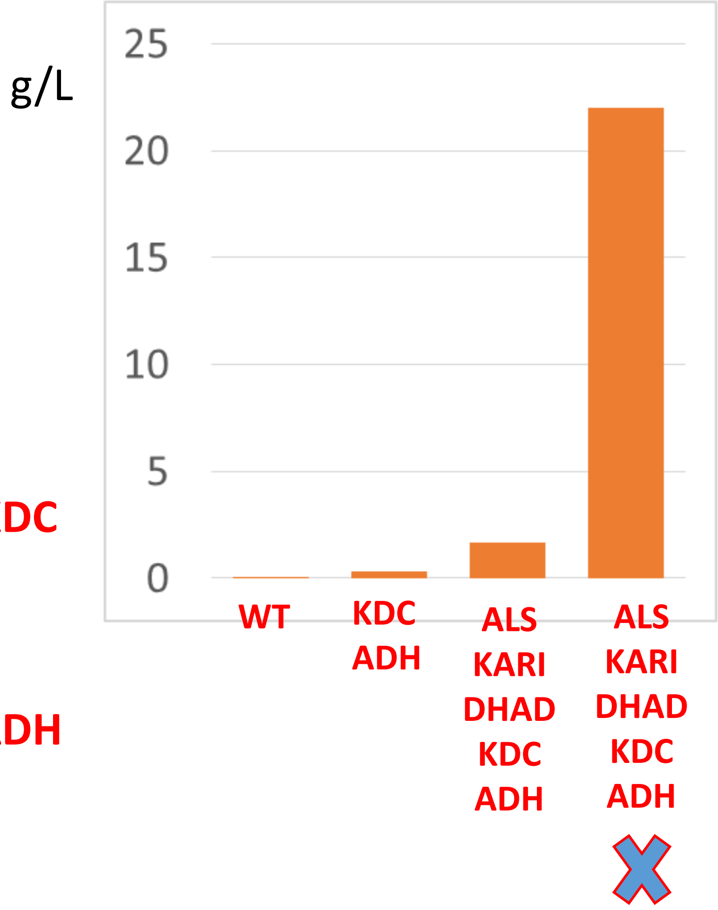
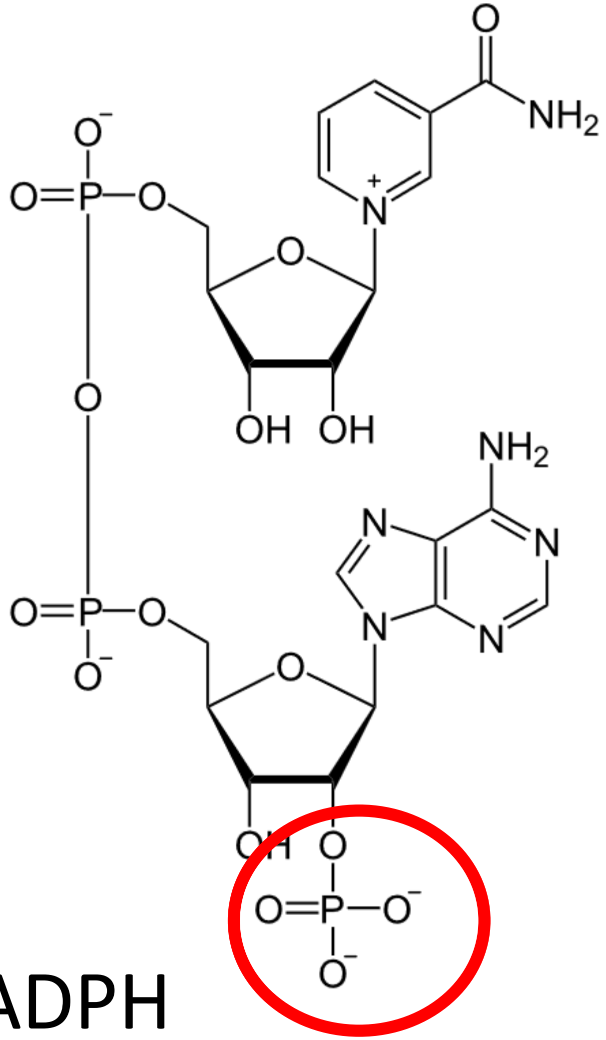
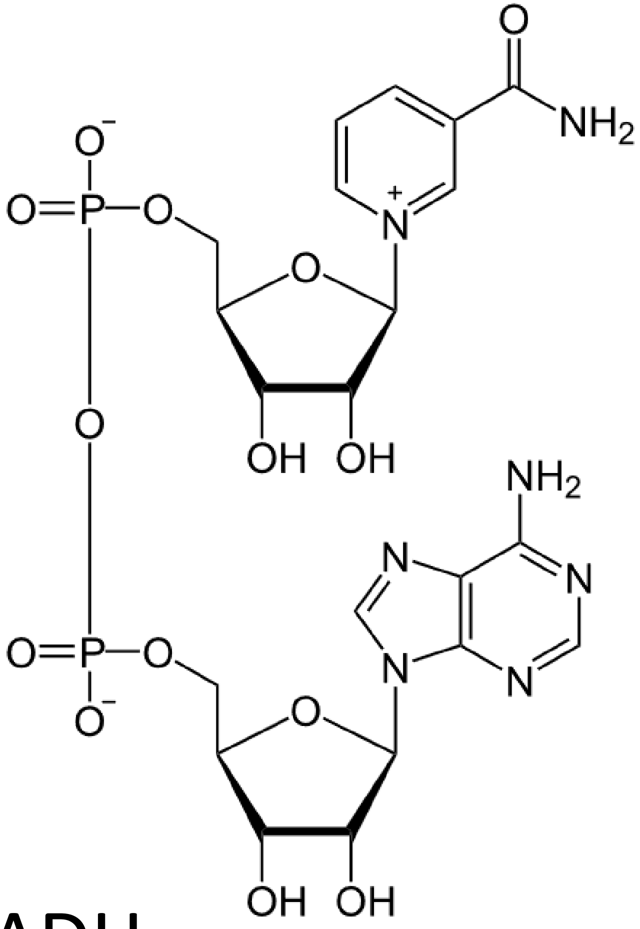




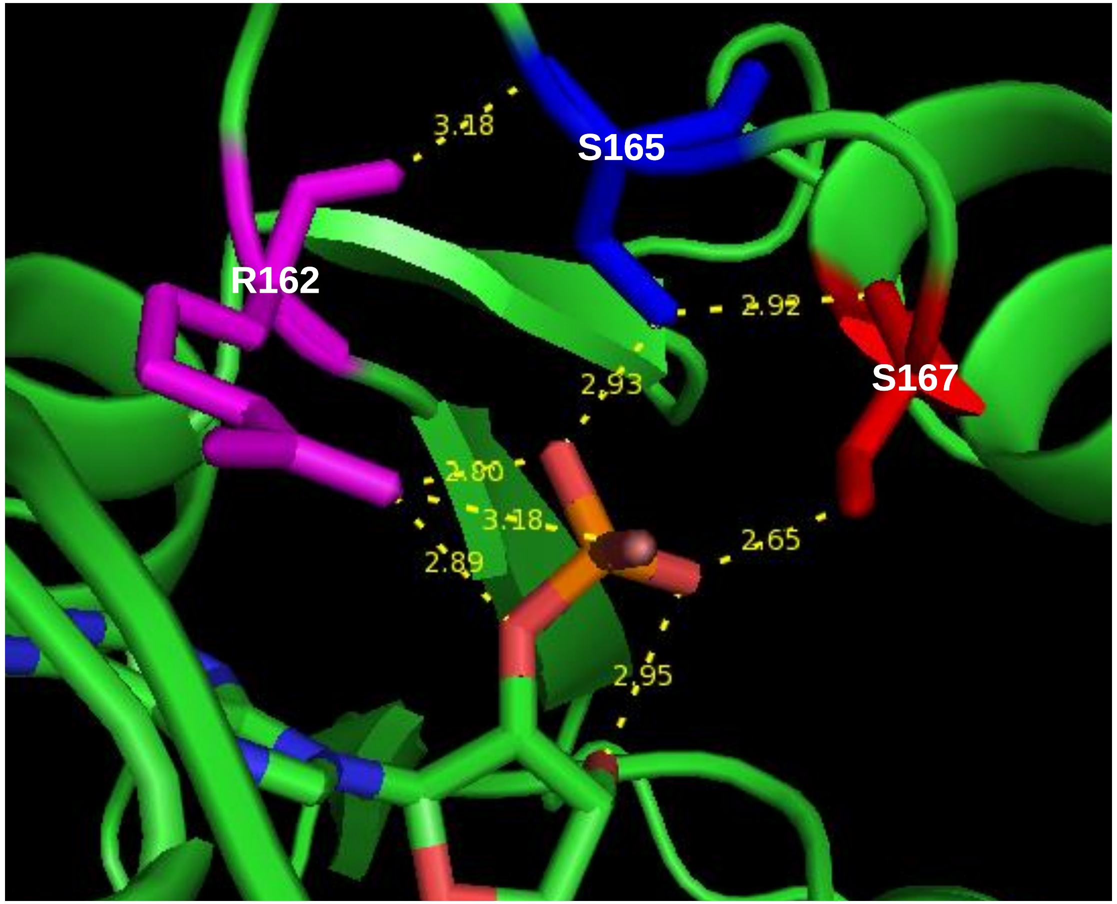


NADPH



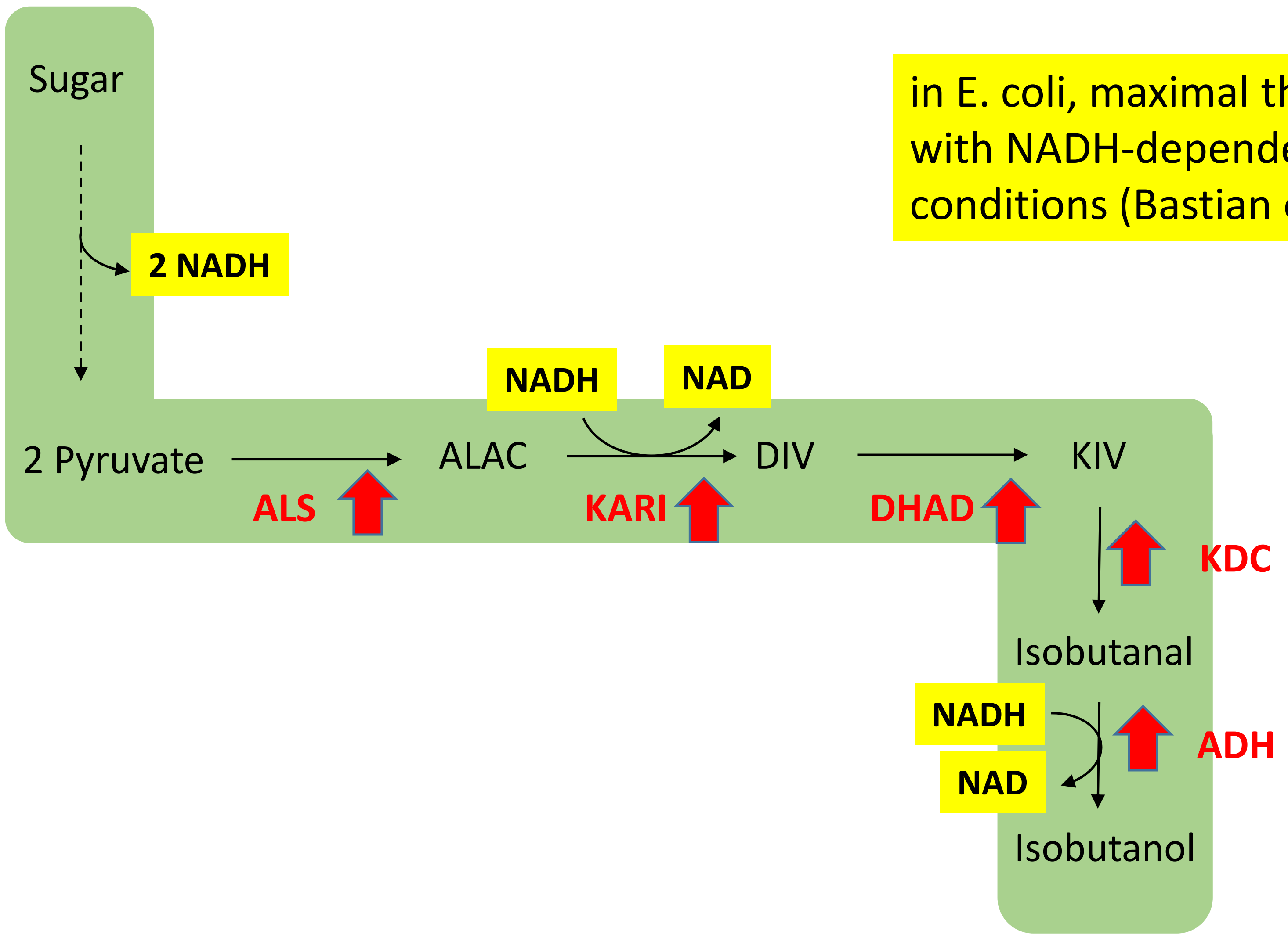
NADH

Protein engineering for metabolic engineering



negative charges of the phosphate group of NADPH are stabilised via electrostatical interactions by highly conserved arginine and serine residues

Redox balanced pathway using engineered KARI

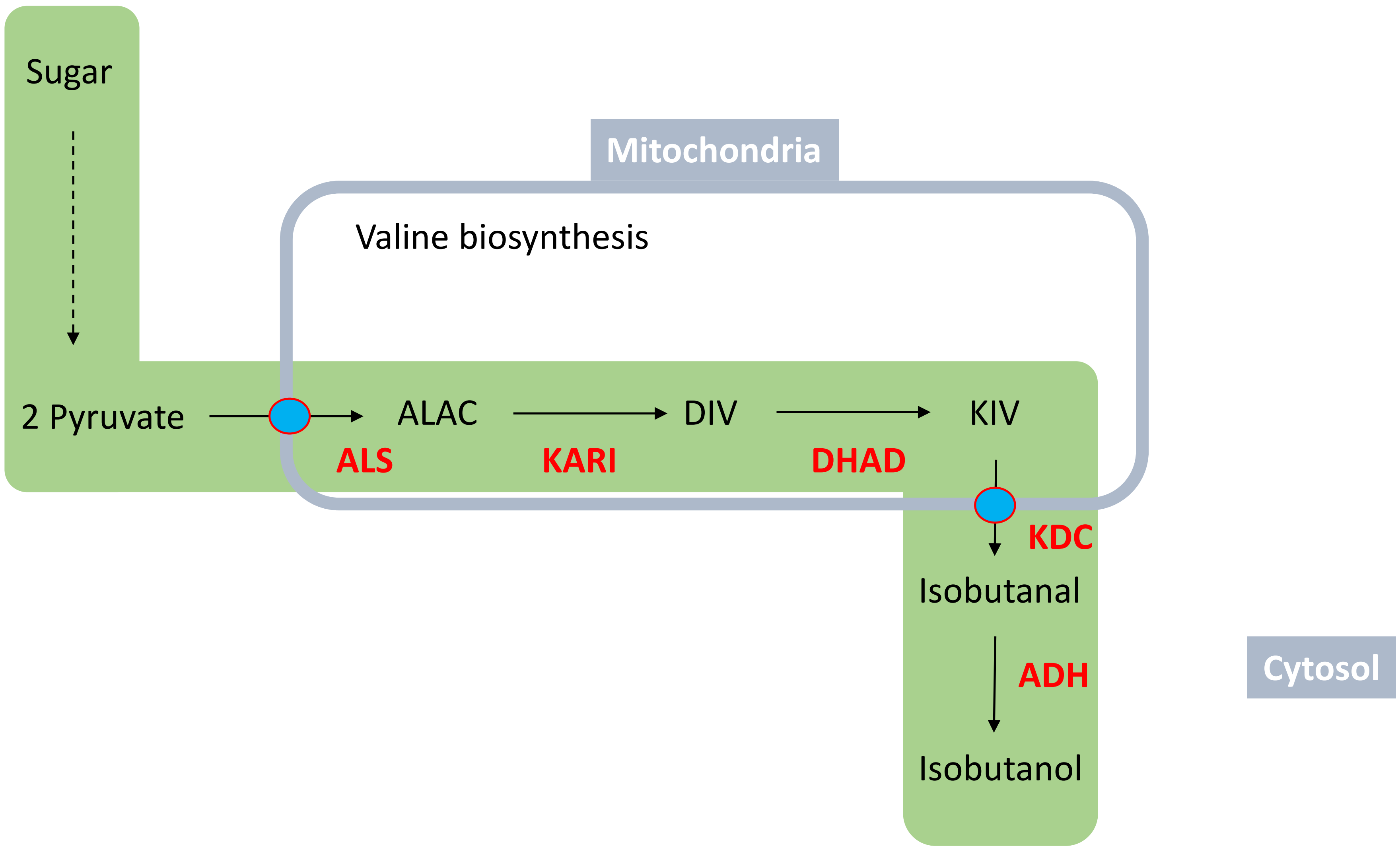


in E. coli, maximal theoretical yield was reached with NADH-dependent KARI under anaerobic conditions (Bastian et al., 2011)

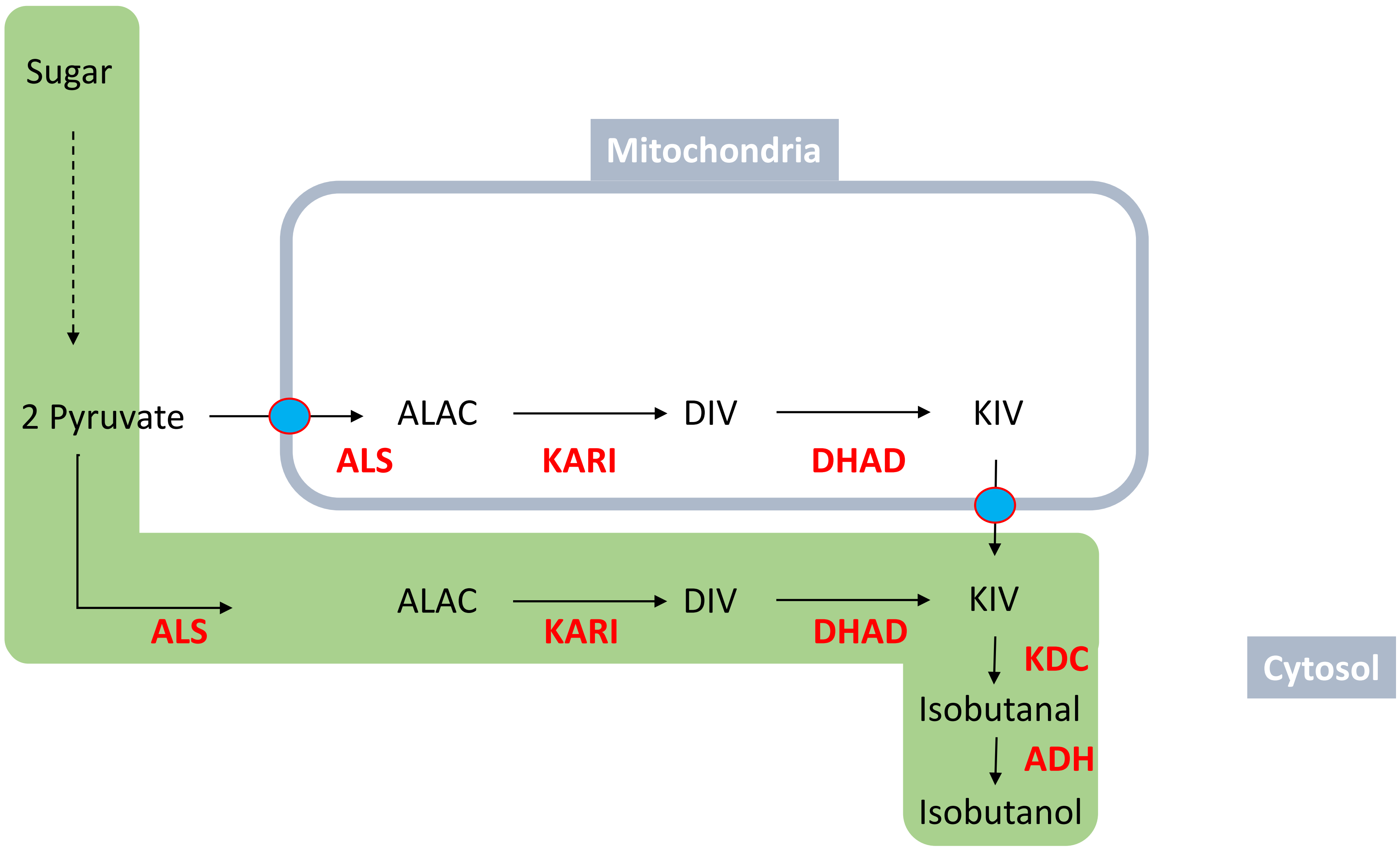
Yeast is a desirable host for biofuel production

- The main ethanol producer
- Good tolerance to alcohols and toxic by-products in lignocellulosic hydrolysates
- Acid-tolerant (important in lignocellulosic hydrolysates)
- Not susceptible to phages

…but more complicated (compartments!)

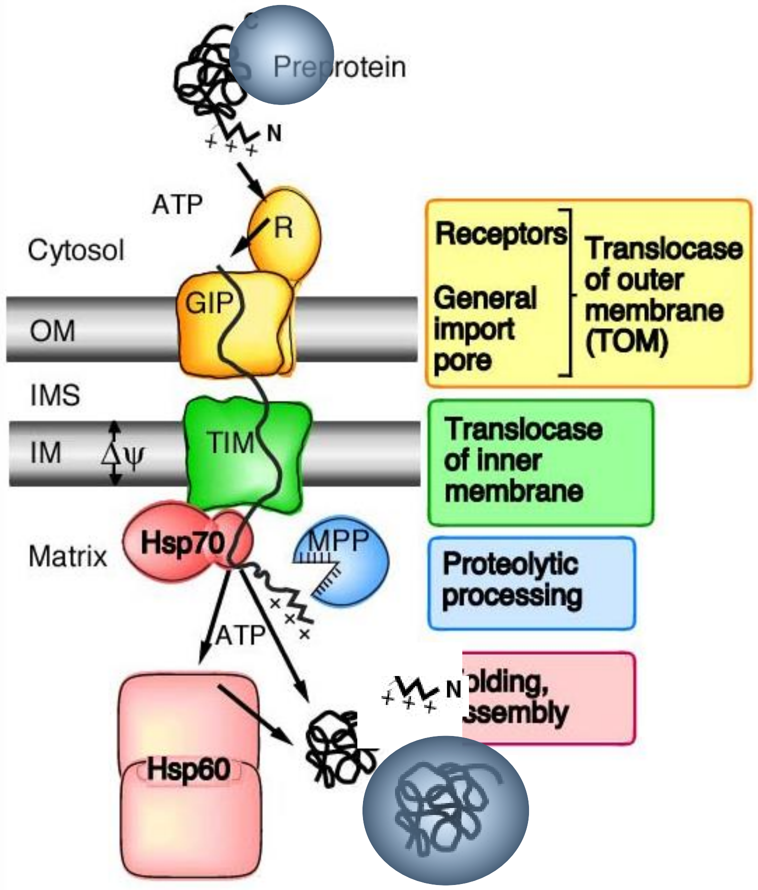


Idea: Expressing the complete pathway in the cytosol

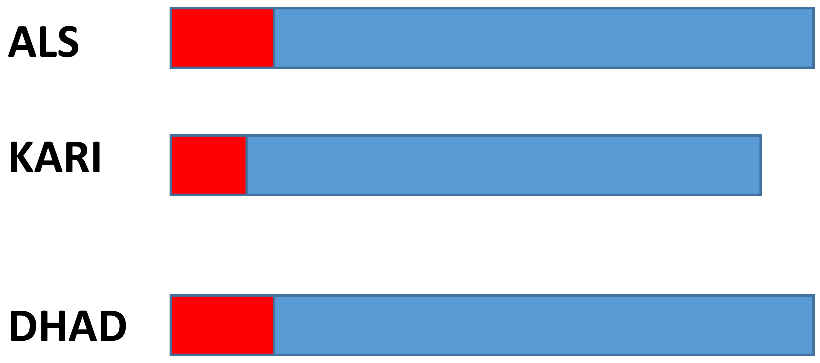


Deletion of N-terminal targeting sequences

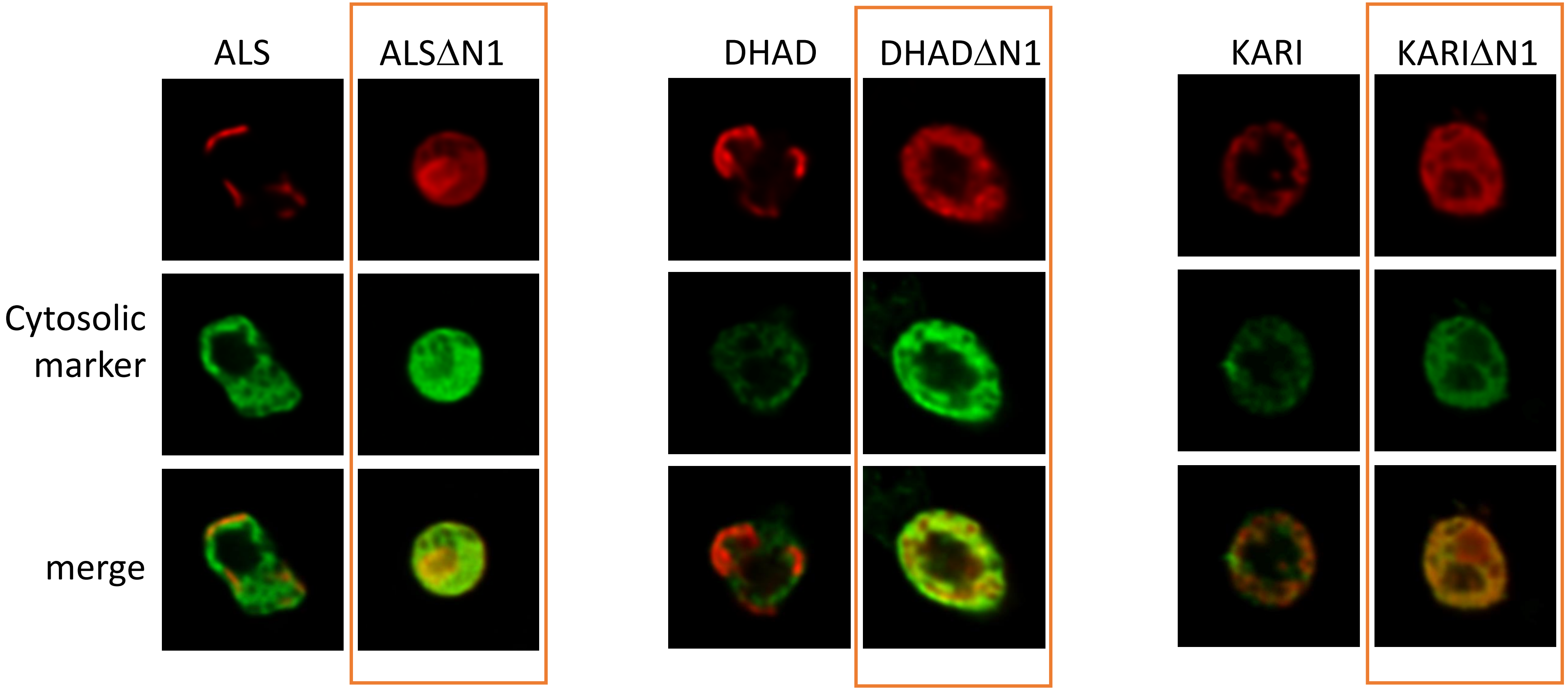
Principles of mitochondrial protein import



Bioinfrmatic prediction of mt. Targeting sequences

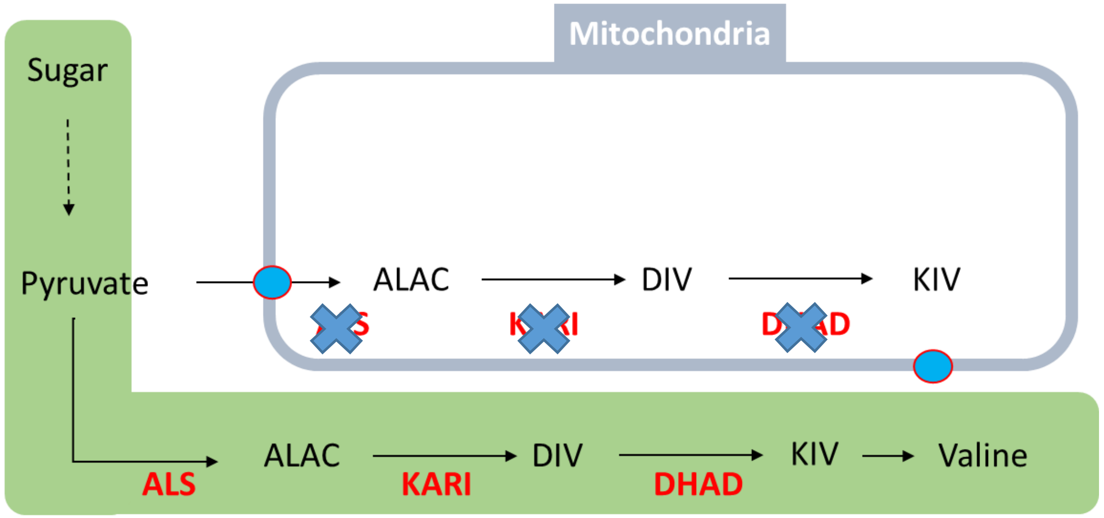


Truncated proteins are cytosolic



Brat et al. (2012)

Are they also functional?



Als, kari, dhad **triple deletion strain**

Brat et al. (2012)

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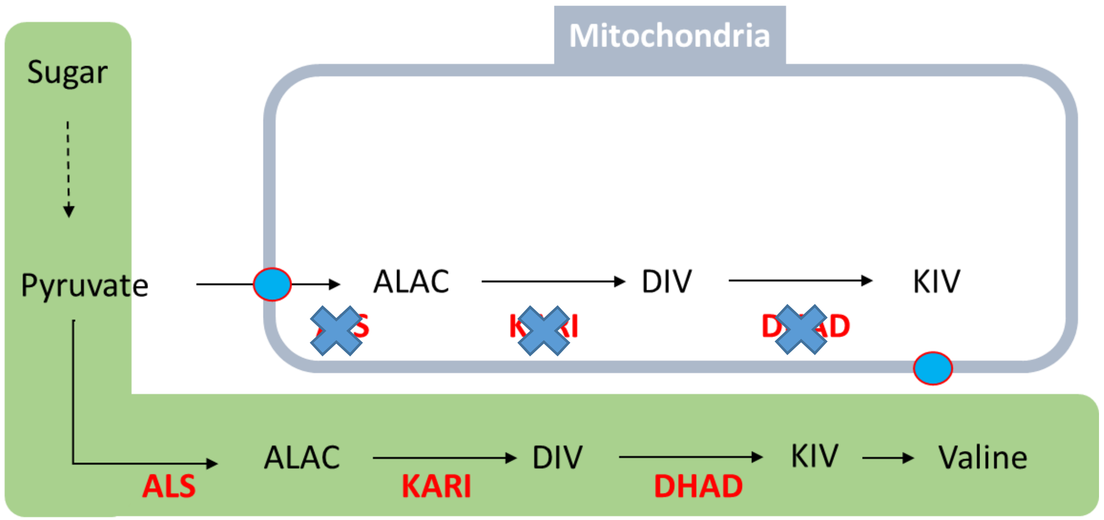
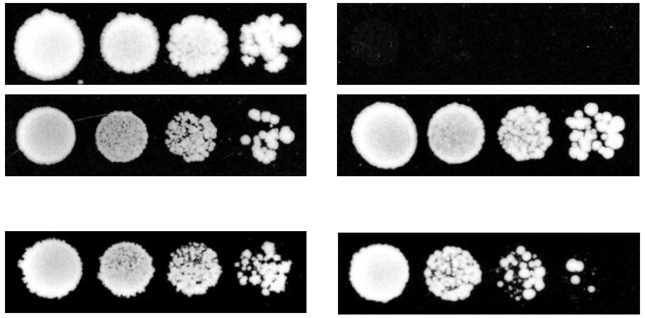


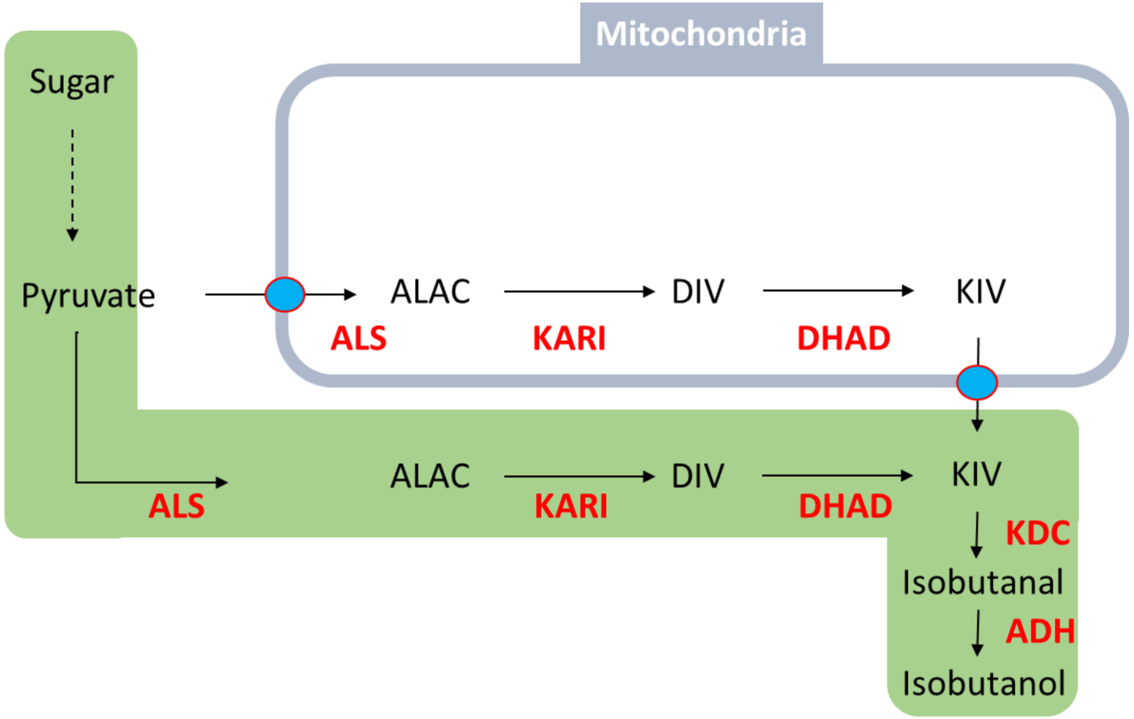
Als, kari, dhad **triple deletion strain**

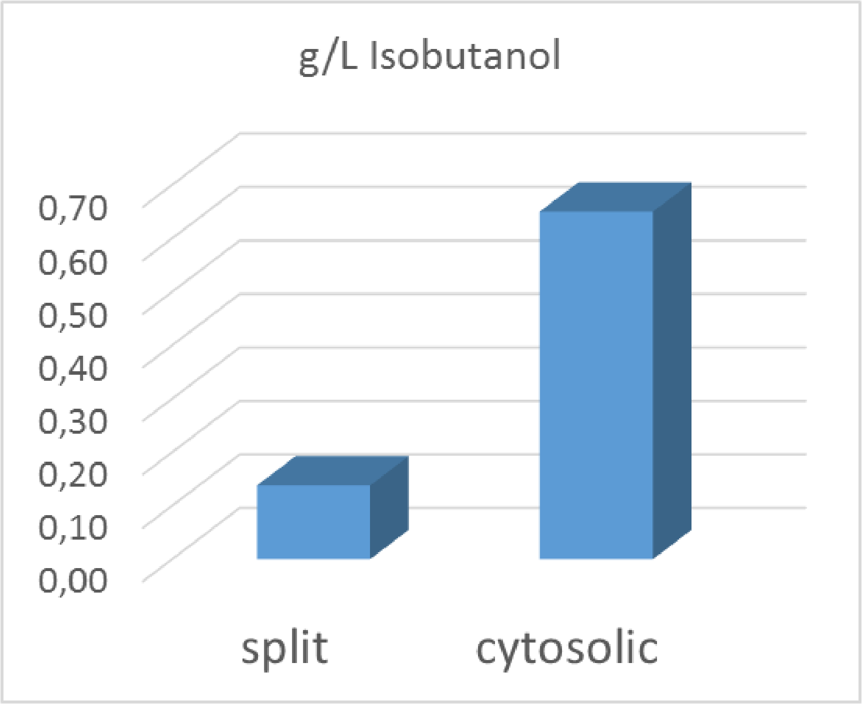
	+val	-val	
Empty vectors			
ALSwt + KARIwt + DHADwt			**mitochondrial**
ALSΔN + KARIΔN + DHADΔN			**cytosolic.**



Brat et al. (2012)

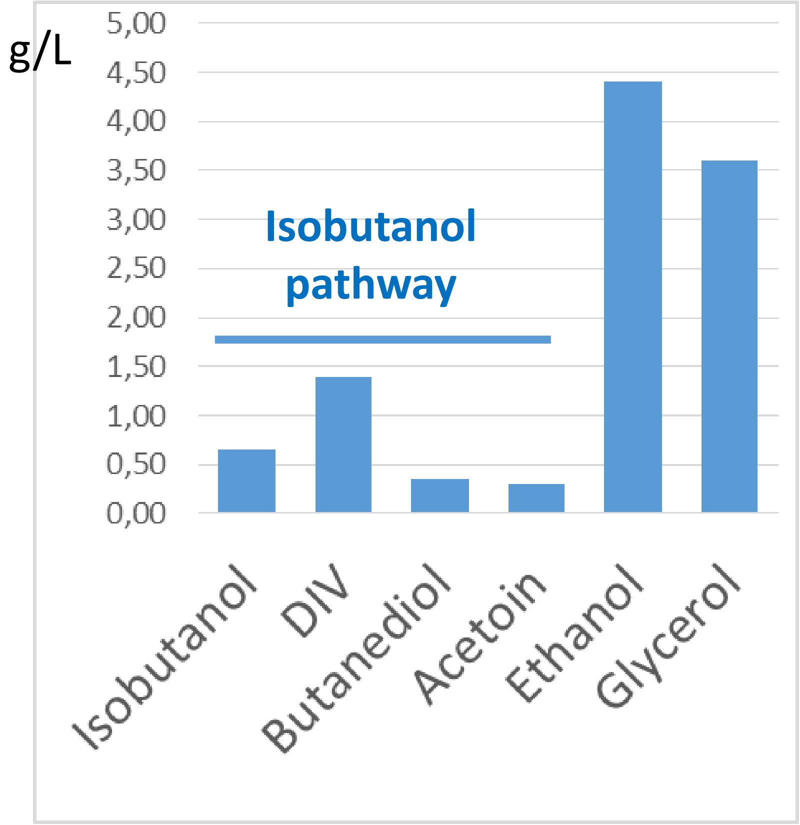
Isobutanol production by the cytosolic pathway is superior to the split pathway

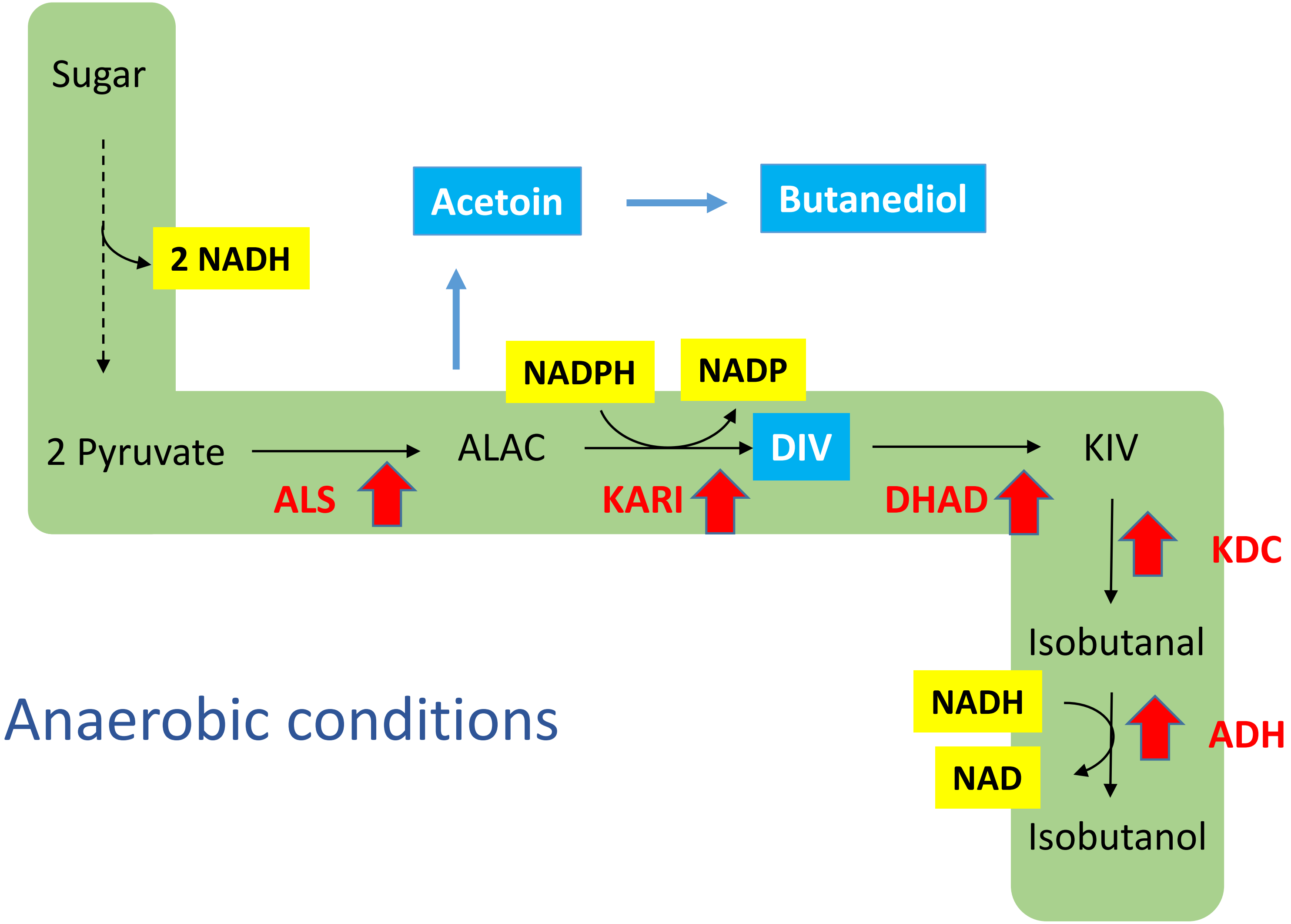




0,65 g/L, 15 mg isoutanol per g Glucose, Brat et al., 2012

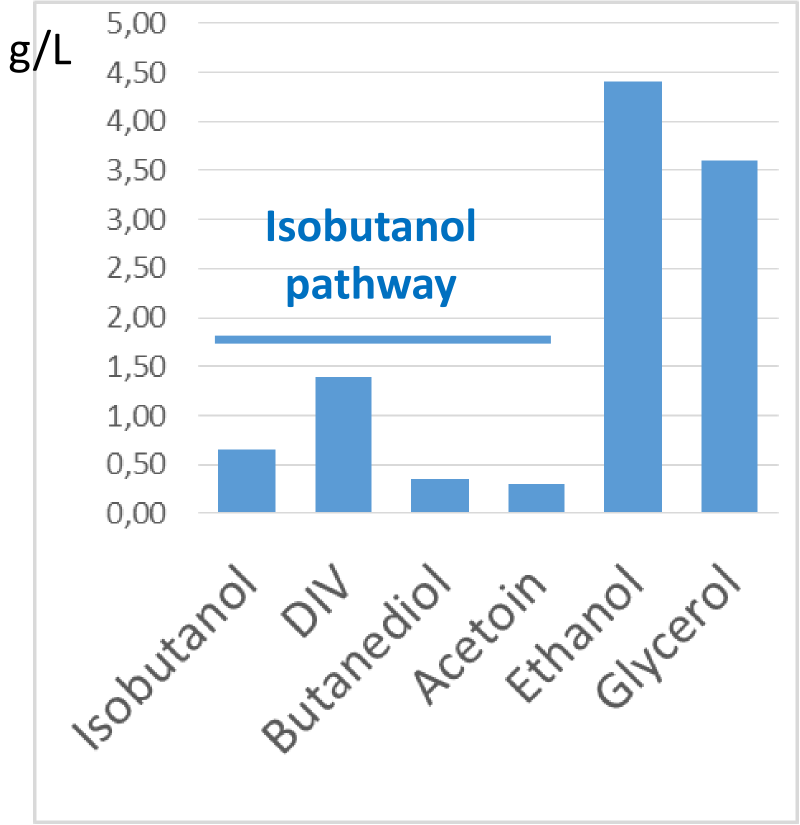
Metabolite analysis identifes bottleneck steps

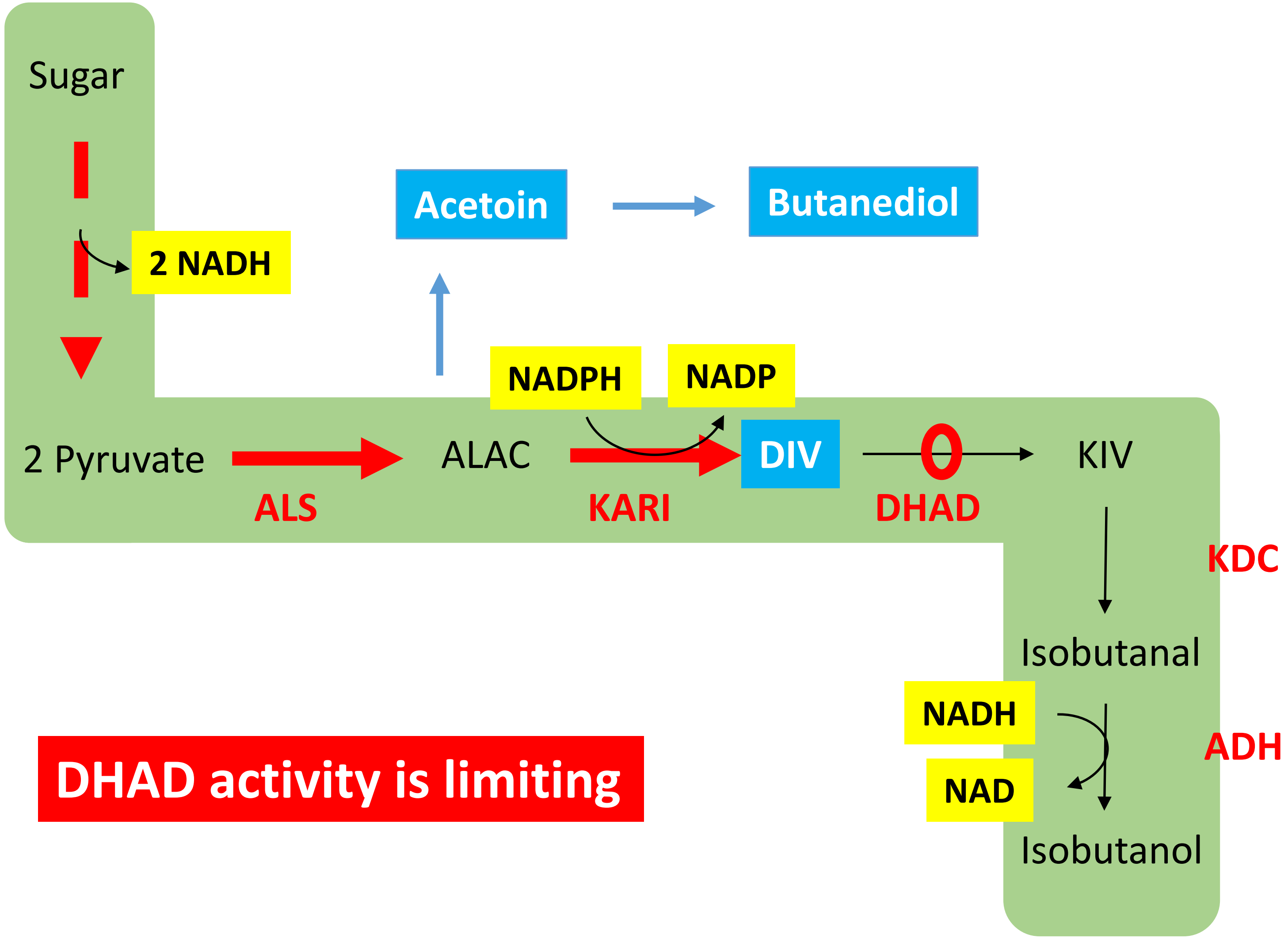




Anaerobic conditions

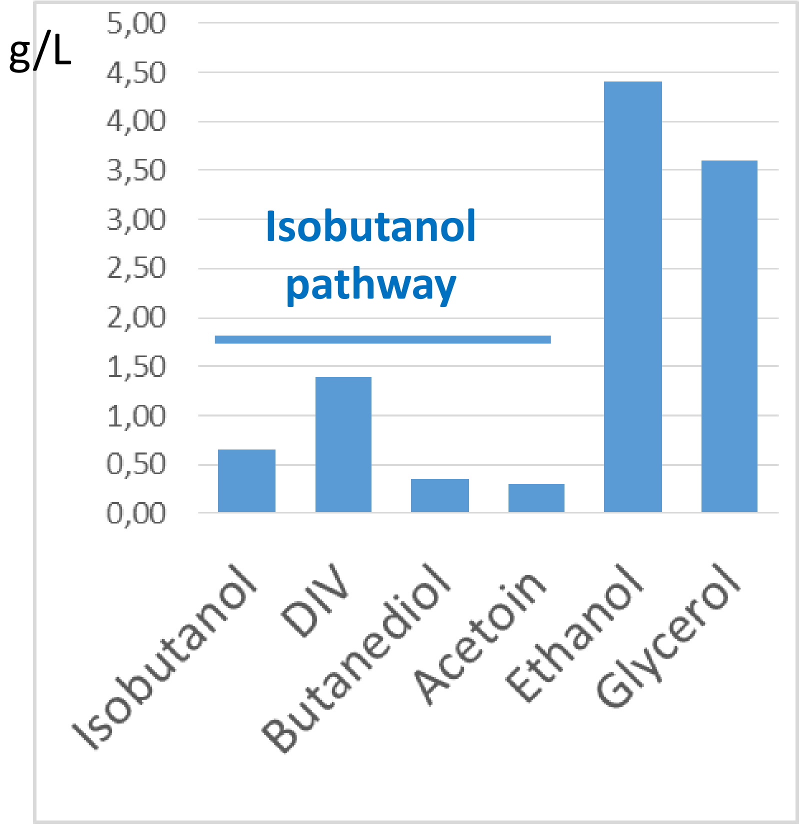
Metabolite analysis identifes bottleneck steps

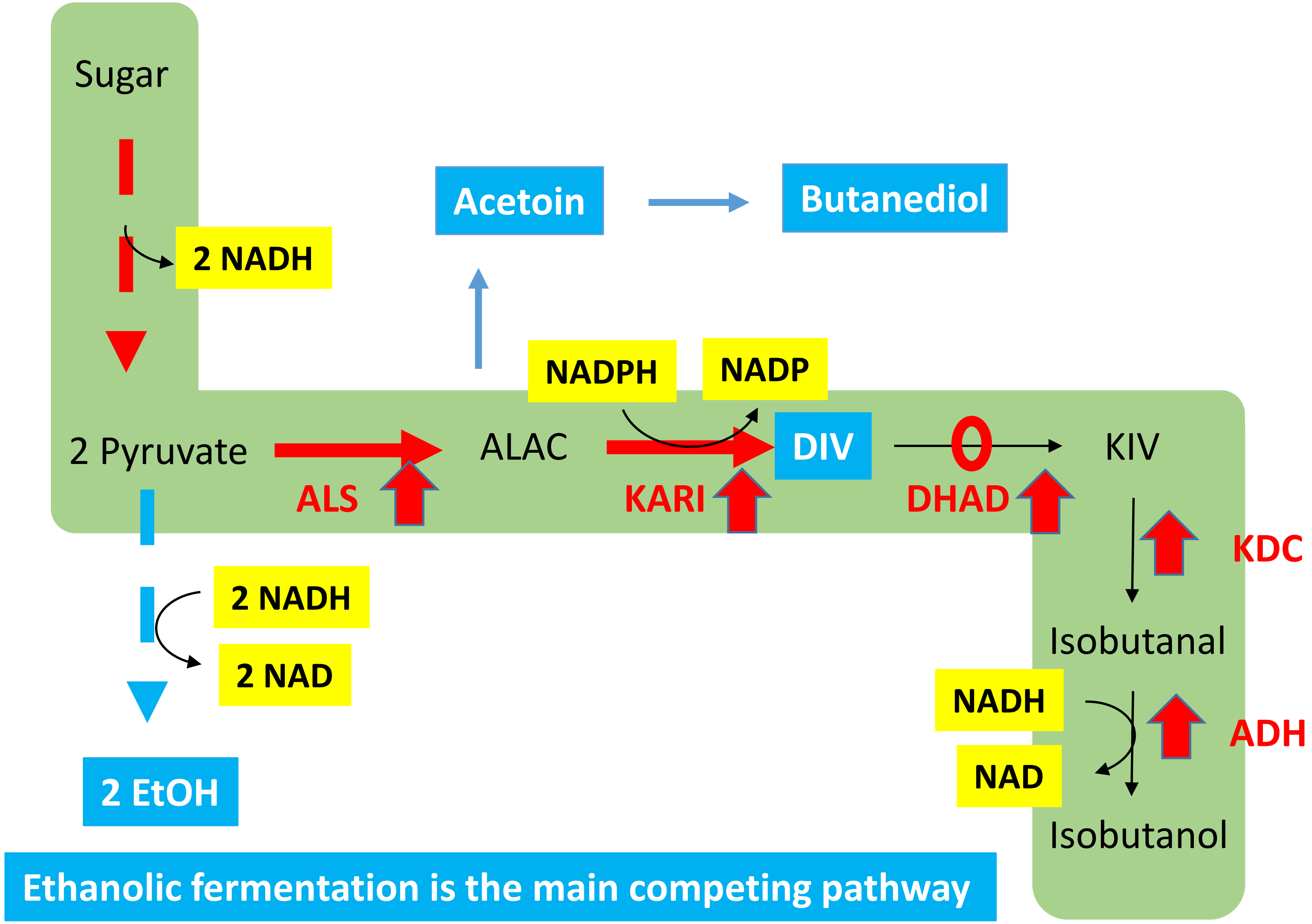




DHAD activity is limiting

Metabolite analysis identifes bottleneck steps





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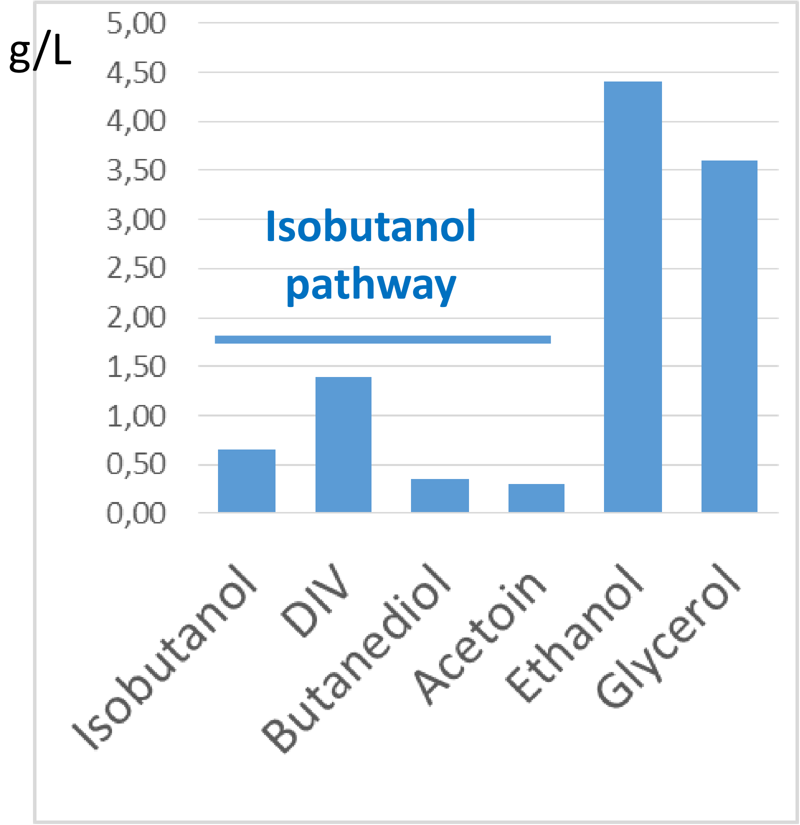
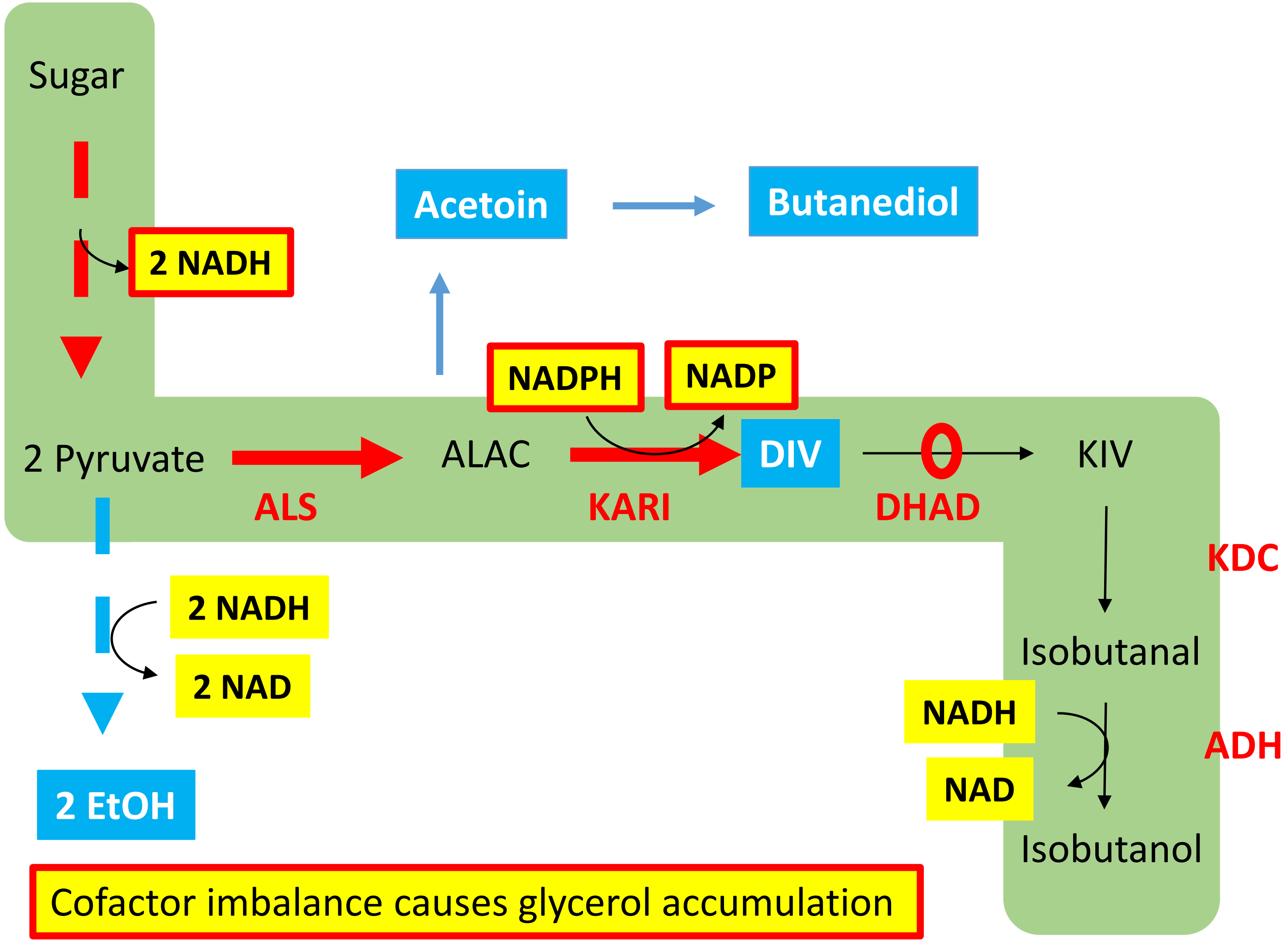
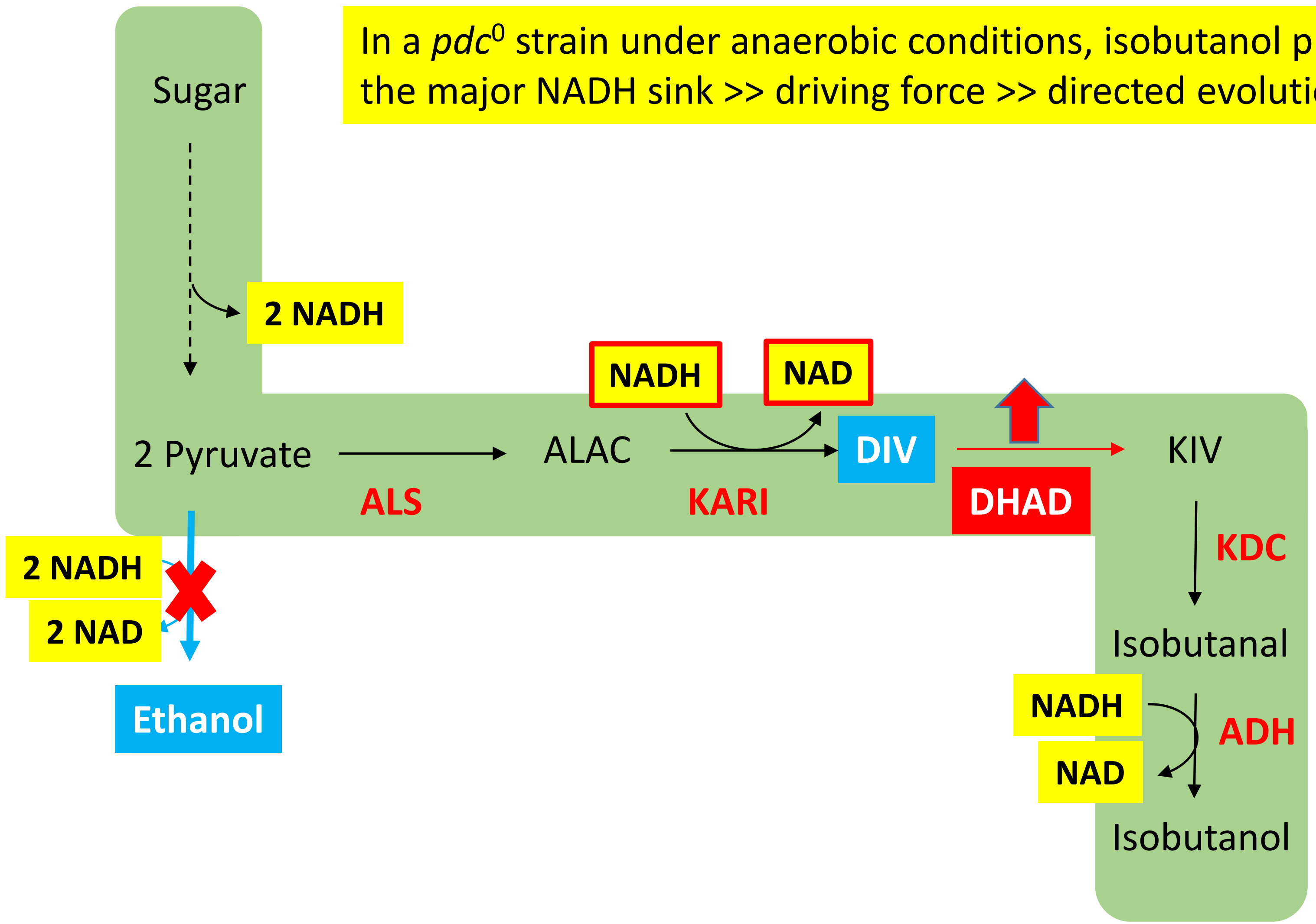




Current work / future prospect

In a *pdc*[0] strain under anaerobic conditions, isobutanol production would be the major NADH sink >> driving force >> directed evolution is possible!



Summary

- Isobutanol (and other higher alcohols) have higher energy density and lower hygroscopicity than ethanol
- Isobutanol can be synthesized from the 2-ketoacid ketoisovalerate
- To produce isobutanol from sugars, valine biosynthesis and Ehrlich pathway must be short-circuited
- In E. coli, 100% of the theoretical yield could be reached
- ME of yeast is more challenging due to compartmentalization, but promising results could be obtained
- Industrial production of isobutanol is entering the commercial phase

Acknowledgements

Prof. Eckhard Boles
Group leader

Wesley Cardoso Generoso
PhD student

CIÊNCIA SEM FRONTEIRAS

Sponsor: FAPESP, FAEPEX UNICAMP

Organization: nipe, UNICAMP

Suport: FEA, Faculdade de Engenharia Química