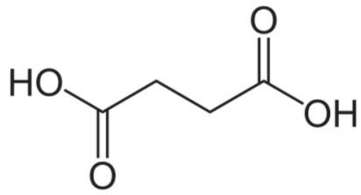
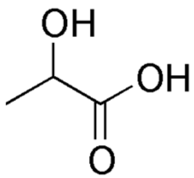
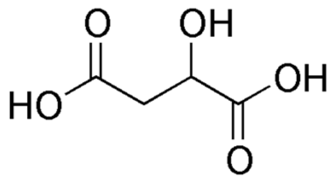
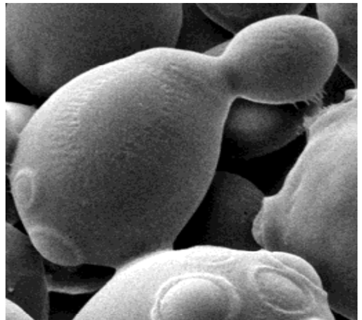
Bioenergy beyond biofuels:
Low-value, high-volume compounds









Ton van Maris

Delft University of Technology

Department of Biotechnology

Section Industrial Microbiology

Delft, the Netherlands

Advanced School Present & Future of Bioenergy

Campinas, October 12th, 2014

TU Delft

What are desirable process characteristics for low-value high-volume products?

-
-
-
-
-
-
-
-
-
-
-
-

TU Delft

What are desirable process characteristics for low-value high-volume products?

- High yield (large contribution of feedstock to cost price)

- High rate (return on investment)

- High titer (savings down-stream processing) or *in situ* removal

- Preferrably anaerobic (aeration and cooling = expensive)
  - Redox co-factor balanced and ATP yielding

- Robust process and microorganism (no stuck fermentations)
- Simple growth requirements (price & purity of media)
- Low by-product formation (e.g. biomass, glycerol)
- Conditions in bioreactors that facilitate DSP (pH, T etc.)

3

TU Delft

Back of the envelope cost-price guesstimation

	(ethanol guess)
Maximum theoretical yield (MTY)	(0.51 g_p/g_s)
% of MTY realised in fermentation	(± 90%)
% of feedstock in cost price	(± 70%)

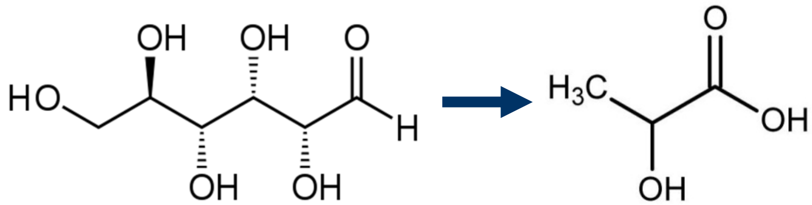
→ (1/(0.51·0.90·0.70) · sugar price (units as sugar price)

How do you determine the maximum theoretical yield of a product?

Law of conversation of mass

No more of an element can end up in the product than was available in the (mixed) substrate.

Production of lactic acid from glucose



1 $C_6H_{12}O_6$			**→ ? $C_3H_6O_3$**			
Substrate(s):			**Product(s):**			
C	**= 1x6**	**= 6**	**C**	**= ?x3**	**= 6**	
H	**= 1x12**	**= 12**	**H**	**= ?x6**	**= 12**	
O	**= 1x6**	**= 6**	**O**	**= ?x3**	**= 6**	

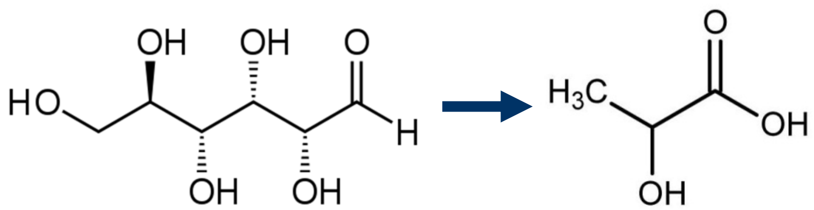
www.gogreeninstages.com
www.iconarchive.com
www.greentechnolog.com

How do you determine the maximum theoretical yield of a product?

Law of conversation of mass

No more of an element can end up in the product than was available in the (mixed) substrate.

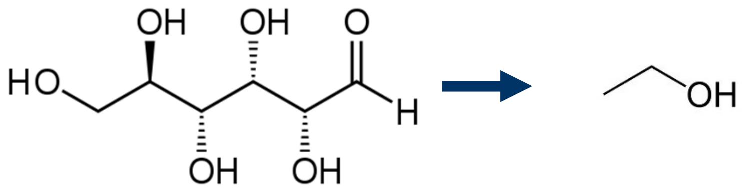
Production of lactic acid from glucose



$1\ C_6H_{12}O_6 \rightarrow 2\ C_3H_6O_3$

Substrate(s):			Product(s):		
C	= 1x6	= 6	C	= 2x3	= 6
H	= 1x12	= 12	H	= 2x6	= 12
O	= 1x6	= 6	O	= 2x3	= 6

Max. theoretical yield of glucose conversion into ethanol?



$1\ C_6H_{12}O_6 \rightarrow 2\ C_2H_6O + 2\ CO_2$

Substrate(s):

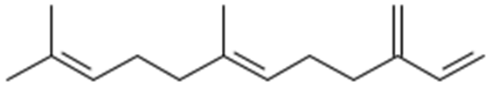
C	= 1x6	= 6
H	= 1x12	= 12
O	= 1x6	= 6

Product(s):

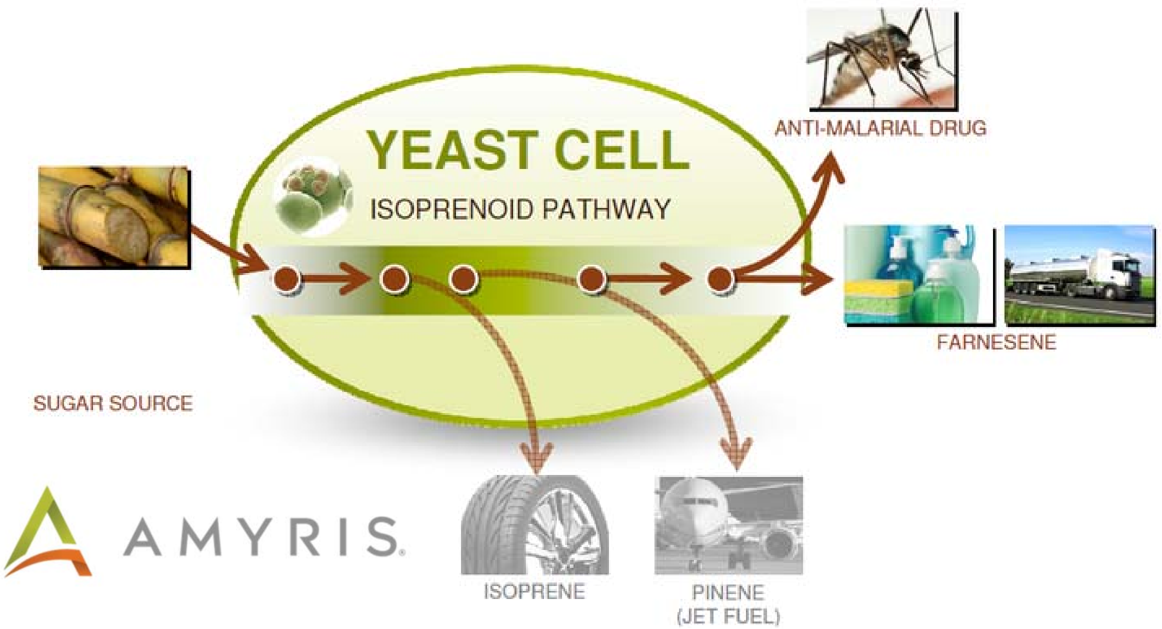
C	= 2x2+2x1	= 6
H	= 2x6+2x0	= 12
O	= 2x1+2x2	= 6

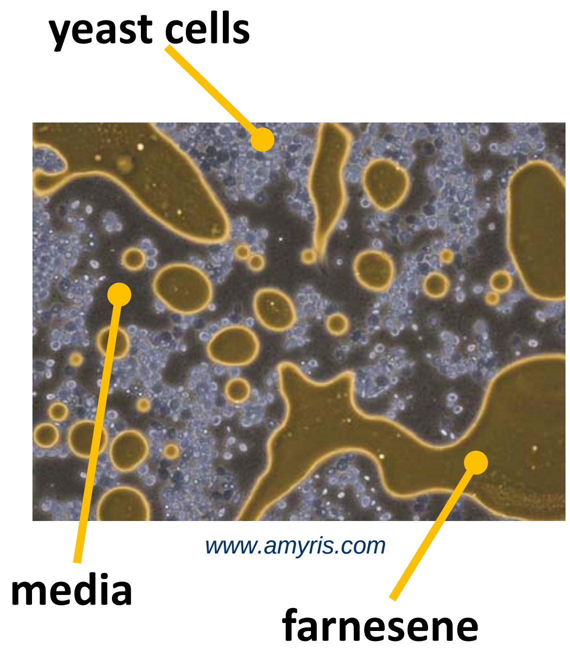
www.360telemetry.com
www.yummy.co.ke
www.wikipedia.org
www.auto.howstuffworks.com

Max. theoretical yield of glucose conversion into farnesene?



farnesene ($C_{15}H_{24}$)





Max. theoretical yield of glucose conversion into farnesene?

? $C_6H_{12}O_6$ $\rightarrow$? $C_{15}H_{24}$ + ? + ?

Difficult!

Can we look at products and substrates from a different perspective?

Knall Gas Reaktion

$$2\ H_2 + 1\ O_2 \rightarrow 2\ H_2O$$

What type of reaction is this?

Redox reaction:

Electron donor reaction:

$H_2 \rightarrow 2\ H^+ + 2\ e^-$ **2x**

Electron acceptor reaction:

$½\ O_2 + 2\ H^+ + 2\ e^- \rightarrow 1\ H_2O$ **2x**

+

$2\ H_2 + 1\ O_2 \rightarrow 2\ H_2O$

Combustion of glucose to CO_2

$1\ C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$

Substrate(s):		Product(s):	
C	= 1x6+6x0 = 6	C	= 6x1+6x0 = 6
H	= 1x12+6x0= 12	H	= 6x0+6x2 = 12
O	= 1x6+6x2 = 18	O	= 6x2+6x1 = 18

Reaction releases (free) energy !

As redox reaction:

Electron donor reaction:

$1\ C_6H_{12}O_6 + 6\ H_2O \rightarrow 6\ CO_2 + 24\ H^+ + 24\ e^-$ 1x

Electron acceptor reaction:

$1\ O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$ 24/4 = 6x

+

$1\ C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$

‘Degree of reduction (γ)’: handy conservation principle

	γ-value
C	4
H	1
O	-2
-	1
+	-1

γ = 0 for **reference compounds:**
CO_2, H_2O, H^+

γ gives insight into available electrons in substrates/products and donor/acceptors

ex. glucose → 2 ethanol + 2 CO_2

glucose	2 ethanol	2 CO_2
24	**2x12**	**2x0**

all electrons in the product!

Note: Simplified for this presentation, system can be extended to N, S, P & metals & can also be used to estimate overall process stoichiometry including not only product [illegible]th and maintanance.

Max. theoretical yield of glucose conversion into farnesene?

$? \, C_6H_{12}O_6 \rightarrow \, ? \, C_{15}H_{24} + ? + ?$

	γ-value
C	4
H	1
O	-2
-	1
+	-1

What is the γ of a mol of farneseen? 84

How much glucose needed if all e^- end up in product? 84/24=3.5

Complete the mass balance.

$3.5 \, C_6H_{12}O_6 \rightarrow 1 \, C_{15}H_{24} + 9 \, H_2O + 6 \, CO_2$

Substrate(s):			Product(s):		
C	= 3.5x6	= 21	C	= 1x15+6x1	= 21
H	= 3.5x12	= 42	H	= 1x24+9x2+6x0	= 42
O	= 3.5x6	= 21	O	= 1x0+9x1+6x2	= 21
γ	= 3.5x24	= 84	γ	= 1x84+9x0+6x0	= 84

Theoretical yield is determined by:

1. Law of conversation of mass

No more of an element can end up in the product than was available in the (mixed) substrate.

2. Metabolic routes native to (or engineered into) the microorganism of choice

Example: production of acetic acid from glucose by native yeast metabolism

n glucose → 2n pyruvate → 2n acetaldehyde → 2n acetic acid

$C_6H_{12}O_6$ → $C_3H_4O_3$ → → $C_2H_4O_2$

2 NADH + 2 ATP; 2 CO_2; 2 NADH

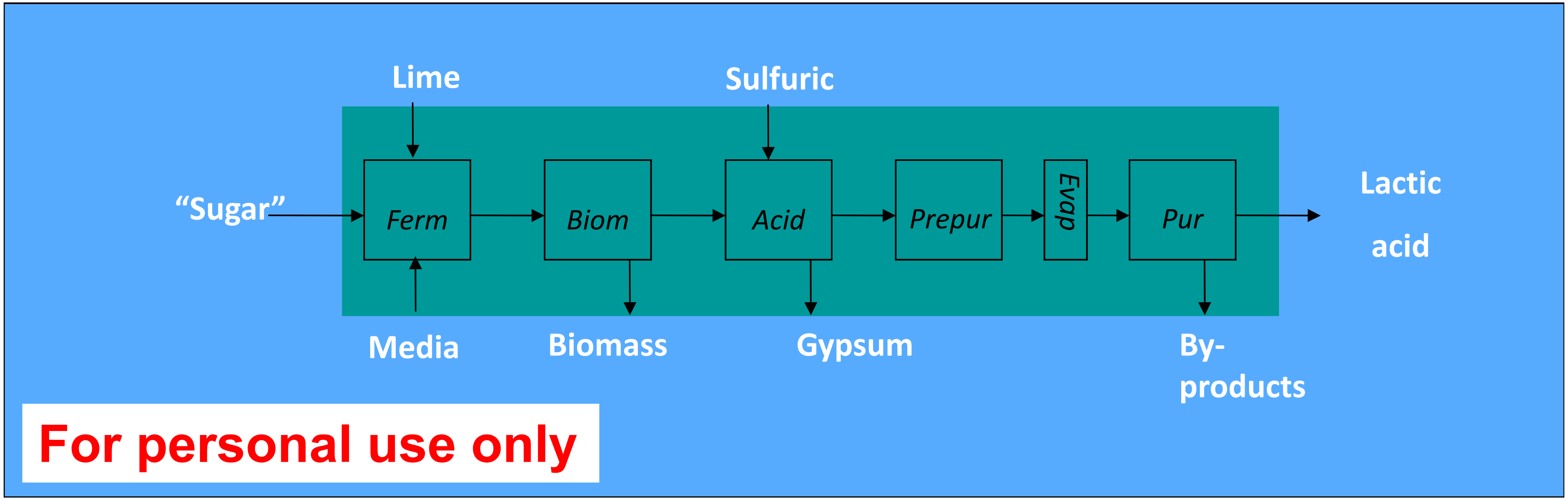
So:

$$1\ C_6H_{12}O_6 + 2\ O_2 \rightarrow 2\ C_2H_4O_2 + 2\ CO_2 + 2\ H_2O \text{ (in yeast)}$$

$$1\ C_6H_{12}O_6 \rightarrow 3\ C_2H_4O_2 \text{ (max. theoretical)}$$

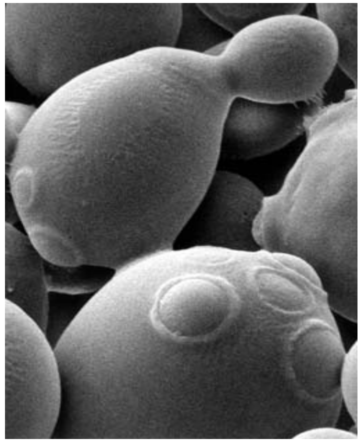
Traditional lactic-acid production process

sugar lime nutrients H_2SO_4 gypsum lactic acid



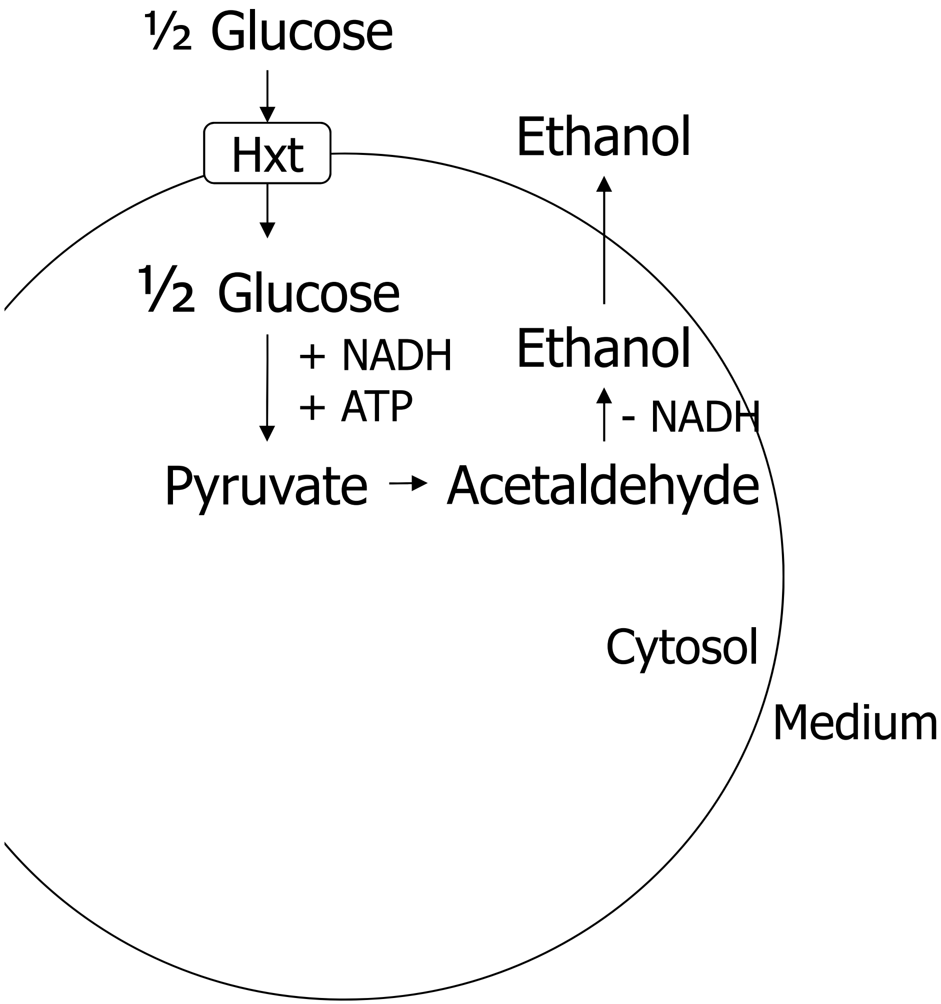
Why *Saccharomyces cerevisiae* ?

- Robust (pH, no phages, morphology)
- Anaerobic growth
- Well studied physiology
- Genetically accessible

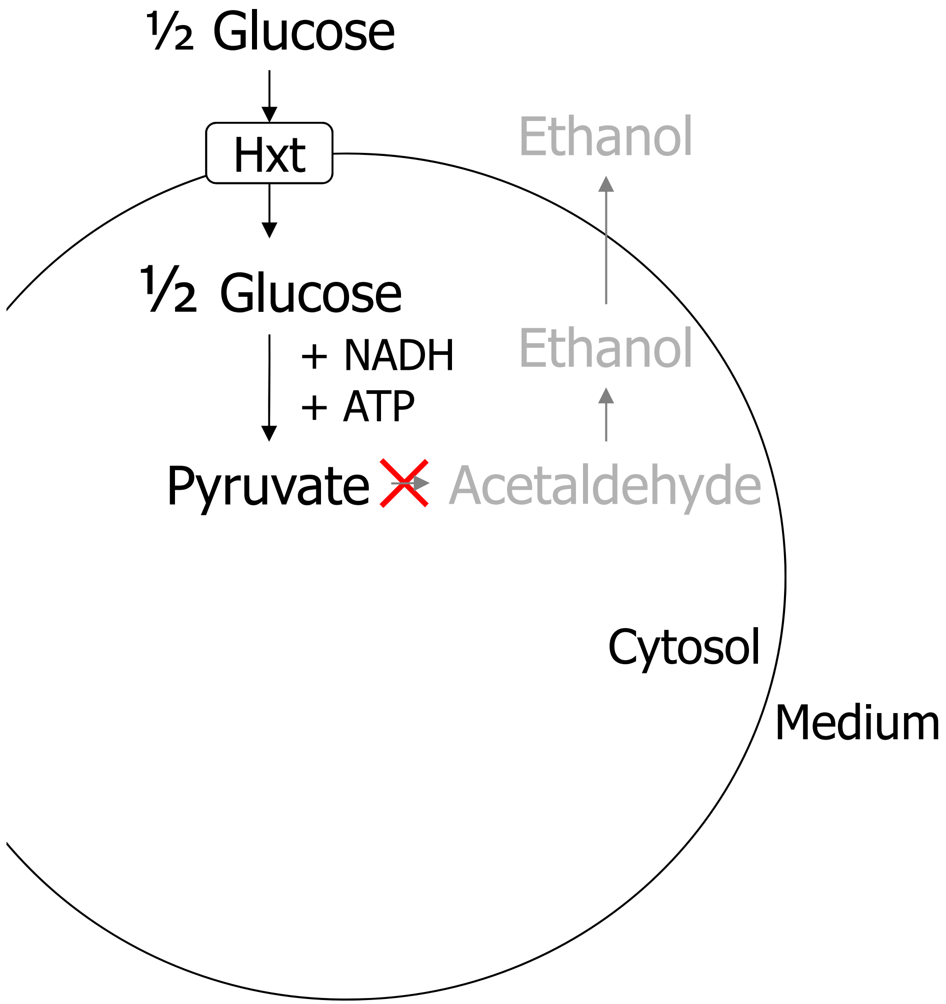


→ House microbe of Delft Industrial Microbiology

Rerouting metabolism for organic acid production

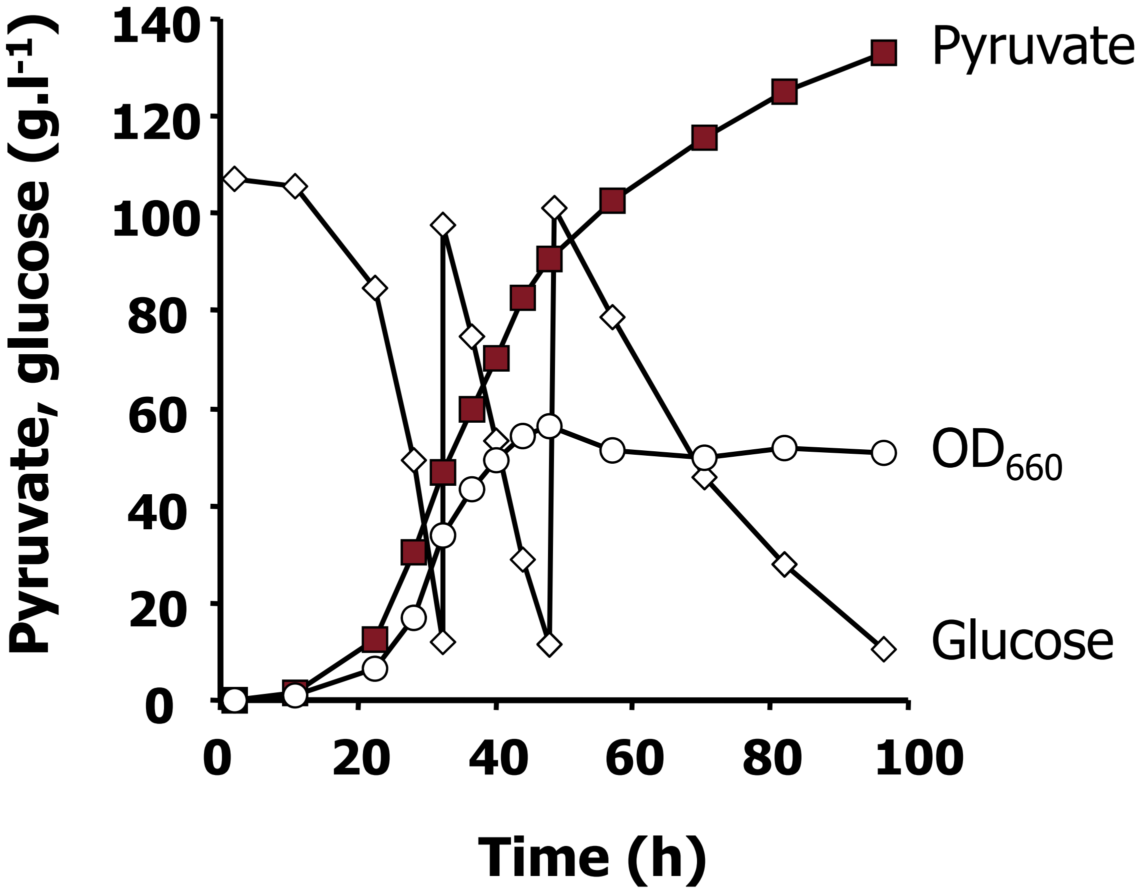


Rerouting metabolism for organic acid production



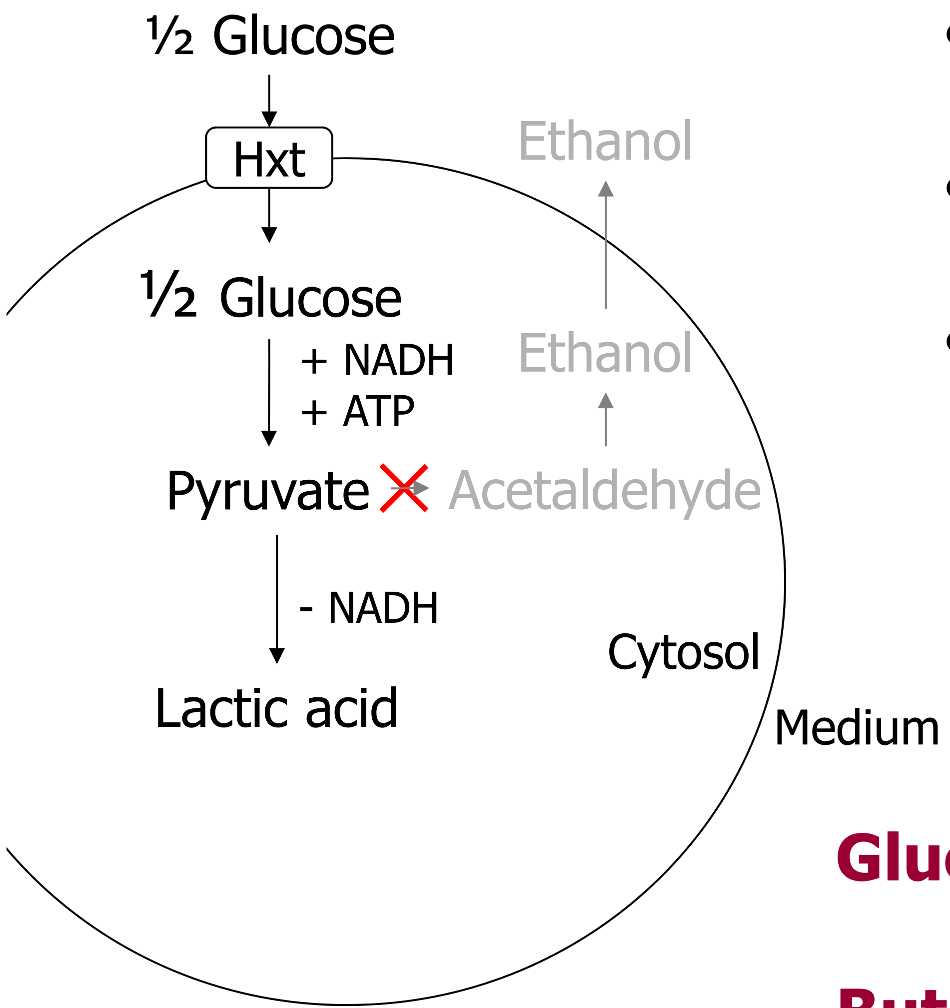
An ethanol-negative, pyruvate producing yeast platform

C_2-independent, glucose-tolerant *pdc1,5,6* strain of *S. cerevisiae*
obtained by evolutionary engineering ("TAM")



[pyruvate] = 135 $g.l^{-1}$
$Y_{pyr/gluc}$ = 0.55 $g.g^{-1}$

Rerouting metabolism for organic acid production

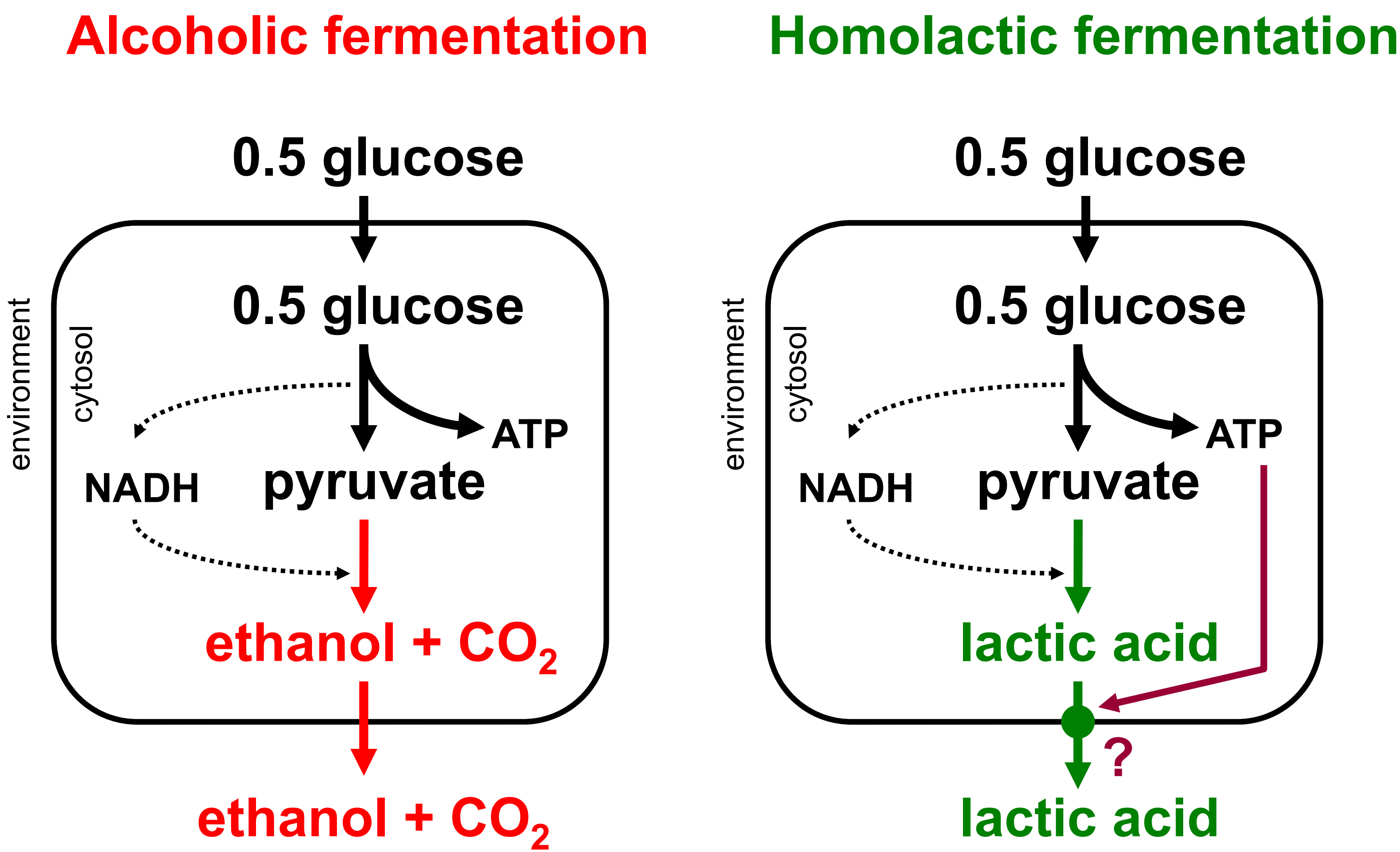


- Rerouting carbon
- Byproduct reduction
- Balanced redox co-factors

Glucose →2 lactic acid + 2 ATP

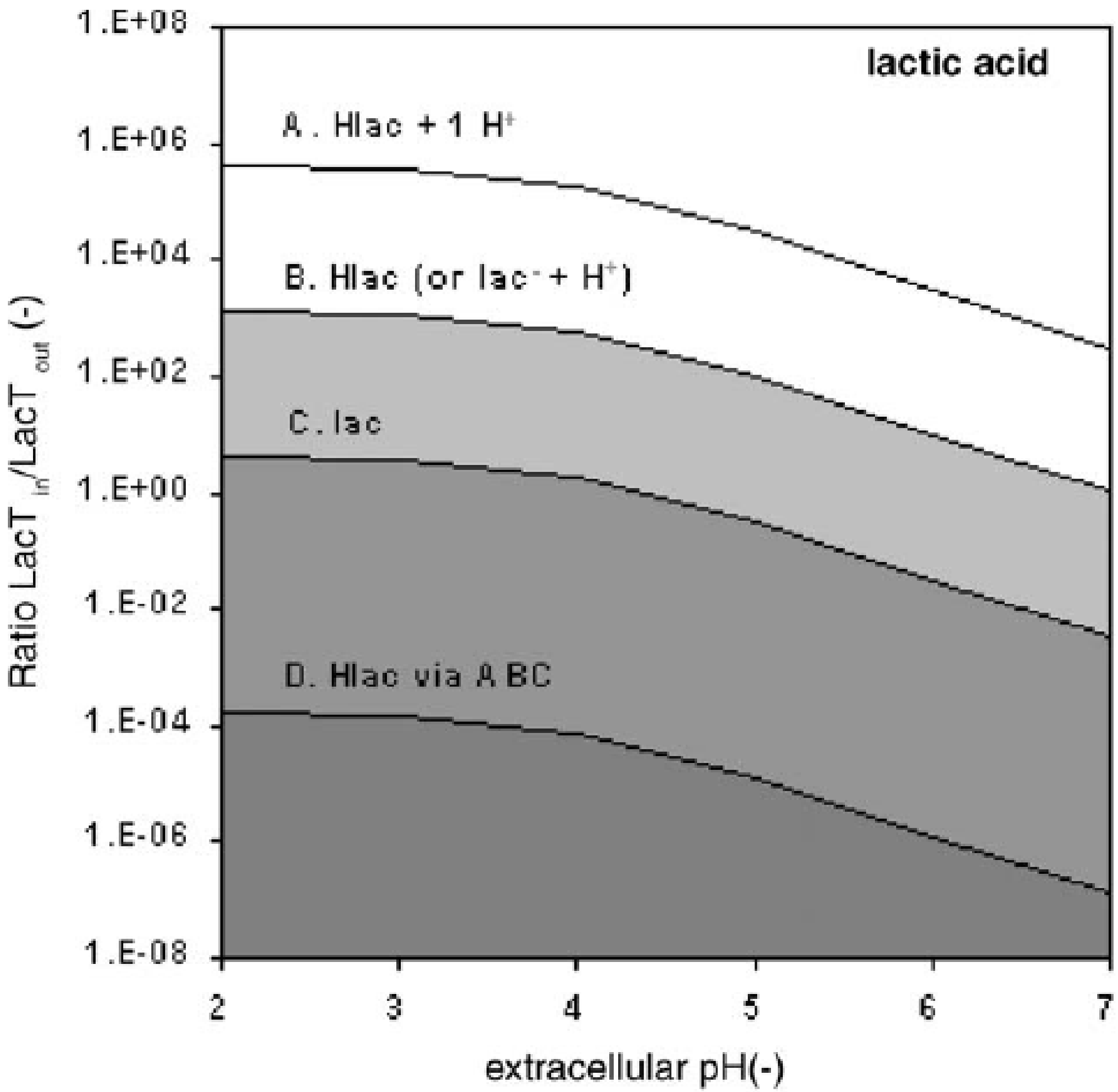
But… no anaerobic growth?

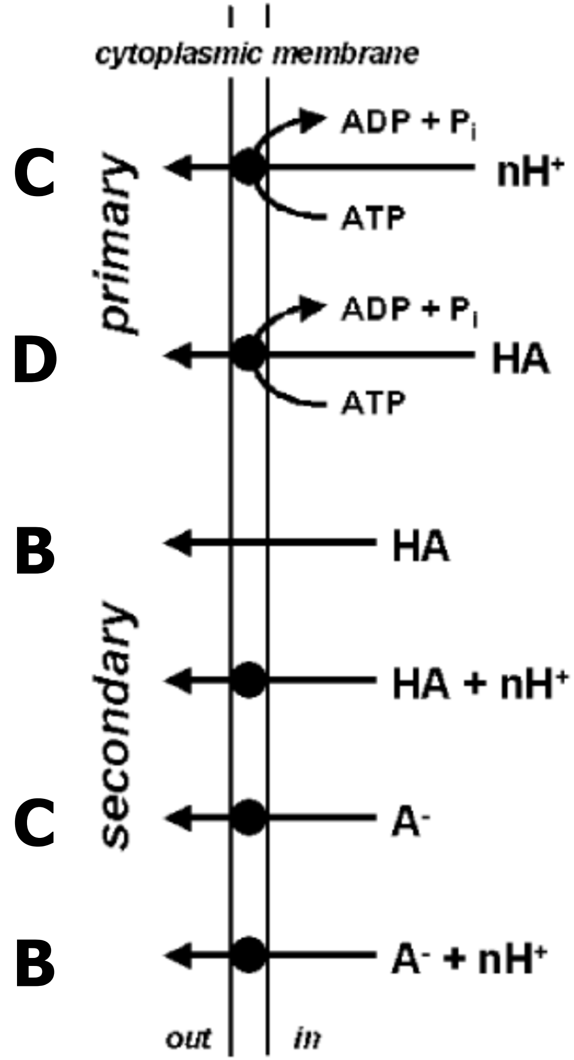
Homolactic fermentation with *S. cerevisiae*



TU Delft

Energetics of lactic acid export



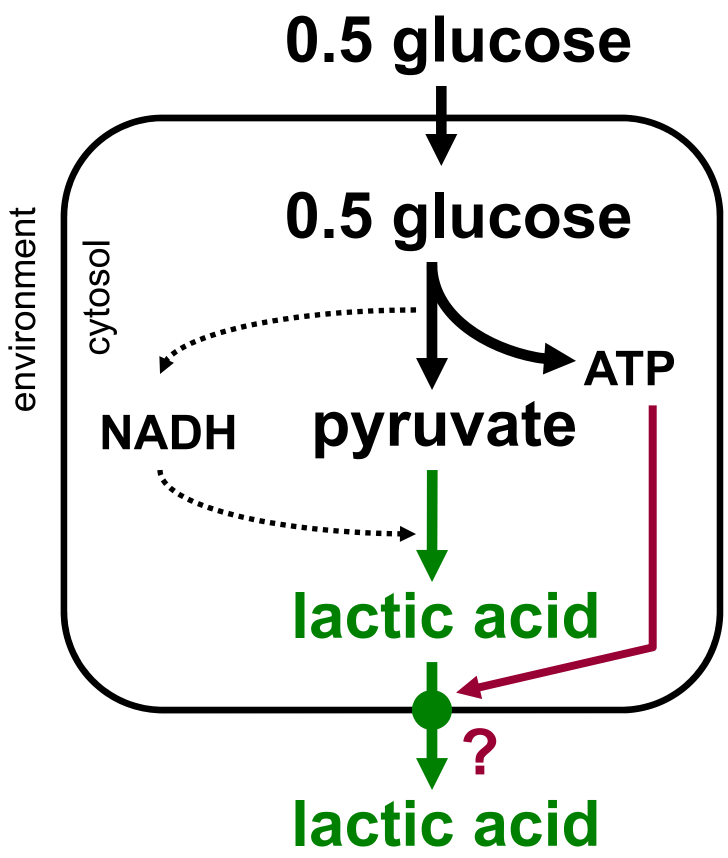


Van Maris et al. (2004) Met. Eng.

TU Delft

Homolactic fermentation with *S. cerevisiae*

Homolactic fermentation



- Apply oxygen limitation (Cargill yeast process)

OR

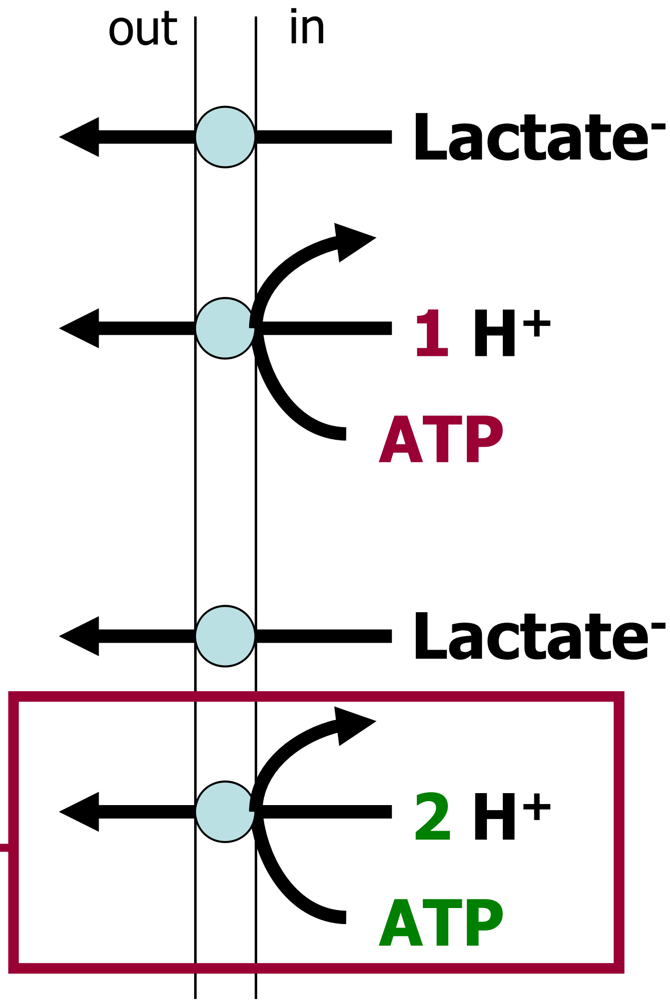
- Engineer free-energy conservation
  - Transport
  - Maltose metabolism
  - Carboxylation reactions
  - Acetyl-CoA

Stoichiometry of plasma membrane ATPase

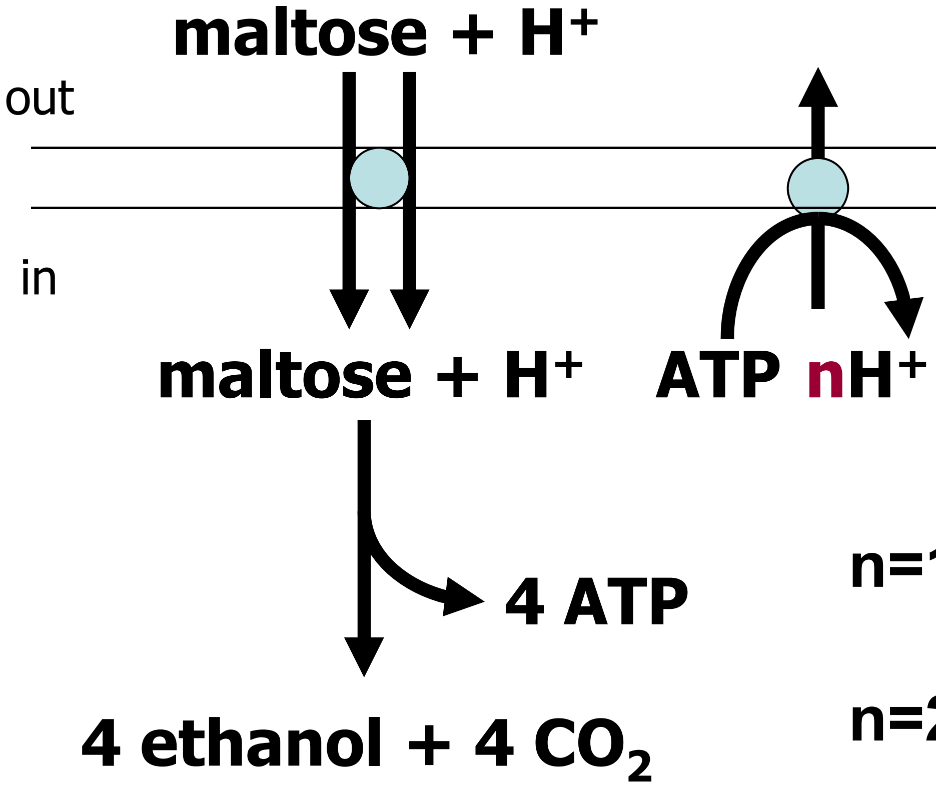
Glucose → 2 lactic acid

Glucose → 2 lactic acid + 1 ATP

Based on *in vitro* studies:
Pma1pSer800Ala (Guerra et al. 2007)
Pma1pGlu803Gln (Petrov et al. 2000)



Stoichiometry of plasma membrane ATPase



n=1 → 3.0 ATP per maltose

n=2 → 3.5 ATP per maltose

This would translate *in vivo* to +17% biomass yield

TU Delft

Stoichiometry of plasma membrane ATPase

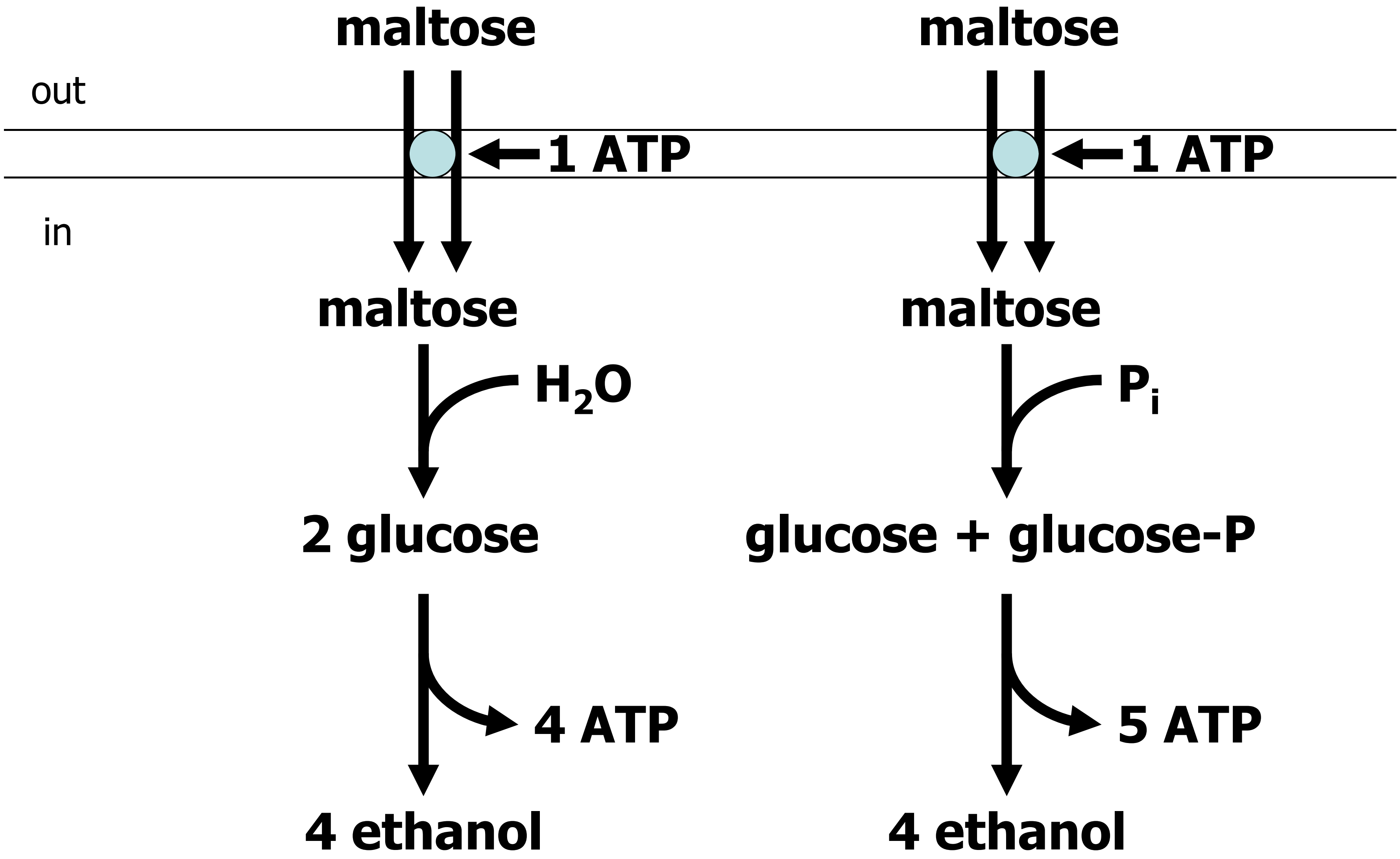
Strain	Relevant genotype	Carbon source	Biomass yield (g·g gluc eq^{-1}) pH 5.0	pH 6.7
CEN.PK113-7D	*PMA1 PMA2*	Glucose	0.095±0.002	0.087±0.001
CEN.PK113-7D	*PMA1 PMA2*	Maltose	0.072±0.000	0.066±0.000
IMK328	*PMA1 pma2Δ*	Maltose	0.072±0.001	0.070±0.000
IMX051B	*PMA1^{Ser800Ala} pma2Δ*	Maltose	0.073±0.001	0.069±0.002
IMX052	*PMA1^{Glu803Gln} pma2Δ*	Maltose	0.073±0.001	0.069±0.001

Regrettably no *in vivo* impact of Ser800Ala and Glu803Gln

Platform to screen for ATPases with altered stoichiometry

De Kok et al. (2012)

Free-enery conservation in disaccharide hydrolysis



 se

4 ATP/maltose

Free-enery conservation in disaccharide hydrolysis

- Creating a strain without maltase activity
- Heterologous expression of MalP from *L. sanfranciscensis*
- Co-expression of β-phosphoglucomutase (βg1p→g6p)

Strain	**IMZ199**	**IMZ226**
Relevant genotype	***malΔ mphΔ + MAL11 + MAL12***	***malΔ mphΔ + MAL11 + mapA + pgmB***
Theoretical biomass yield (g gluc eq^{-1})	**0.066**	**0.088**
Observed biomass yield (g gluc eq^{-1})	**0.067±0.000**	**0.085±0.000**

From anaerobic maltose-limited chemostat cultures at a dilution rate of 0.05 h^{-1}.

De Kok et al. (2011)

Design a process, not just a microorganism!

- What would the process ideally look like? DSP, fermentation, feedstock?
- What would be the max. theoretical yield for product X be on substrate Y?
- Can I (or my software) come up with a pathway with that yield? Or close to it?
    - Redox-cofactor balances (O_2 or engineering?)
    - Positive ATP yield (O_2 or engineering?)
- Which microorganism can (potentially) fulfil those requirements?

Acknowledgements

Rintze Zelle (Mascoma)

Stefan de Kok (Amyris → TUD)

TATE & LYLE: Jeff Lievense, Chi-Li Liu
Stan Bower

MICROBIA: Jake Harrison
Josh Trueheart
Kevin Madden

BIRD ENGINEERING: Hans van Dijken
Ron Winkler

WAGENINGEN UR: Cor Dijkema
Pieter de Waard

Wouter van Winden (then TUDelft, now DSM)

Industrial Microbiology Delft

Marinka Almering
Ishwar Bajaj
Nuria Barrajon Simancas
Mark Bisschops
Irina Bolat
Lizanne Bosman
Marcel van den Broek
Barbara Crimi
Jean-Marc Daran
Pascale Daran-Lapujade
Victor Guadalupe
Marit Heblij
Eline Huisjes
Erik de Hulster
Frank Koopman
Barbara Kozak
Niels Kuijpers
Marijke Luttik
Ruben Martinez
Benjamin Metz
Nick Milne
Jurgen Nijkamp
Bart Oud
Jack Pronk
Daniel Gonzalez Ramos
Gabriele Romagnoli
Harmen van Rossum
Daniel Solis Escalante
Tim Vos
Duygu Yilmaz

Kluyver Centre for Genomics Of Industrial Fermentation
Netherlands Genomics Initiative
TU Delft