PQI 5888
Fisiologia e Biotecnologia de Leveduras

Prof. Thiago Basso

15 de abril de 2020

[Aula 3 EaD]

Leveduras – Aplicações Industriais

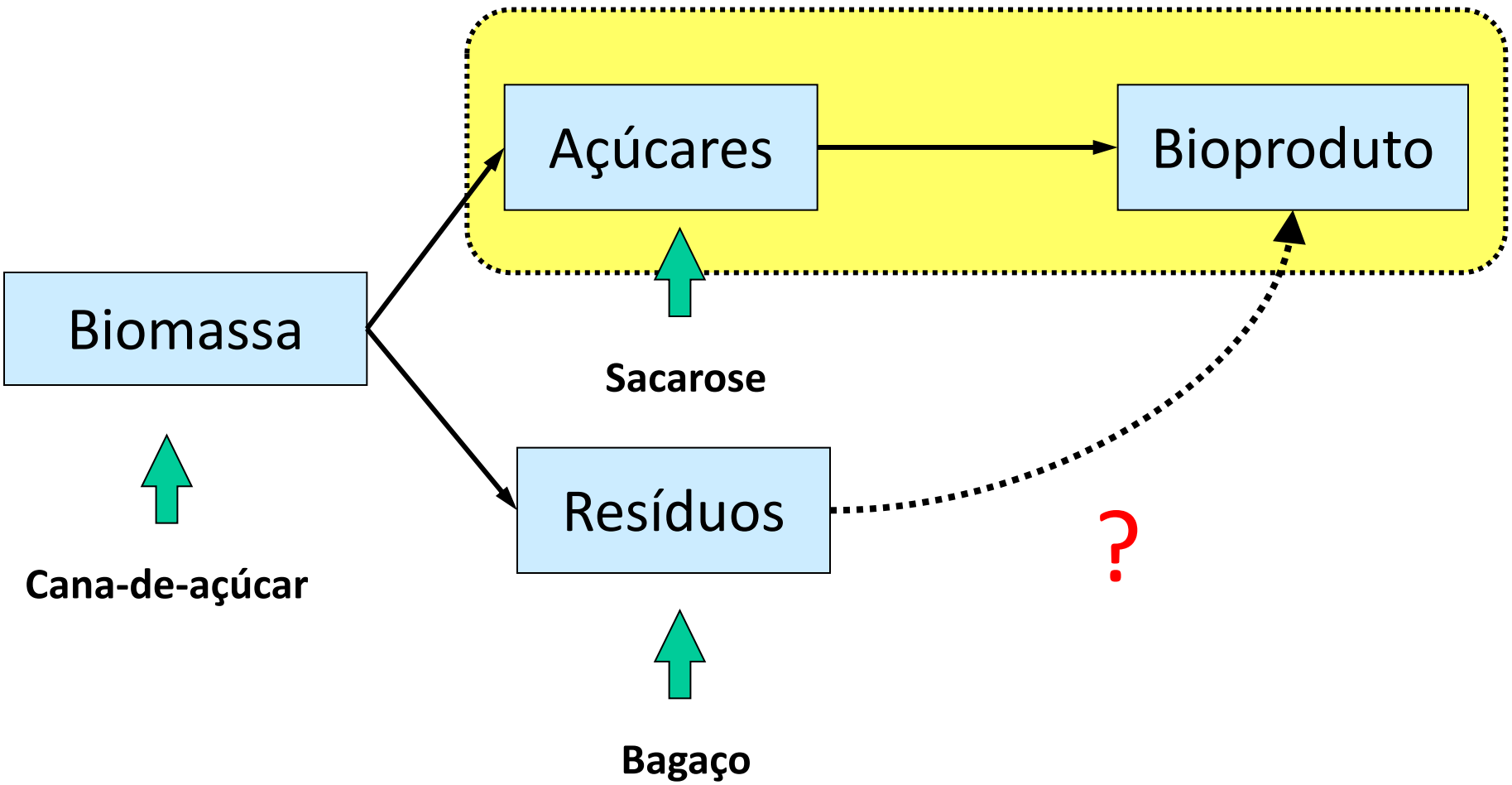
(Bioprocessos Lignocelulósicos)

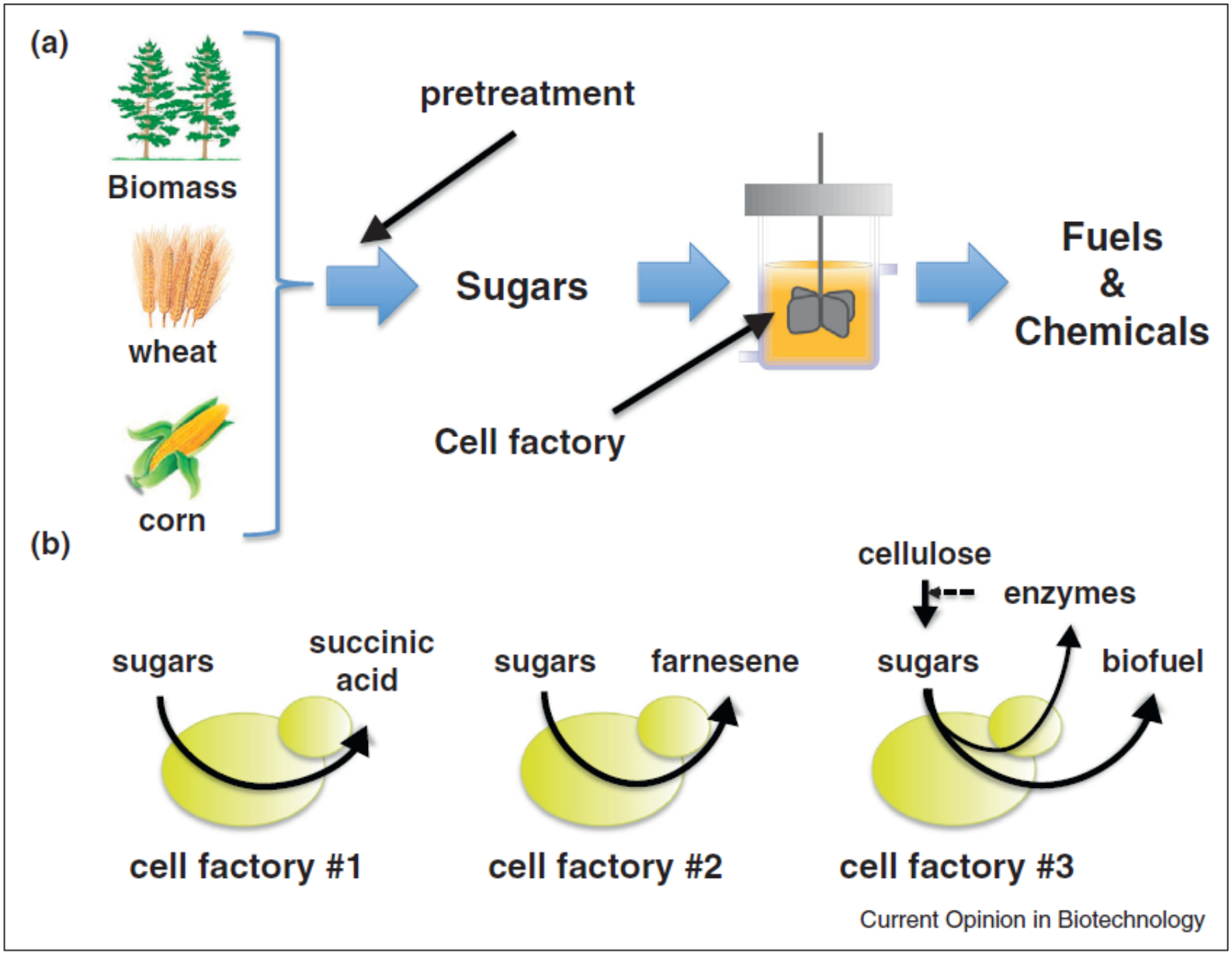
(b)

BIOPROCESSOS de SEGUNDA GERAÇÃO (2G)

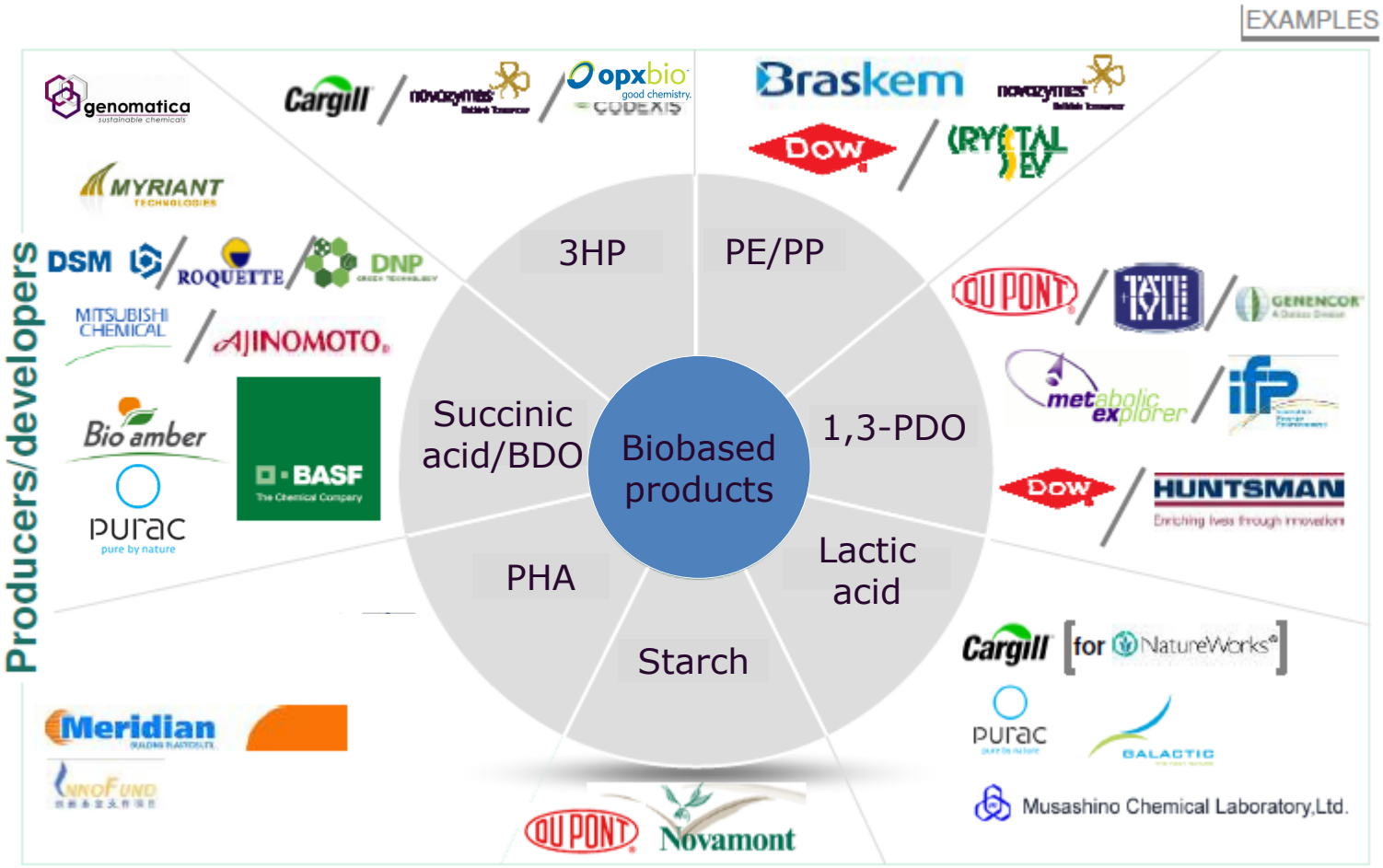
DESAFIOS E PERSPECTIVAS

Bioprocessos 2G



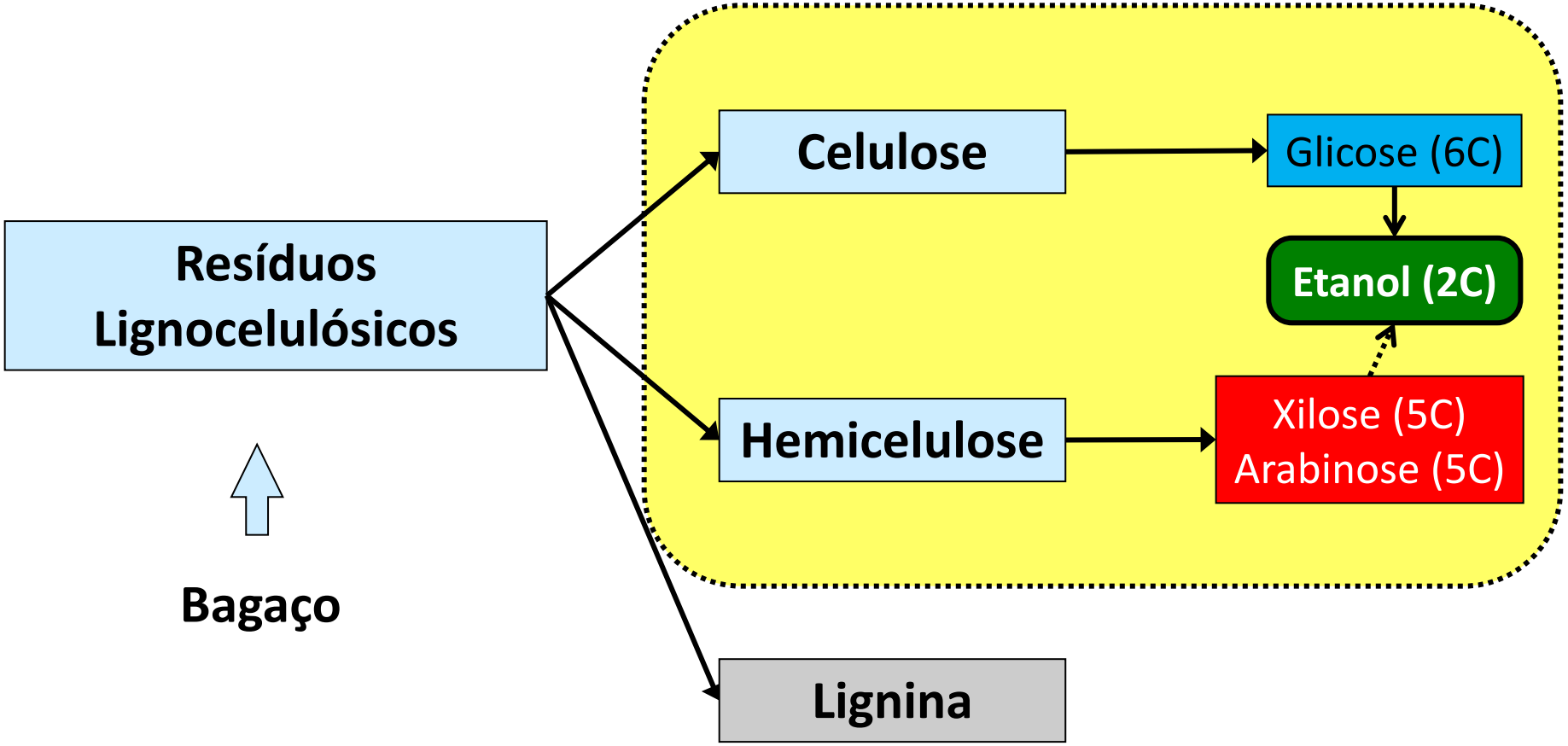

(a)
Biomass
wheat
corn
pretreatment
Sugars
Cell factory
Fuels
&
Chemicals
(b)
sugars
succinic
acid
cell factory #1
sugars
farnesene
cell factory #2
cellulose
enzymes
sugars
biofuel
cell factory #3
Current Opinion in Biotechnology

Example of activities - green plastics and intermediates



Source: McKinsey 2011 and press search

Etanol de Lignocelulósicos



BIOMASSA PARA O ETANOL 2G

Cana-de-açúcar (BAGAÇO)

Sorgo

Eucalyptus

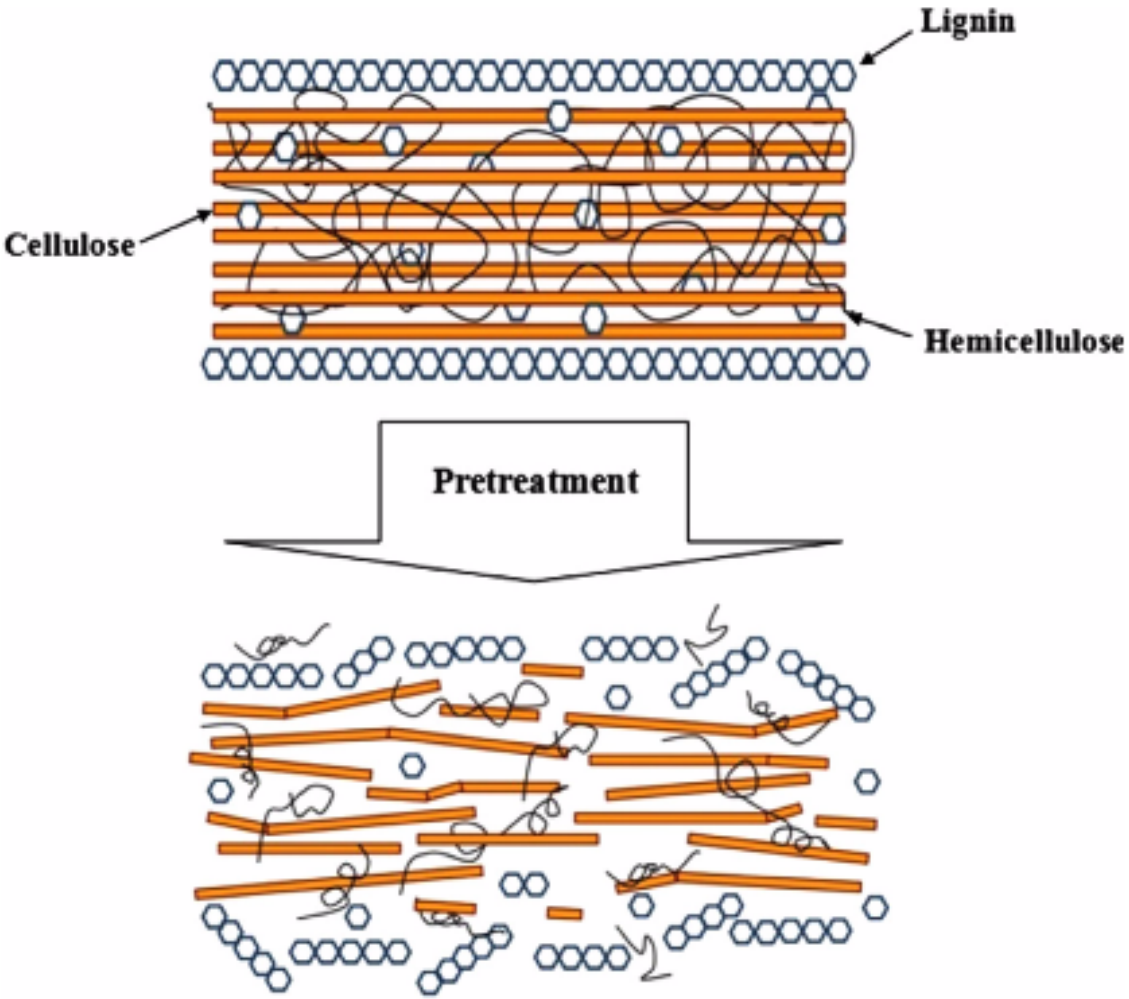
Milho (SABUGO)

Cana-energia

LIGNOCELULOSE

Genome Management Information System/ORNL

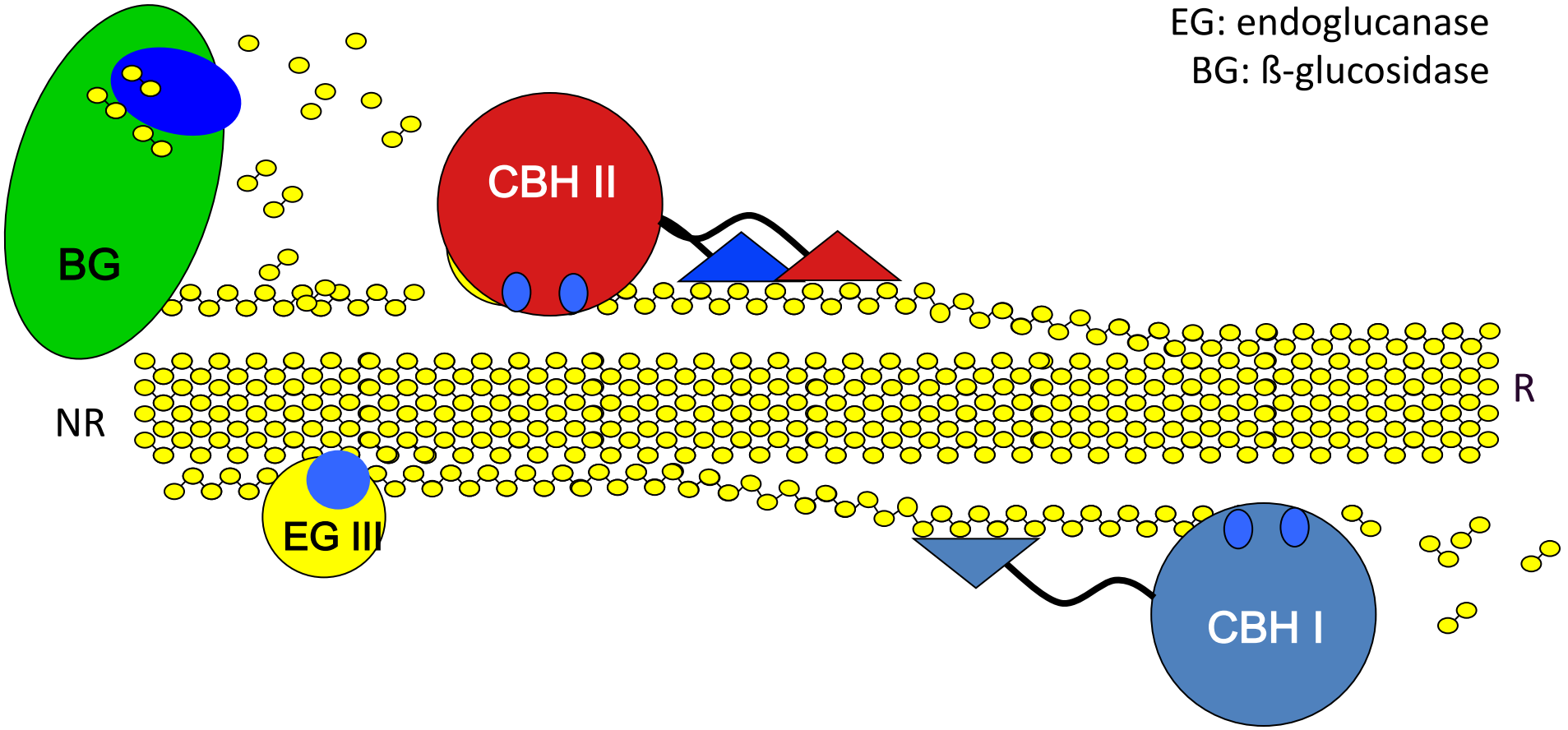
PRÉ-TRATAMENTO



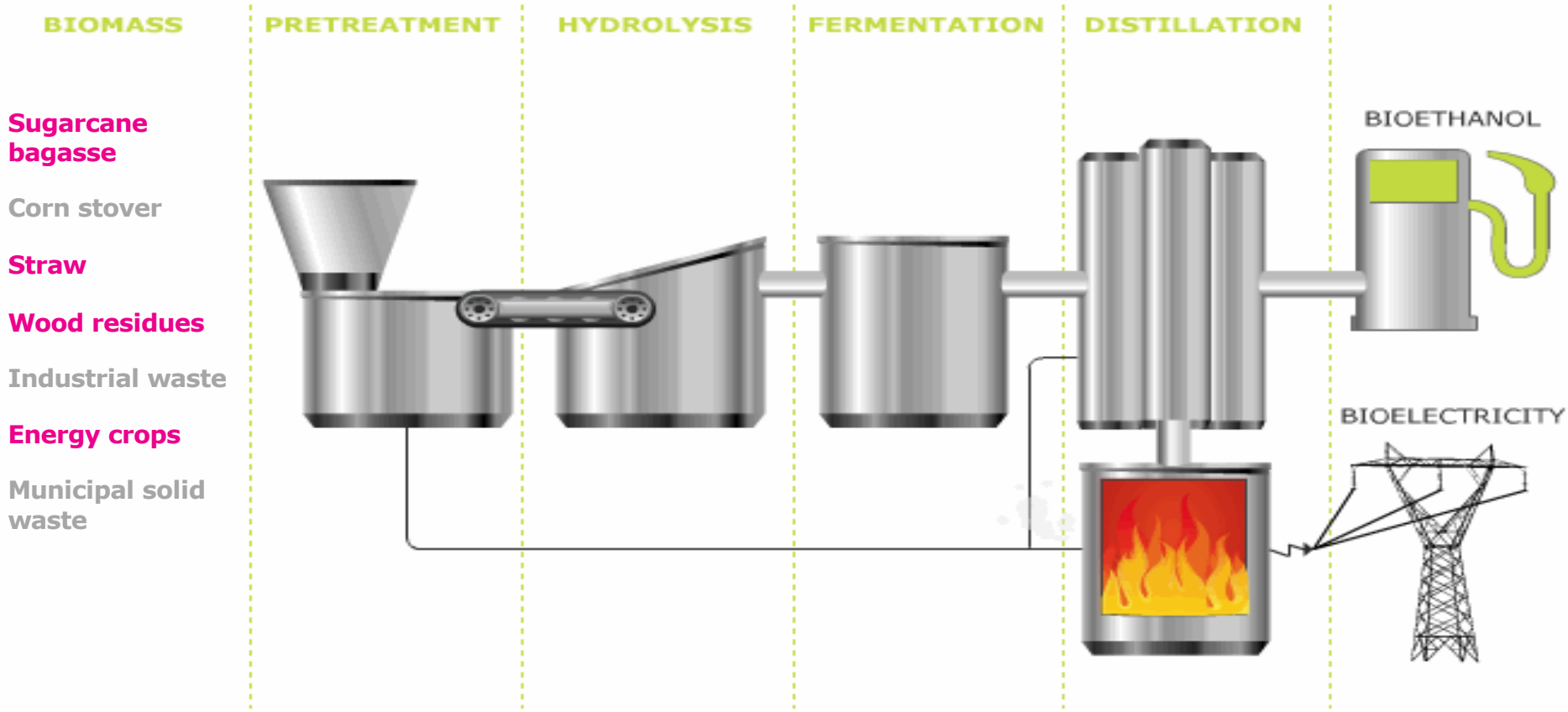
PRÉ-TRATAMENTO E HIDRÓLISE

HIDRÓLISE ENZIMÁTICA DA CELULOSE

CBH: exocellobiohydrolsae
EG: endoglucanase
BG: ß-glucosidase

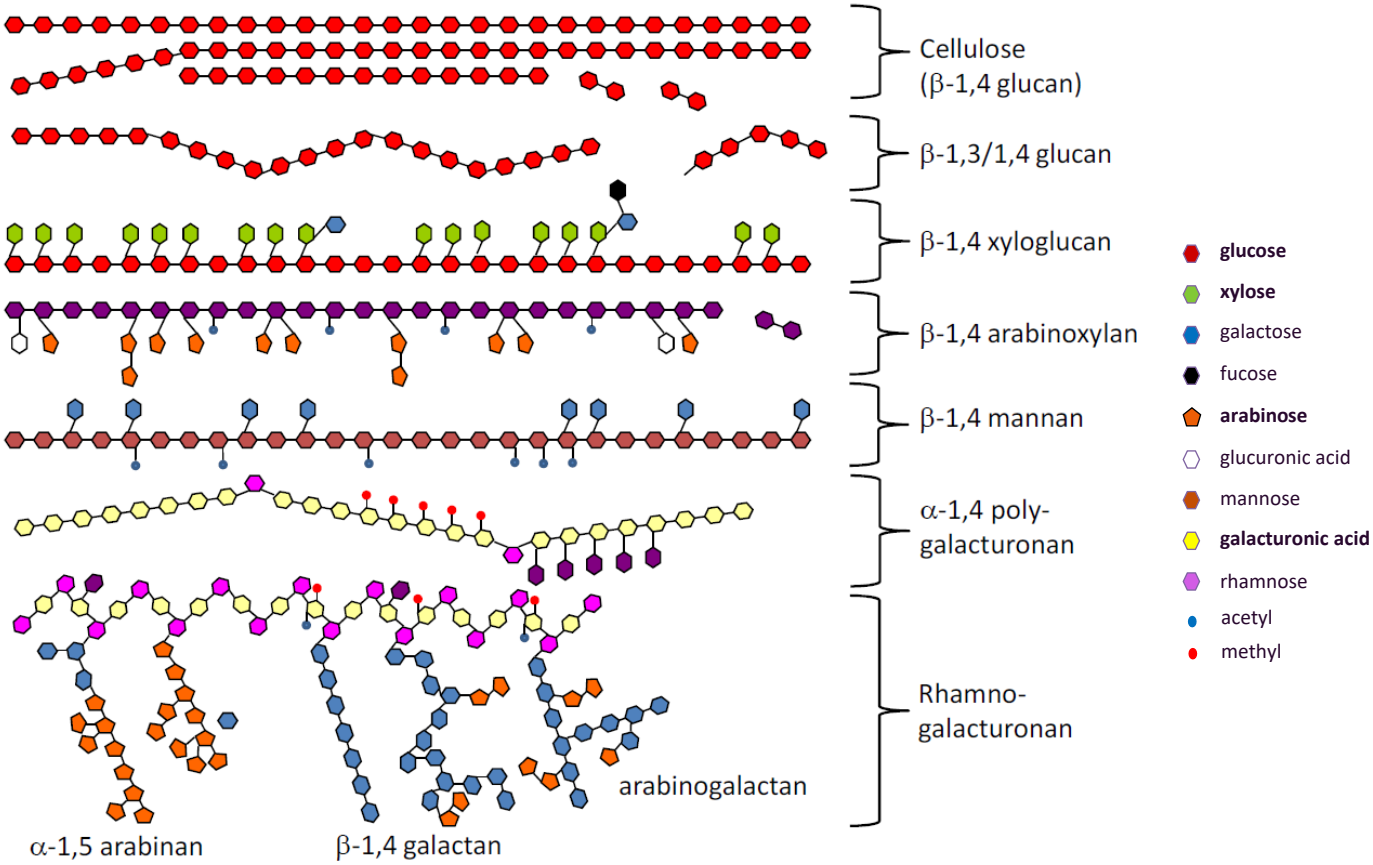


TECNOLOGIA 2G



PRODUTOS DA HIDRÓLISE

Sugarcane bagasse



Phalip et al., 2012

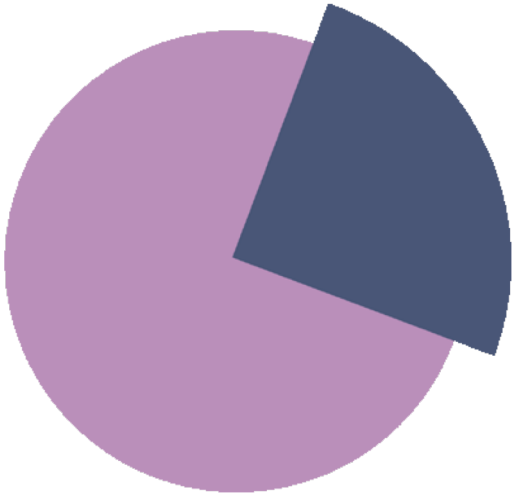
Bagaço

Table 2 Composition (major sugars) of common agricultural lignocellulosic feedstocks (adapted from Grohmann and Bothast 1994; Lee 1997; http://www.ecn.nl/phyllis/)

	Corn stover	Wheat straw	Bagasse	Cotton gin	Sugar beet pulp	Switch grass
Carbohydrate (%)						
Glucose	34.6	32.6	39.0	37.1	24.1	31.0
Mannose	0.4	0.3	0.4	1.1	4.6	0.2
Galactose	1.0	0.8	0.5	2.4	0.9	0.9
Xylose	19.3	19.2	22.1	9.4	18.2	0.4
Arabinose	2.5	2.4	2.1	2.3	1.5	2.8
Uronic acids	3.2	2.2	2.2	NA	20.7	1.2
Non-carbohydrate (%)						
Lignin	17.7	16.9	23.1	28.8	1.5	17.6
Extractives	7.7	13.0	3.8	7.7	NA	17.0
Ash	10.4	10.2	3.7	10.5	8.2	5.8

Etanol de Lignocelulósicos

Bagaço de cana



Xilose (5C)

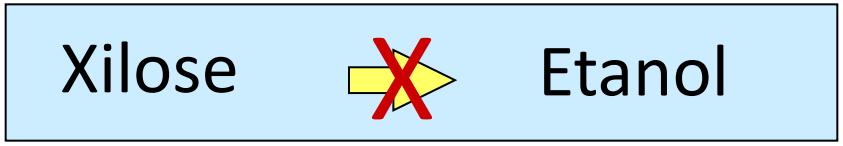
~ 20%

1 ton cana seca → 600kg bagaço → 120kg xilose → 60L etanol

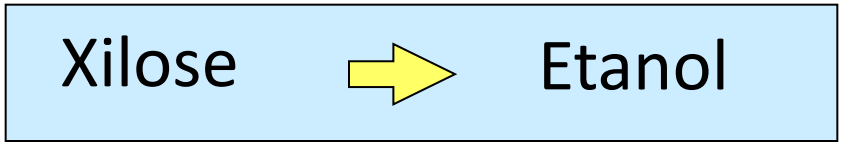
300 Milhões de Ton Cana-de-açúcar ⇢ 18 Bilhões de L ***extra***-etanol

Limitações na fermentação de xilose

A levedura ***S. cerevisiae***...



Leveduras fermentadoras de xilose (*P. stipitis*, *C. shehatae*)...

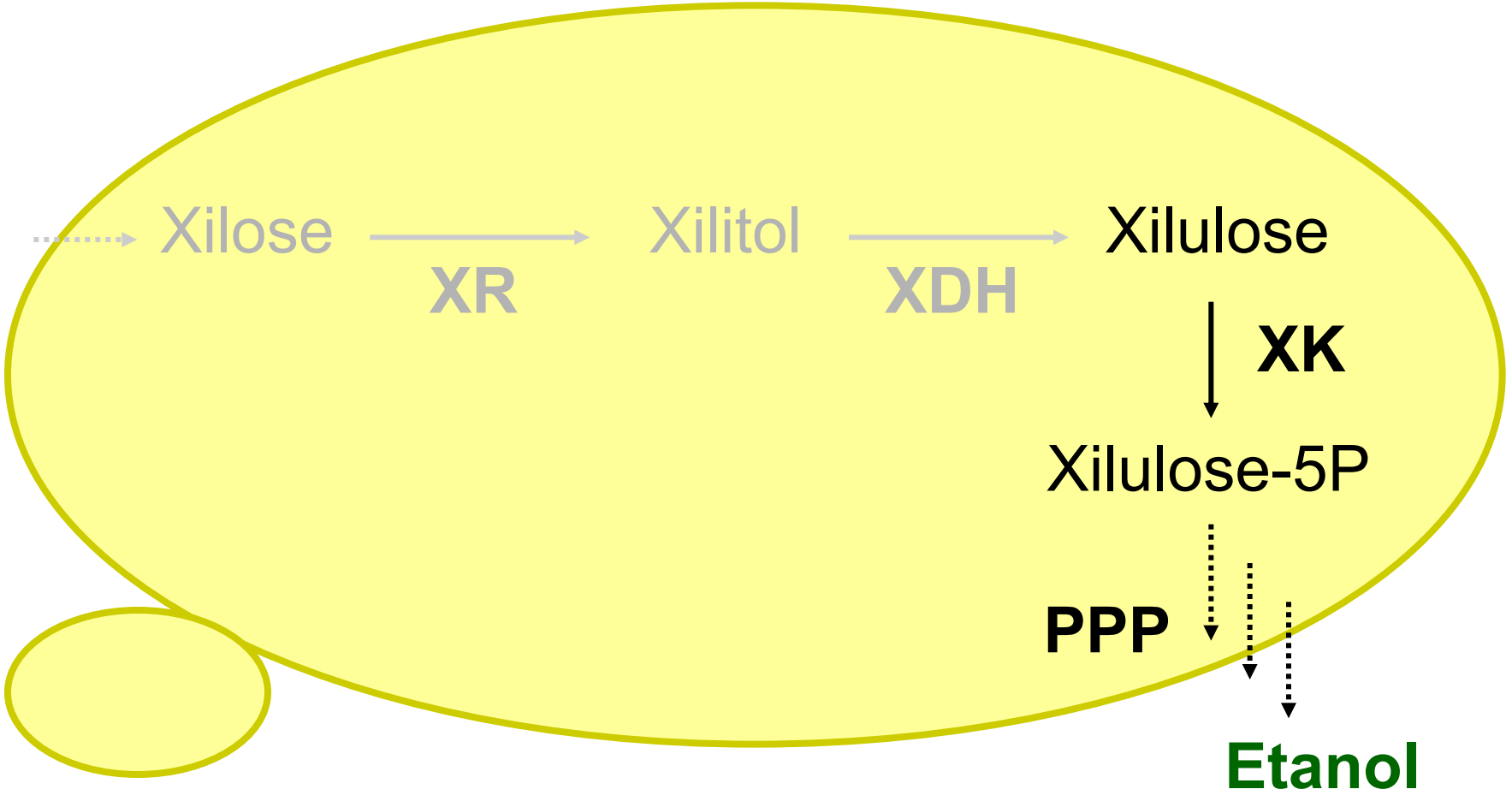


Poucas são **capazes** de fermentar xilose em anaerobiose...

...**não** suportariam o ambiente industrial (estresses)!

Metabolismo da Xilose em *S. cerevisiae*

Fonte: Kuyper et al., 2004



Metabolismo da Xilose em *S. cerevisiae*

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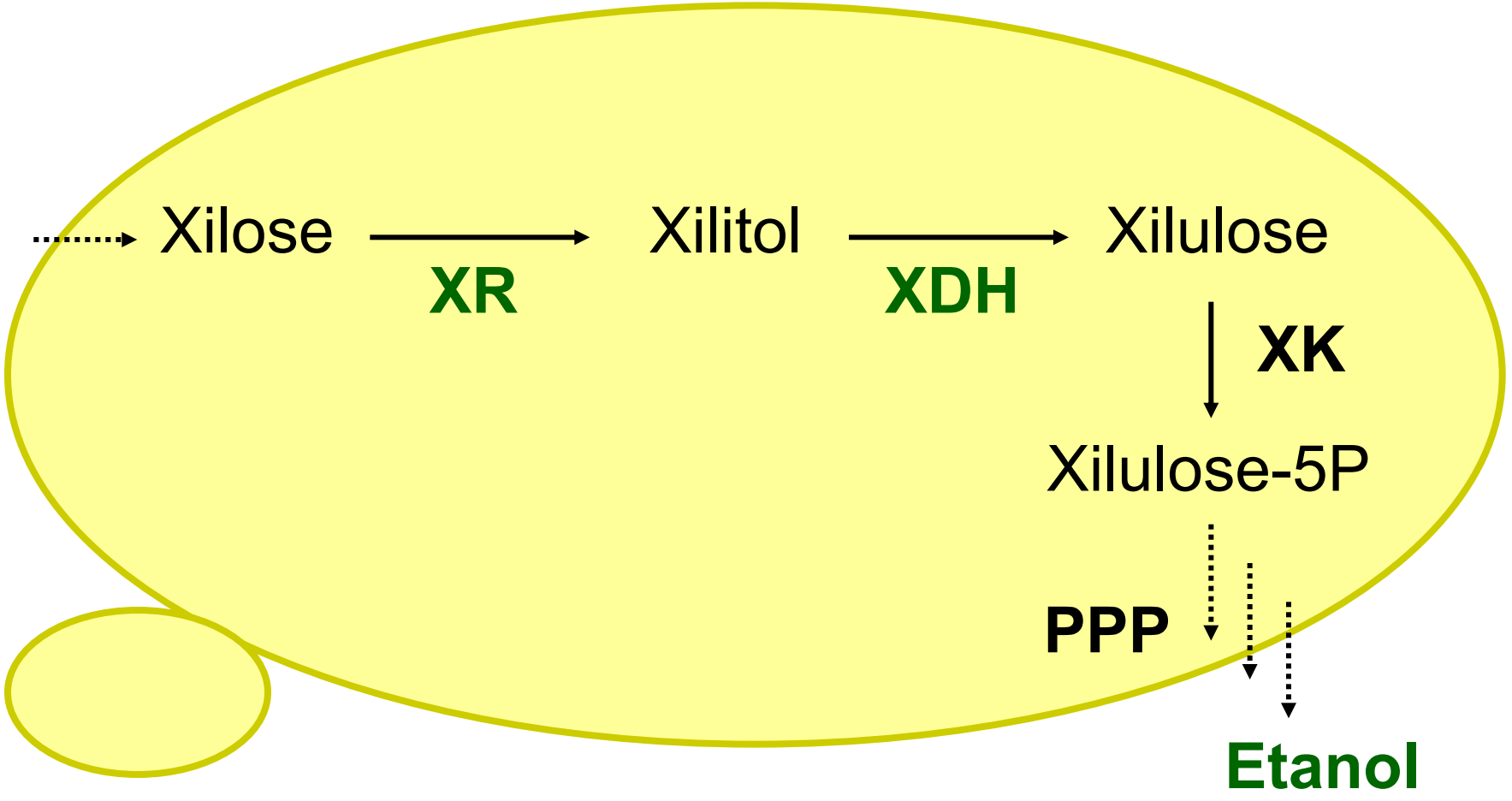

Metabolismo da Xilose em *S. cerevisiae*

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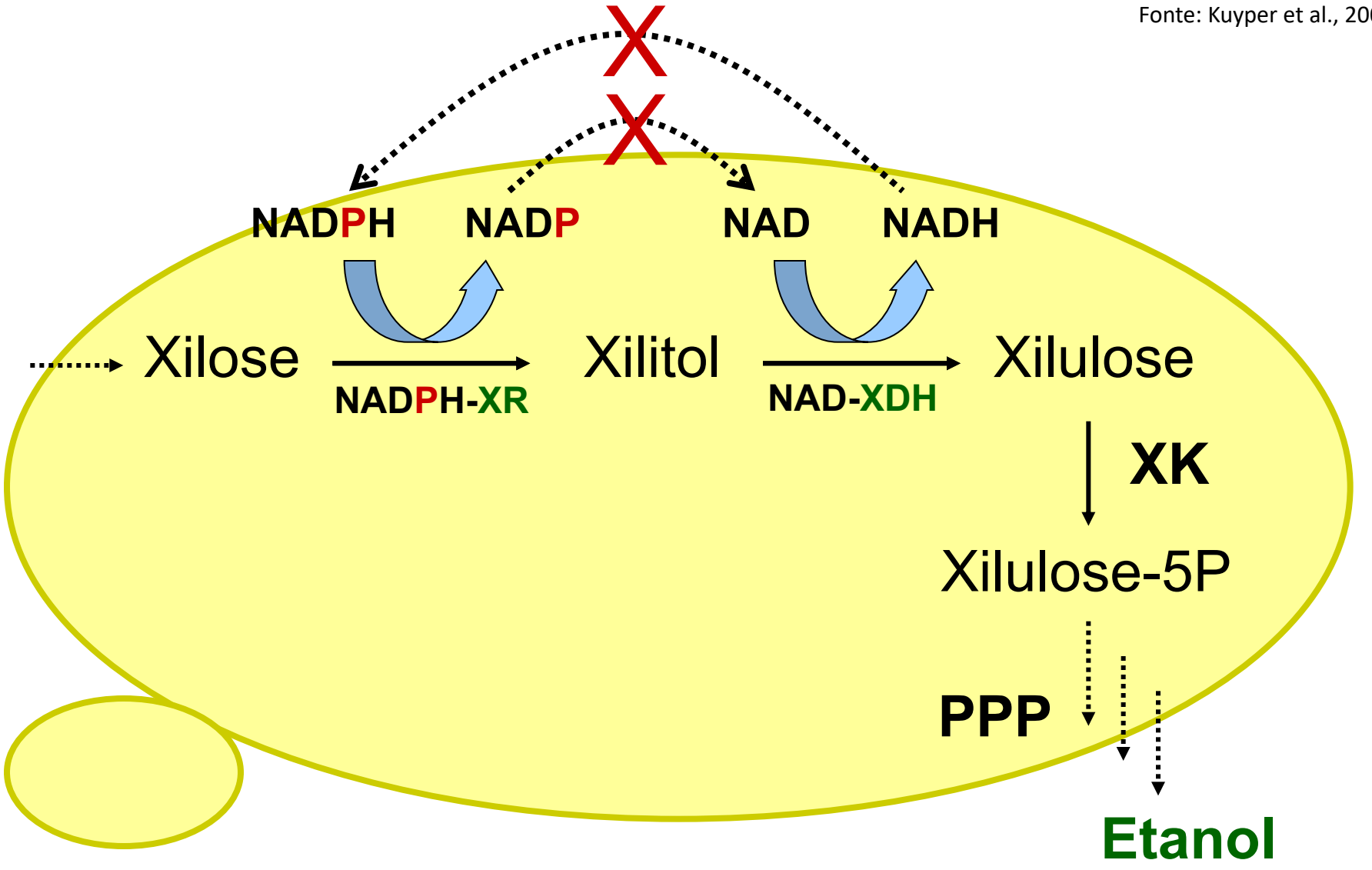

Metabolismo da Xilose em *S. cerevisiae*

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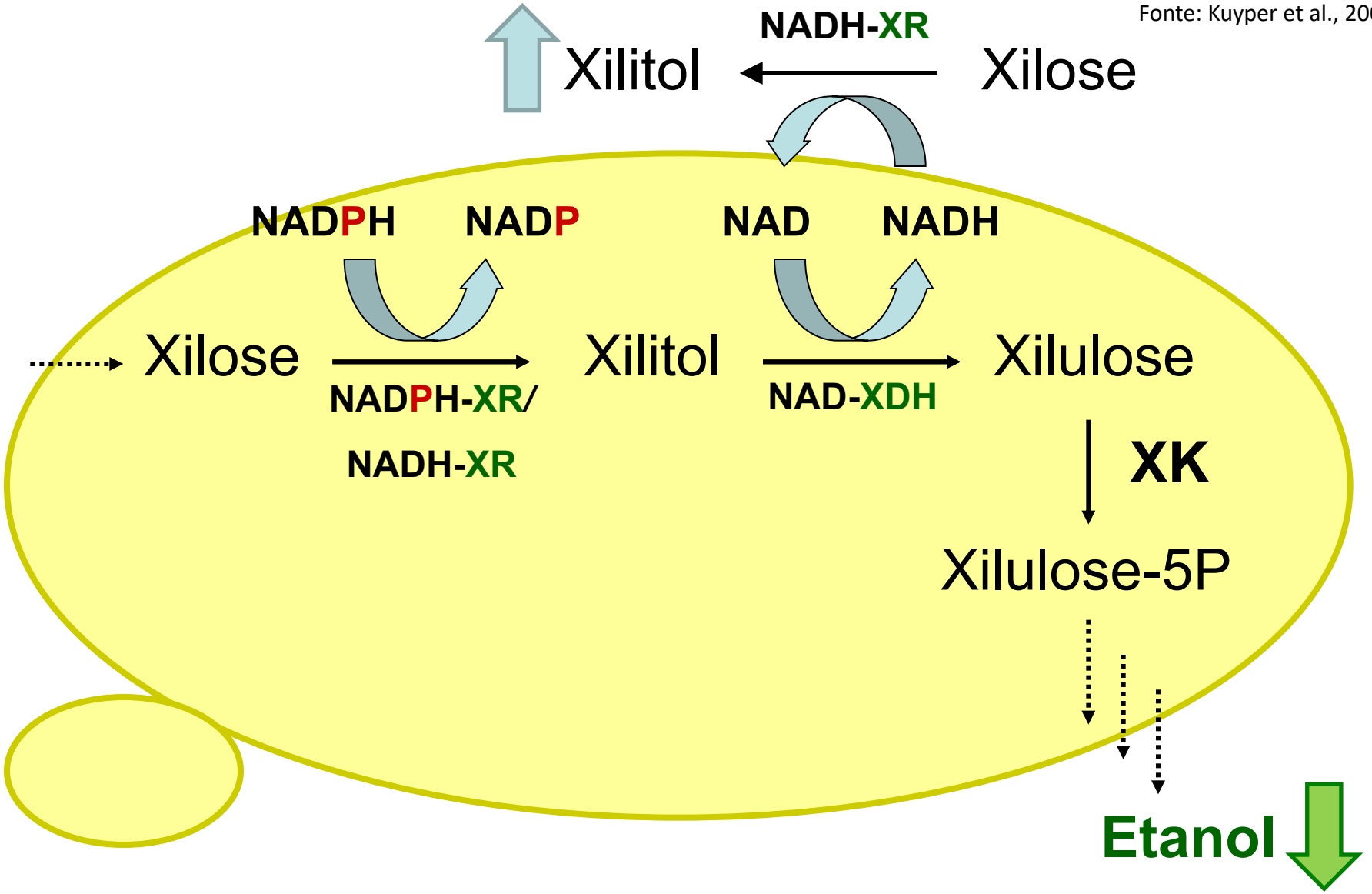
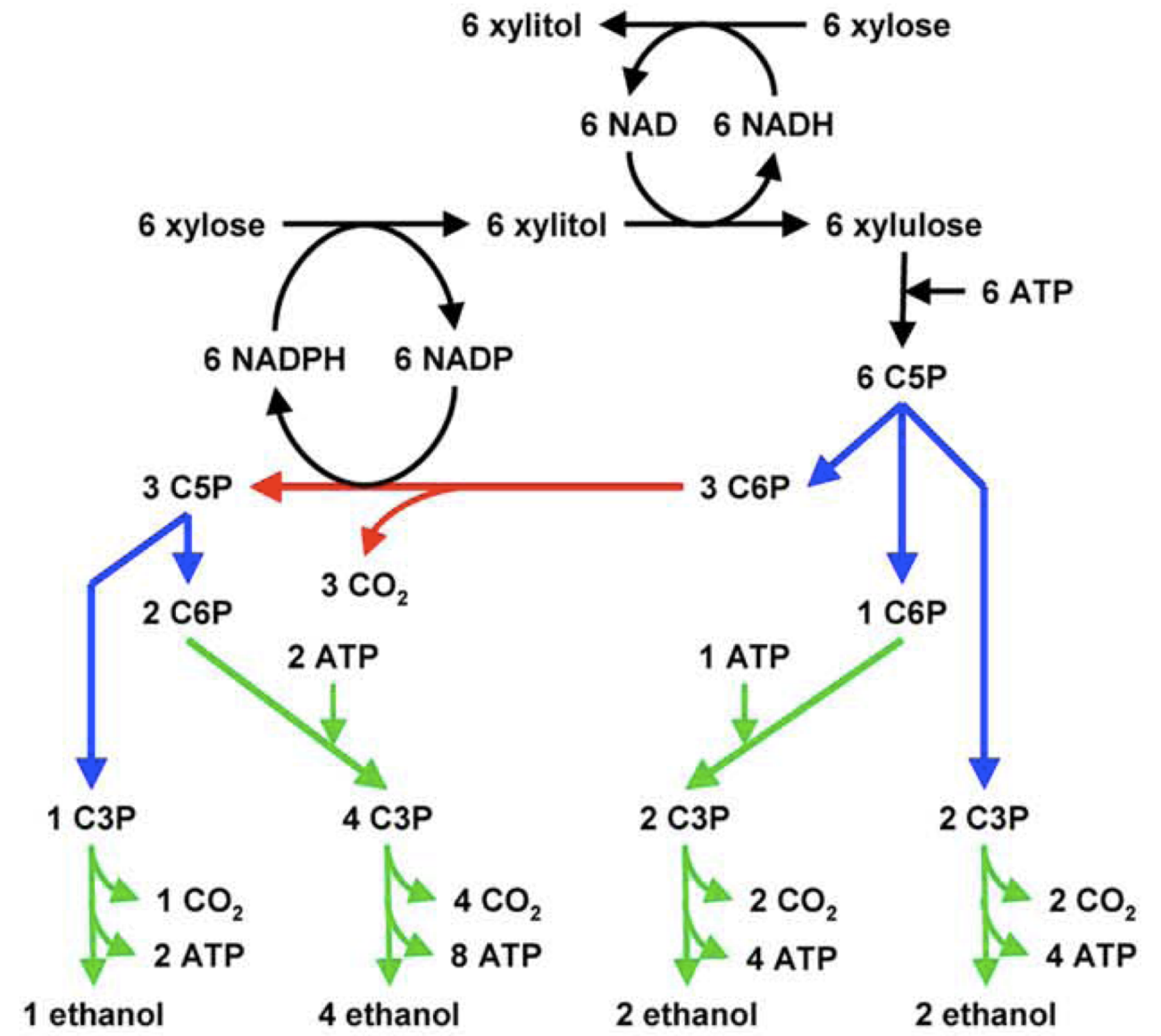
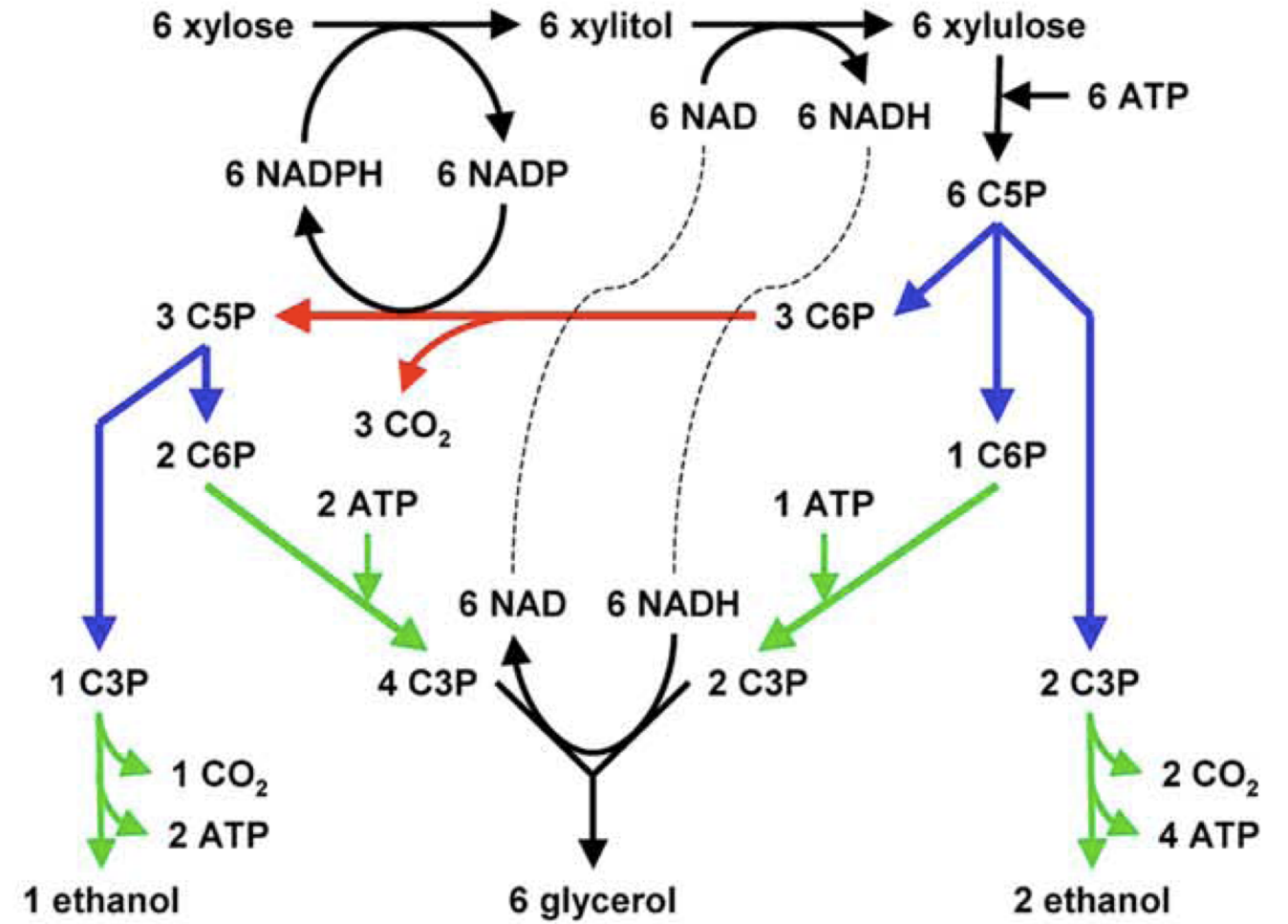


(c)



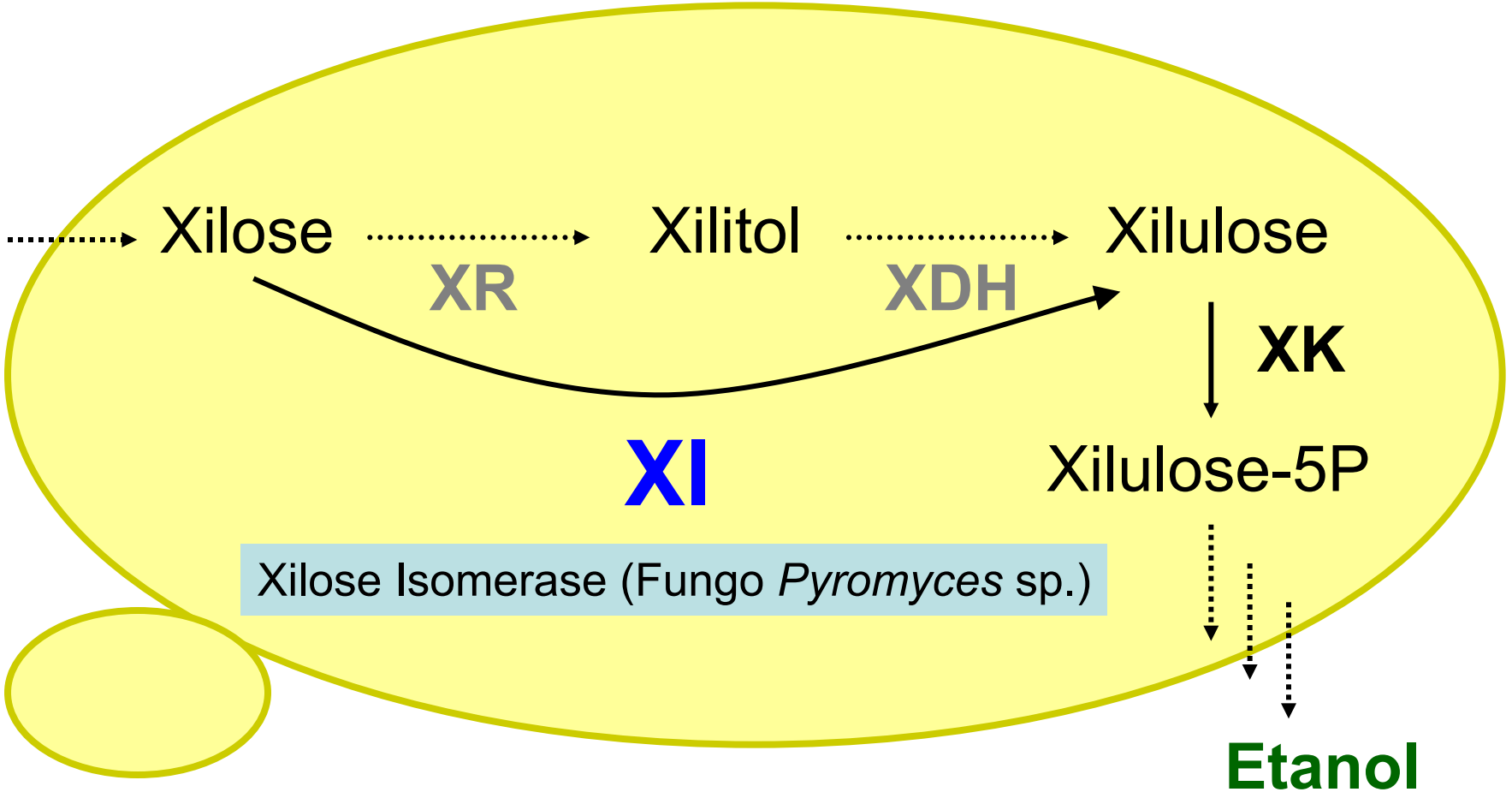
12 xylose → 9 ethanol + 12 CO_2 + 9 ATP + 6 xylitol

(b)



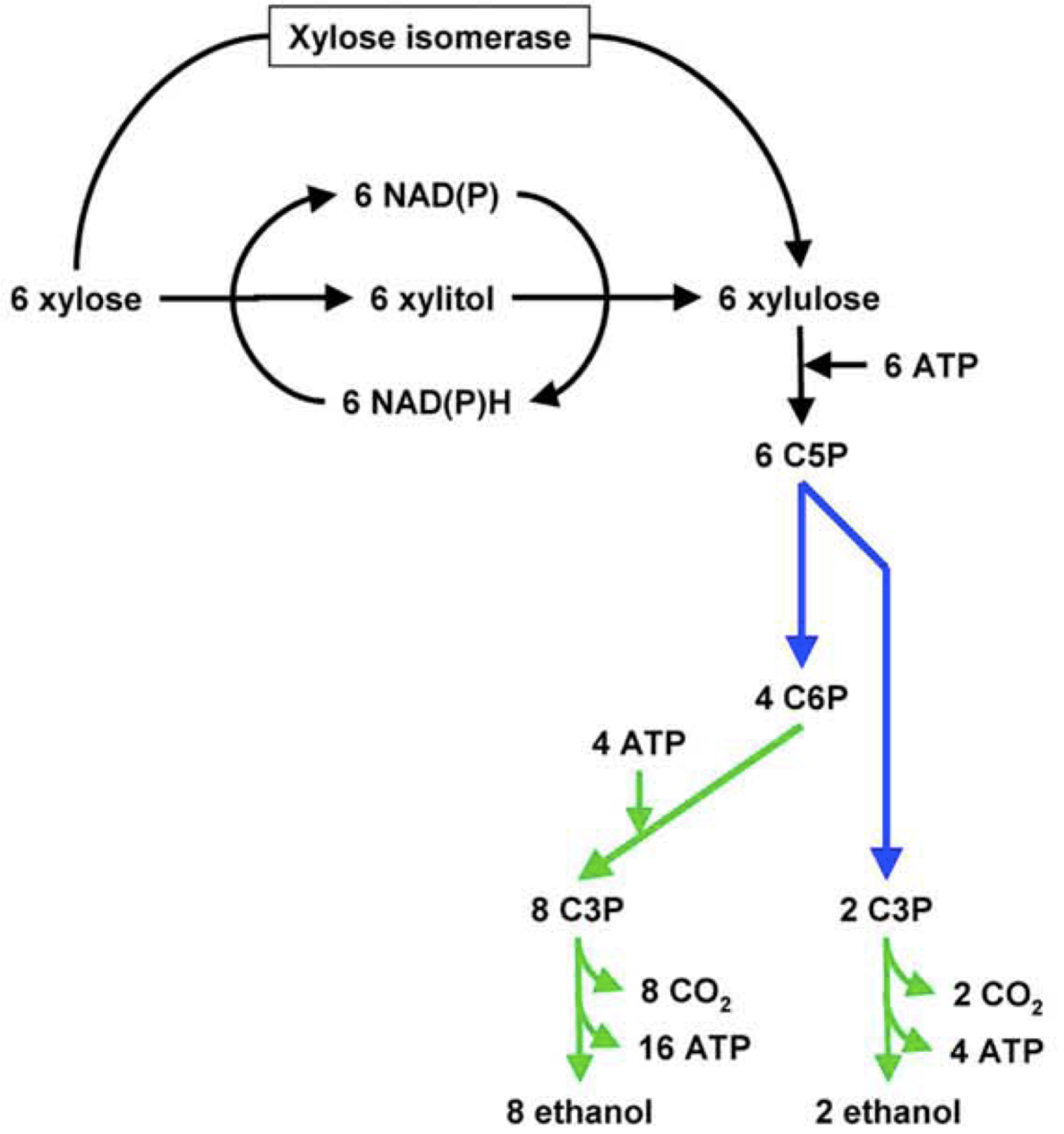
6 xylose + 3 ATP → 3 ethanol + 6 glycerol + 6 CO_2

Metabolismo da Xilose em *S. cerevisiae*

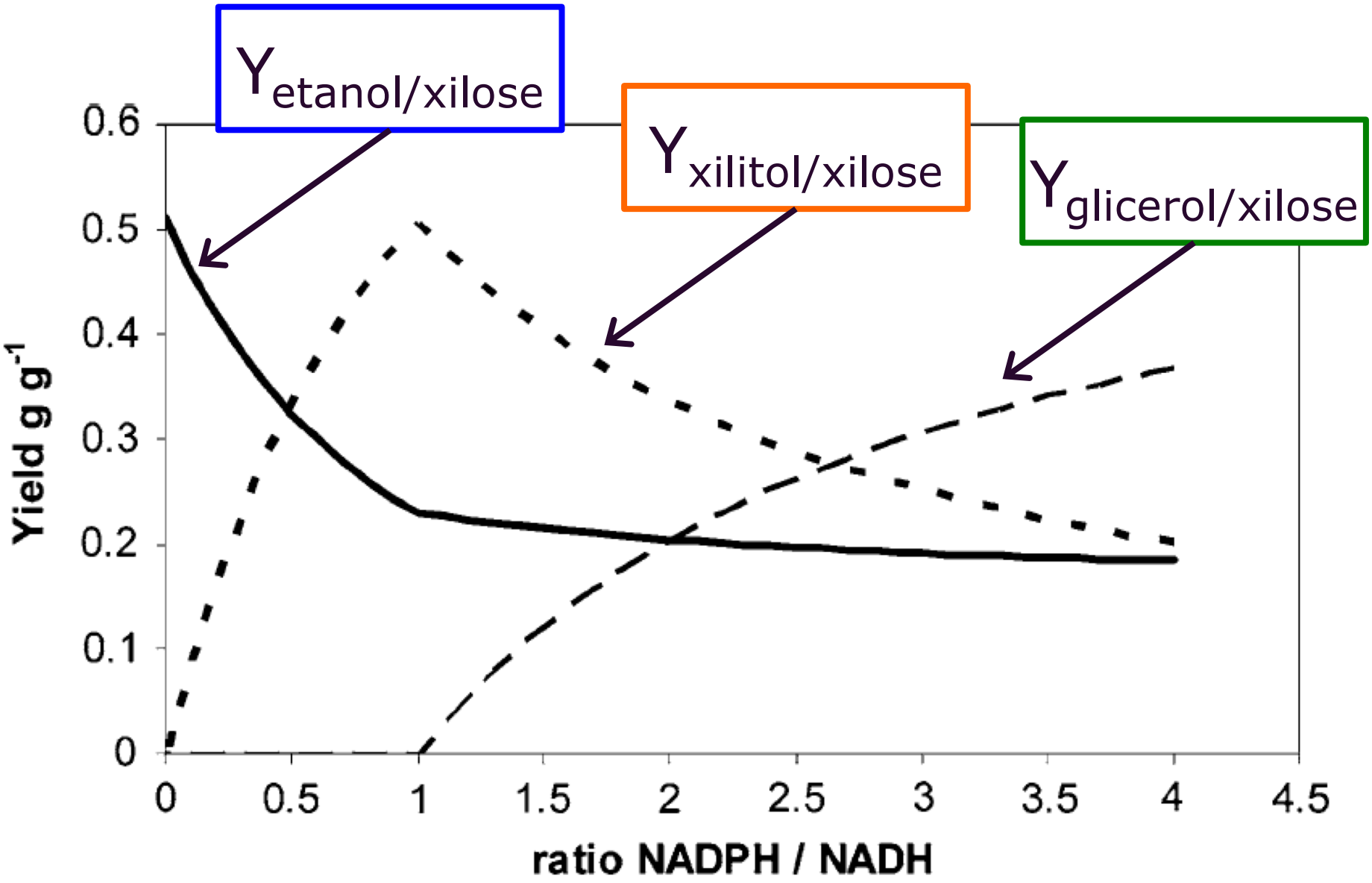


Fonte: Kuyper et al., 2004

(d)



6 xylose → 10 ethanol + 10 CO_2 + 10 ATP


$Y_{etanol/xilose}$
$Y_{xilitol/xilose}$
$Y_{glicerol/xilose}$
Yield g g-1
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
ratio NADPH / NADH

Antonie van Leeuwenhoek (2006) 90:391–418
DOI 10.1007/s10482-006-9085-7

ORIGINAL PAPER

Alcoholic fermentation of carbon sources in biomass hydrolysates by *Saccharomyces cerevisiae*: current status

Antonius J. A. van Maris · Derek A. Abbott · Eleonora Bellissimi · Joost van den Brink · Marko Kuyper · Marijke A. H. Luttik · H. Wouter Wisselink · W. Alexander Scheffers · Johannes P. van Dijken · Jack T. Pronk

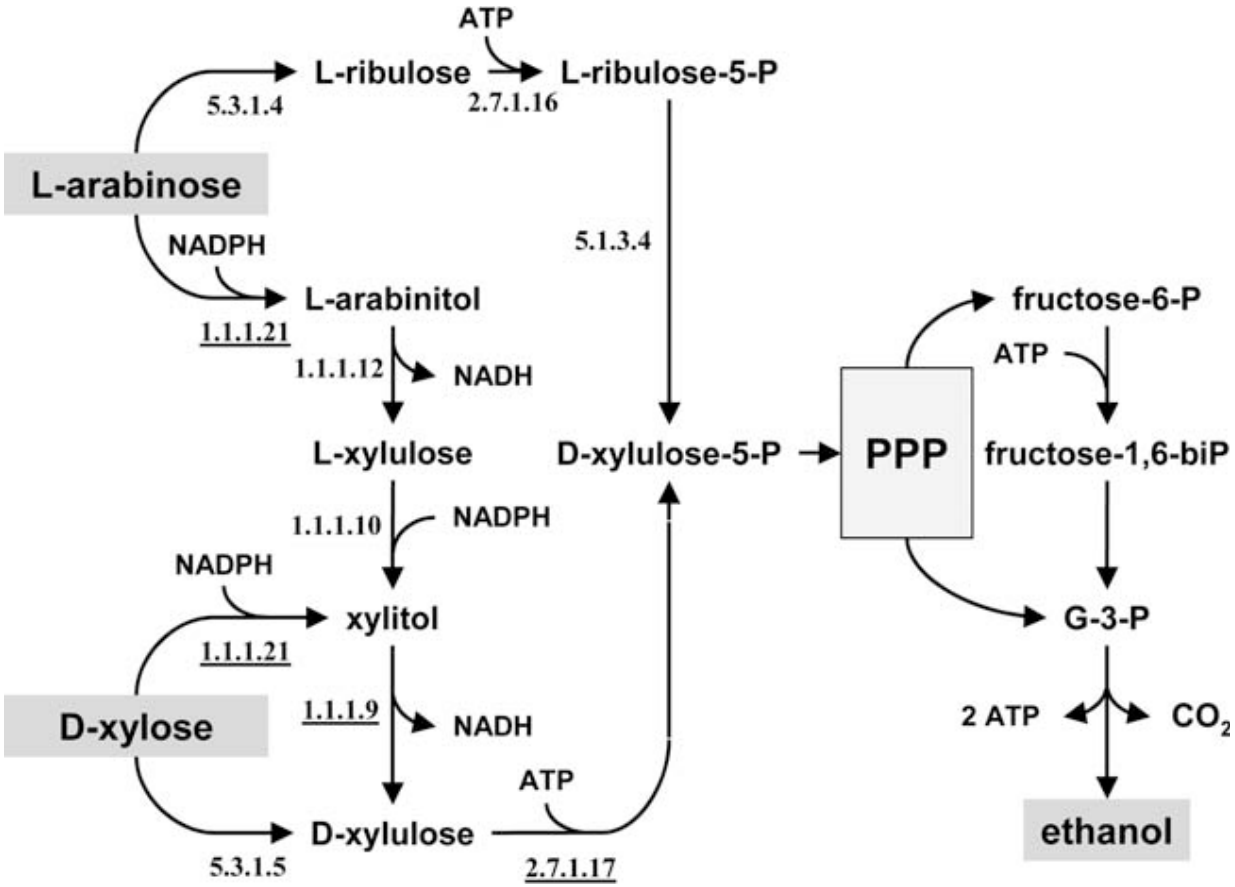


Fig. 3 D-Xylose and L-arabinose catabolism in metabolically engineered *S. cerevisiae* strains. Underlined EC numbers represent enzymes/steps present in 'wild-type' *S. cerevisiae* metabolism. The gene names corresponding to the enzymes are given in parentheses in the legend of this figure. 1.1.1.21, aldose/xylose reductase (*GRE3*/*xyl1*); 1.1.1.9, xylitol dehydrogenase (*XYL2/xyl2*); 2.7.1.17, xylulokinase (*XKS1*/*xyl3*); 5.3.1.5, xylose isomerase (*xylA*); 1.1.1.12, arabinitol 4-dehydrogenase (*lad1*); 1.1.1.10, L-xylulose reductase (*lxr1*); 5.3.1.4, L-arabinose isomerase (*araA*); 2.7.1.16, L-ribulokinase (*araB*); 5.1.3.4, L-ribulose-5-phosphate 4-epimerase (*araD*). G-3-P, glyceraldehyde-3-phosphate; PPP, pentose phosphate pathway

Combinação da fermentação 1G com 2G nas usinas

- **Separação sólido-líquido (reciclo de células)**
- **Contaminação bacteriana - xilose pode ser consumida por várias bactérias**
- **Presença de inibidores**

PLANTAS INDUSTRIAIS PARA PRODUÇÃO DE ETANOL 2G

BIOFLEX / GRANBIO - São Miguel dos Campos - Alagoas

Cellulosic ethanol plants

Plant	Location	Nameplate Capacity (mLpa/gpa)	Technology	Enzymes and Yeast	Feedstock	Shareholders	Start up
Dupont	Iowa	113.6 (30.00)	Dupont (enzymatic hydrolysis)	Enzymes: Accellerase (Dupont) Ethanol produced by bacteria (not yeast)	Corn stover (leaves and stalks) and corn cobs	Dupont Danisco	2014
Abengoa	Hugoton/Kansas	87.1 (23.00)	Abengoa (enzymatic hydrolysis)	Enzymes: Abengoa (with a microorganism licensed from Dyadic)	Corn stover, wheat straw and cotton residue	Abengoa Bioenergy Inc	2014
Bioflex/ GranBio	São Miguel dos Campos	82 (21.66)	Proesa (enzymatic hydrolysis)	Enzymes: Novozymes Yeast: DSM	Sugarcane straw	GranBio Investimentos	2014 September
Poet DSM	Emmetsburg/ Iowa	75.7 (20.00) 94.6 (25.00)	Poet DSM (enzymatic hydrolysis)	Enzymes: Novozymes (to be replaced by DSM)	Kemels and corn cobs	Poet and Royal DSM	September 3, 2014
Chemtex	Crescentino/Italy	50.7 (13.4)	Proesa (enzymatic hydrolysis)	Enzymes: Novozymes Yeast: Leaf	Kemels and corn cobs	Syngenta and Cellulosic Ethanol Tech (Quad County)	3Q, 2013
Raizen	Piracicaba/Brazil	40.00 (10.57)	Iogen (enzymatic hydrolysis)	Enzymes: Novozymes Yeast: TBC	Sugarcane straw and bagasse	Raizen and Iogen	
Enerkem Inc.	Edmonton/ Canada	37.78 (10.00)	Gasification	N/A	Municipal solid waste - MSW	RHO Ventures, Valero, Braemar Venture and others	June 2014
INEOS Bio	Vero Beach/Florida	30.28 (8.00)	Gasification	N/A (INEOS uses a proprietary bacteria)	Wood waste, agri residues, etc	INEOS	August, 2013 (Test) Returned to production on sep/14.
Quad County	Galva/Iowa	7.57 (2.00)	Cellerate	Enzymes: Enogen (Syngenta) Yeast: TBC	Kemels and corn cobs	Syngenta and Cellulosic Ethanl Tech (Quad County)	July, 2014
Anhui Guozhen CO	Fuyang City/China	~200 (52.80)	Proesa (enzymatic hydrolysis)	Enzymes: Novozymes Yeast: TBC	Rice straw	M&G (70%) and Anhui Guozhen CO (30%)	TBC
Energo chemica	Strazske, Slovak Republic	70.0 (18.4)	Proesa (enzymatic hydrolysis)	Enzymes: Novozymes Yeast: Leaf	Non-food biomass	Energochemica	First-half 2017