What's for lunch? Microorganisms for biorefineries

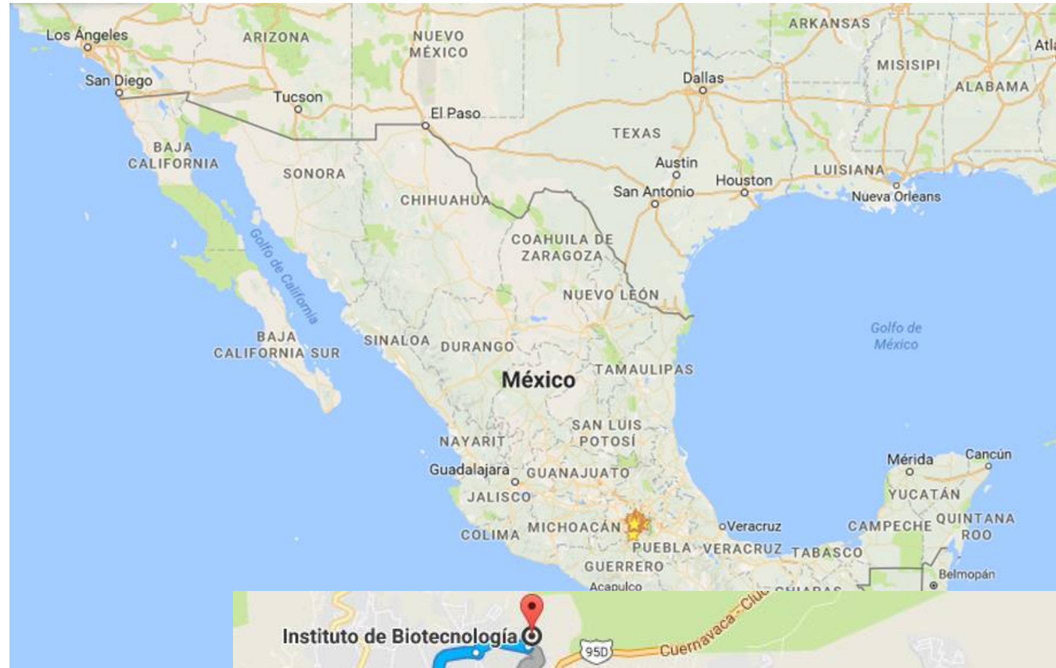
Alfredo Martinez
alfredo@ibt.unam.mx
Biotechnology Institute
National University of Mexico

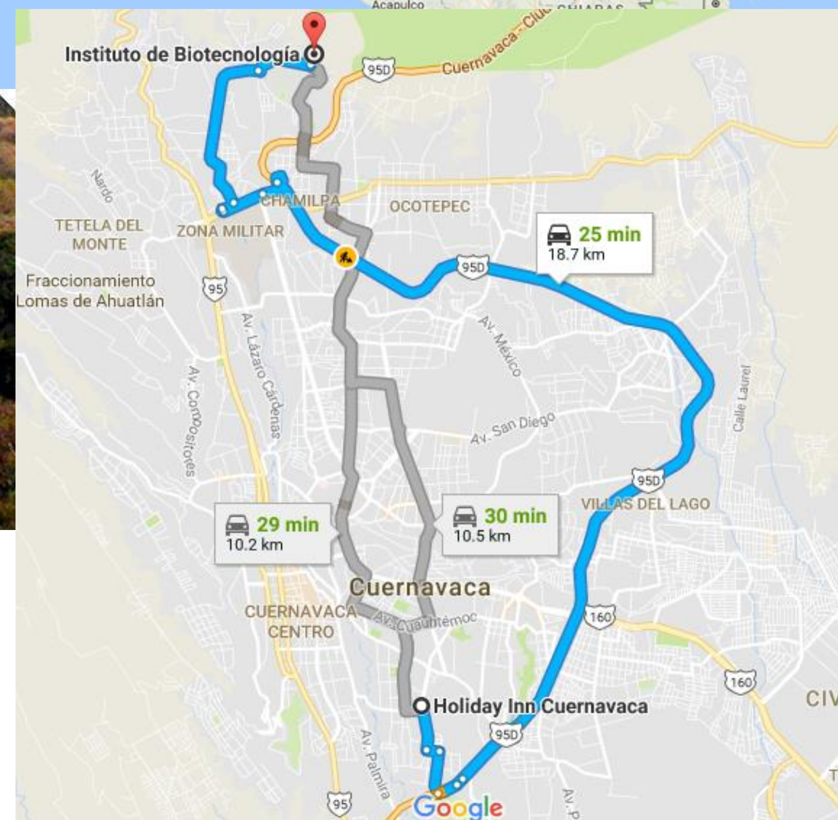
3rd SMIBIO
Cuernavaca, Morelos
14/Nov/2017

Where we are:

Morelos Campus
www.ibt.unam.mx

Cuernavaca: The Eternal Spring City





What's for lunch?

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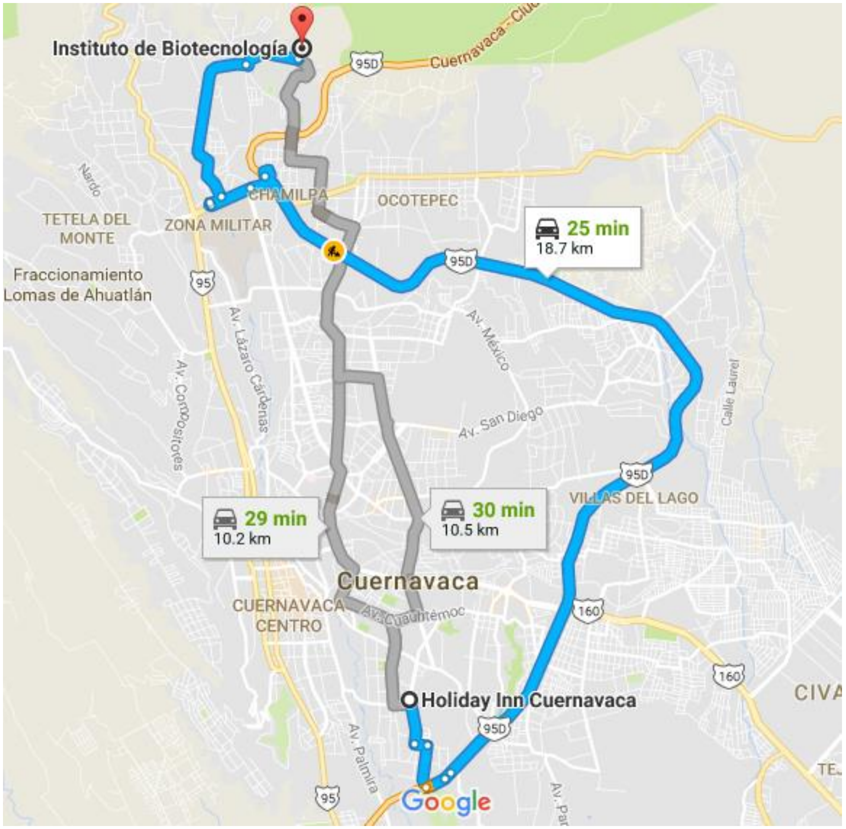
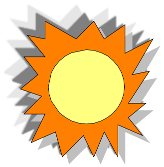


What's for lunch?
Ácido Láctico: Yogurt
Ác. Succínico
Ác. Pirúvico: Piruvato de Creatinina
Etanol: Mezcal

Biorefineries: Biofuels and Chemicals from Lignocellulose



The Sun

Agricultural Residues
Bagasses and Stovers

Artificial CO_2 cycle

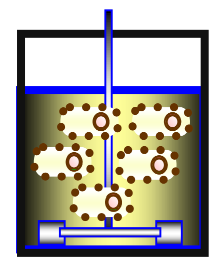
CO_2

Xylose
Glucose
Cellobiose
Mannose
Galactose

Sugars

Hydrolysis

Chemicals
BioFuels



Fermentation

Purpose: Design microorganism and process to transform ALL the SUGARS contained into **lignocellulose (cellulose: glucose & hemicellulose: pentoses, hexoses, disaccharides) to biofuels or chemicals with homofermentative strains**

Carreón Rodríguez et al., 2009

BIO-REFINERÍA

Sol + CO_2 → Biomasa → Combustibles: Sólidos, Gaseosos y Líquidos

Productos de Fermentación

Bio-Refinería →

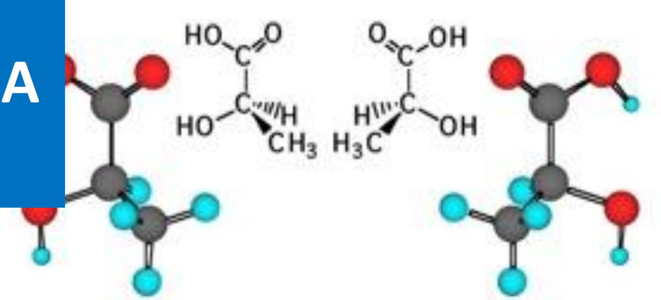
- Bio-Combustibles
- Bio-Plásticos
- Bio-Polímeros
- Bio-Resinas
- Bio-Químicos

¡~ 1 año!
vs Petróleo
Costos

Bio-Plásticos
Son 3 R

BIODEGRADABLE
AMIGABLE CON EL MA
RENOVABLE

Bio-Etanol



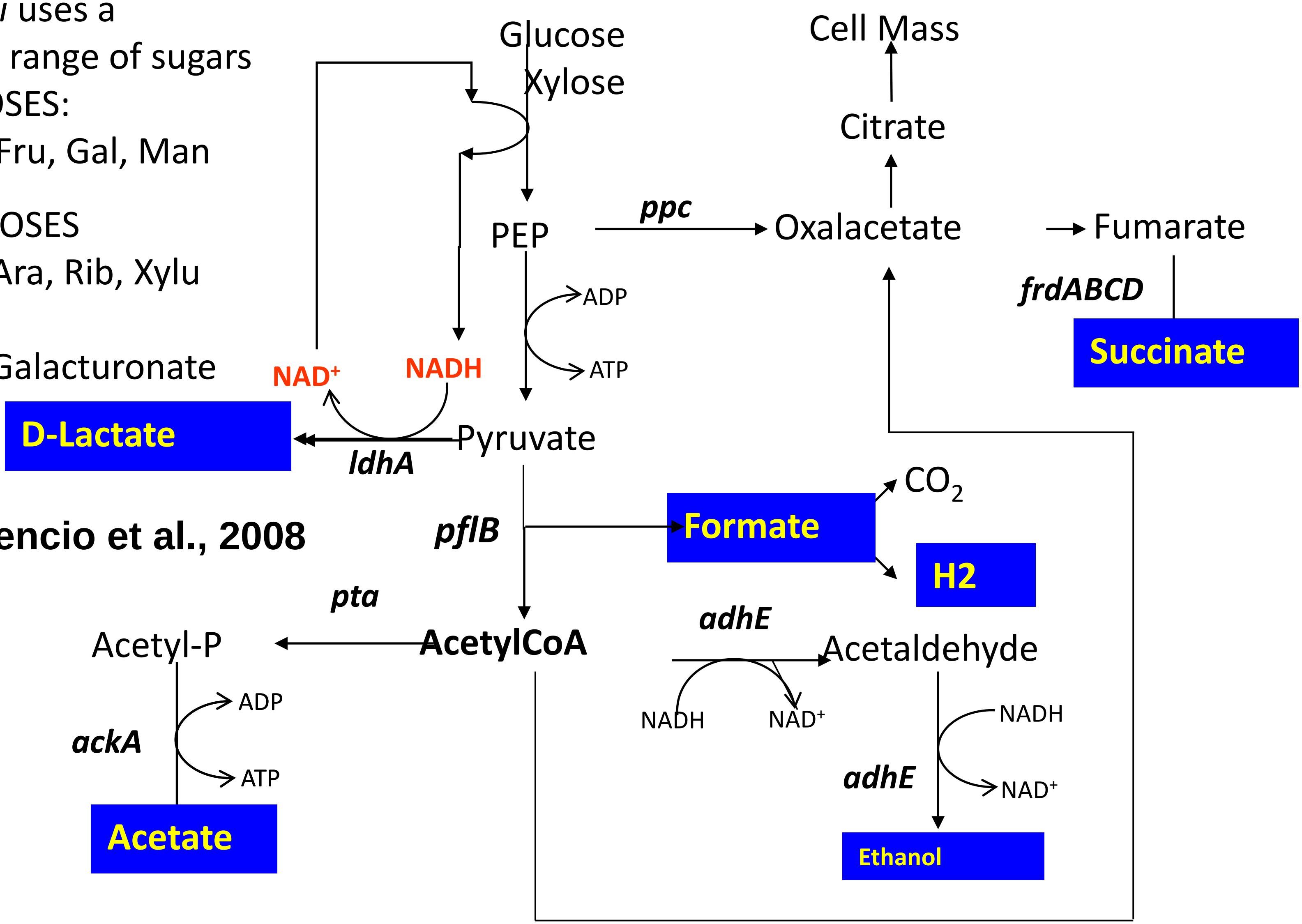
Martínez 2009

Fermentation Products *Escherichia coli*

E. coli uses a
Wide range of sugars
HEXOSES:
Glc, Fru, Gal, Man

PENTOSES
Xyl, Ara, Rib, Xylu

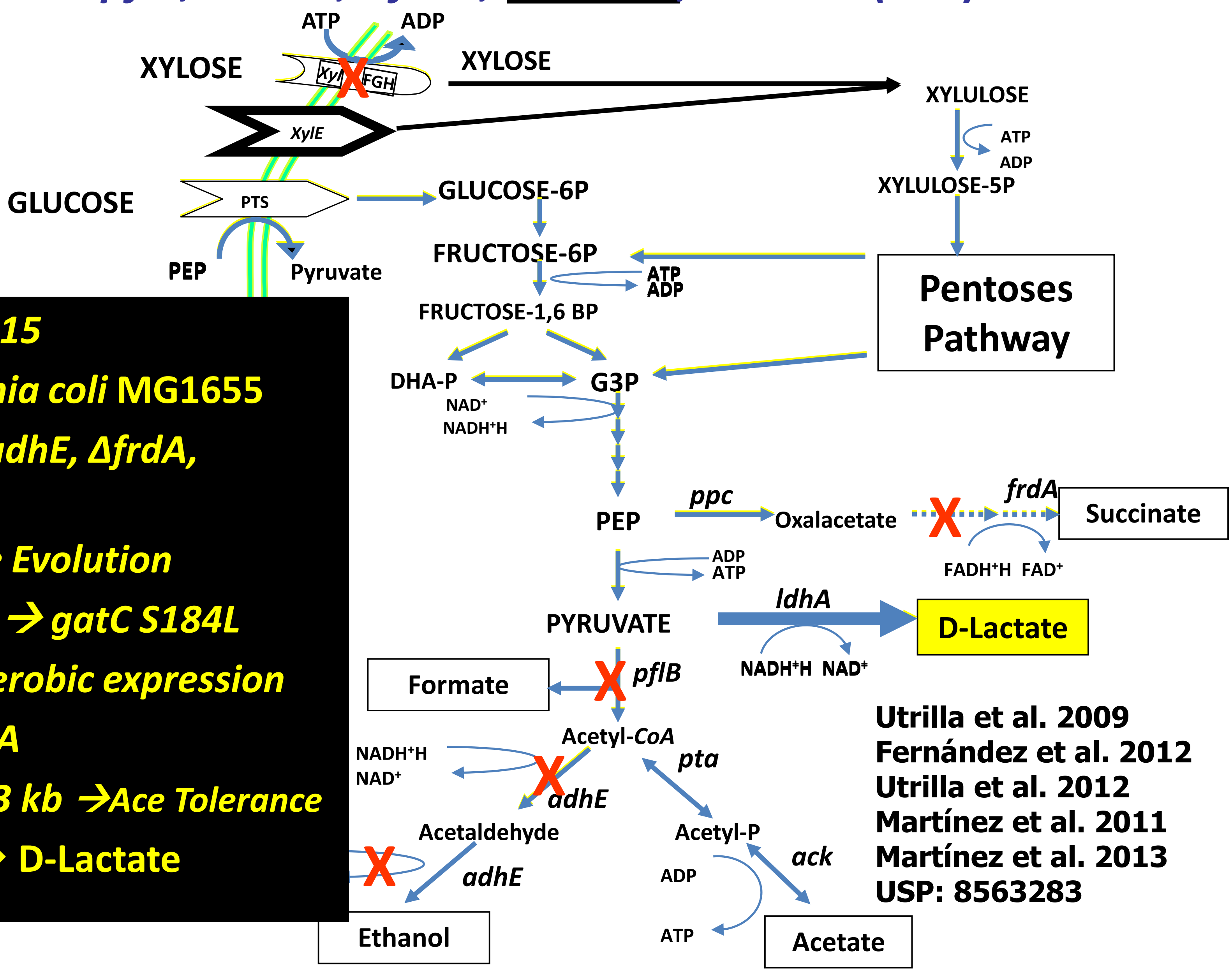
And Galacturonate



Orencio et al., 2008

and make a mix of fermentation products→ Homo Fermentative

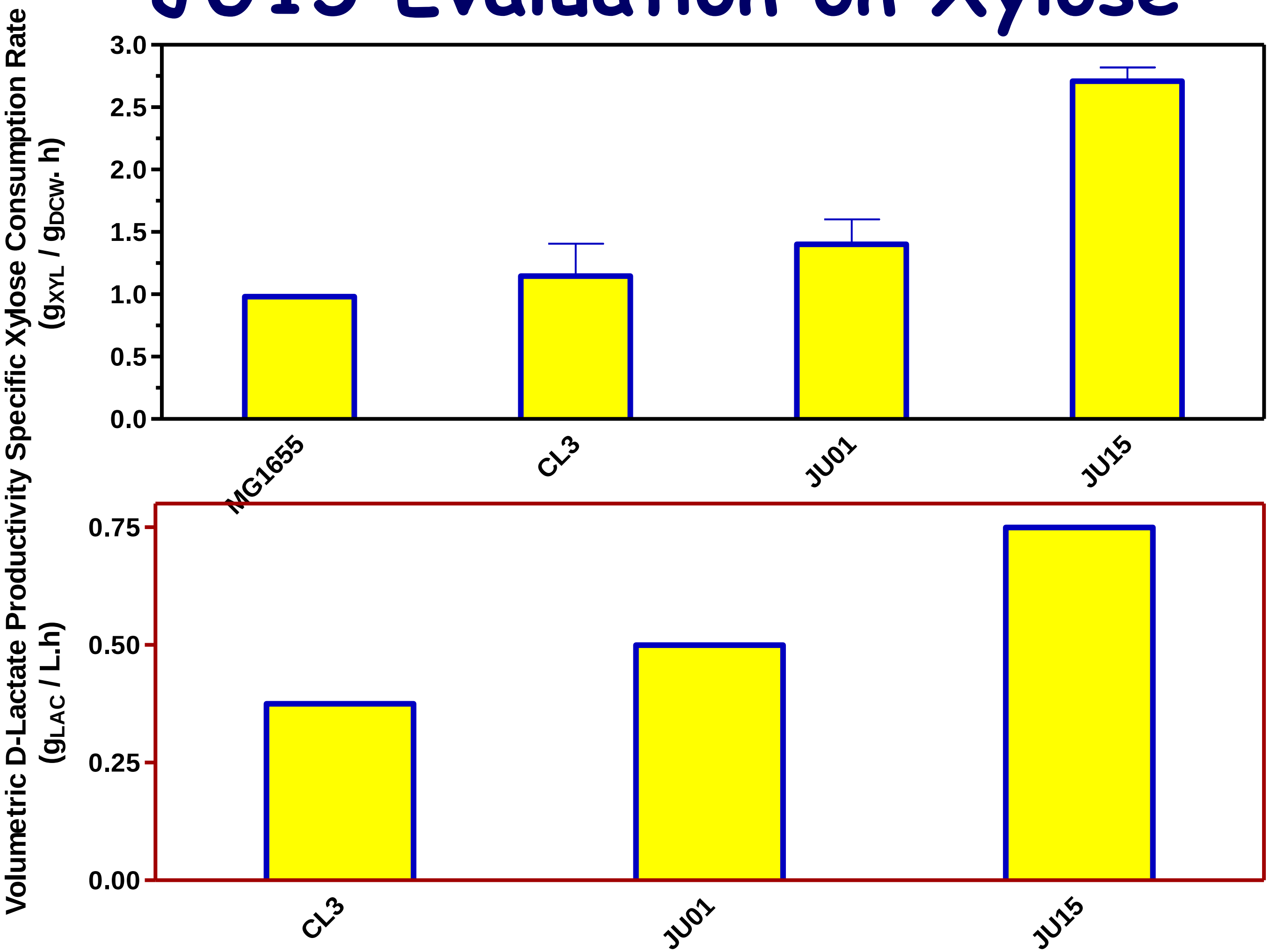
D-Lactogenic *E. coli* strain to use pentose-hexose mixtures
MG1655: *ΔpflB, ΔadhE, ΔfrdA,* ***<u>ΔxylFGH</u>****, Evolved (ALE)*



- *Strain JU15*
- *Escherichia coli* MG1655
- *ΔpflB, ΔadhE, ΔfrdA, ΔxylFGH*
- *Adaptive Evolution*
- *ΔxylFGH → gatC S184L*
- *Pdh anaerobic expression*
- *ΔmidarpA*
- *Δreg 27.3 kb →Ace Tolerance*
- Xylose → D-Lactate

Utrilla et al. 2009
Fernández et al. 2012
Utrilla et al. 2012
Martínez et al. 2011
Martínez et al. 2013
USP: 8563283

JU15 Evaluation on Xylose



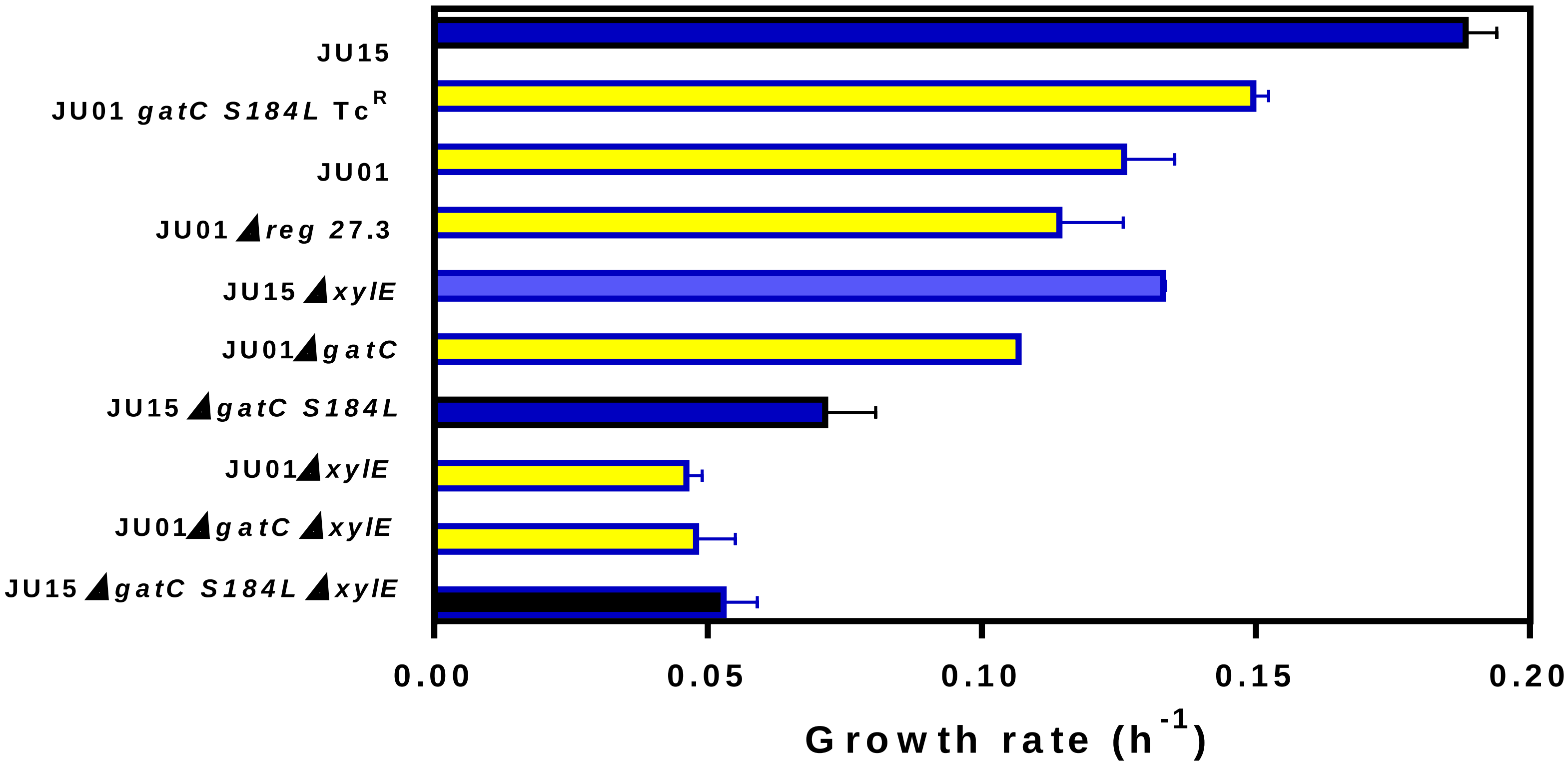
Utrilla et al. JIM&B 2009
Utrilla et al. Met Eng. 2012

CL3 (MG1655Δ*pflB* Δ*adhE* Δ*frdA*)

JU01 (MG1655Δ*pflB* Δ*adhE* Δ*frdA* Δ*xylFGH::Km*)

JU15 (MG1655Δ*pflB* Δ*adhE* Δ*frdA* Δ*xylFGH::Km Evolved*)

JU15 and JU01 *gatC xylE* mutants

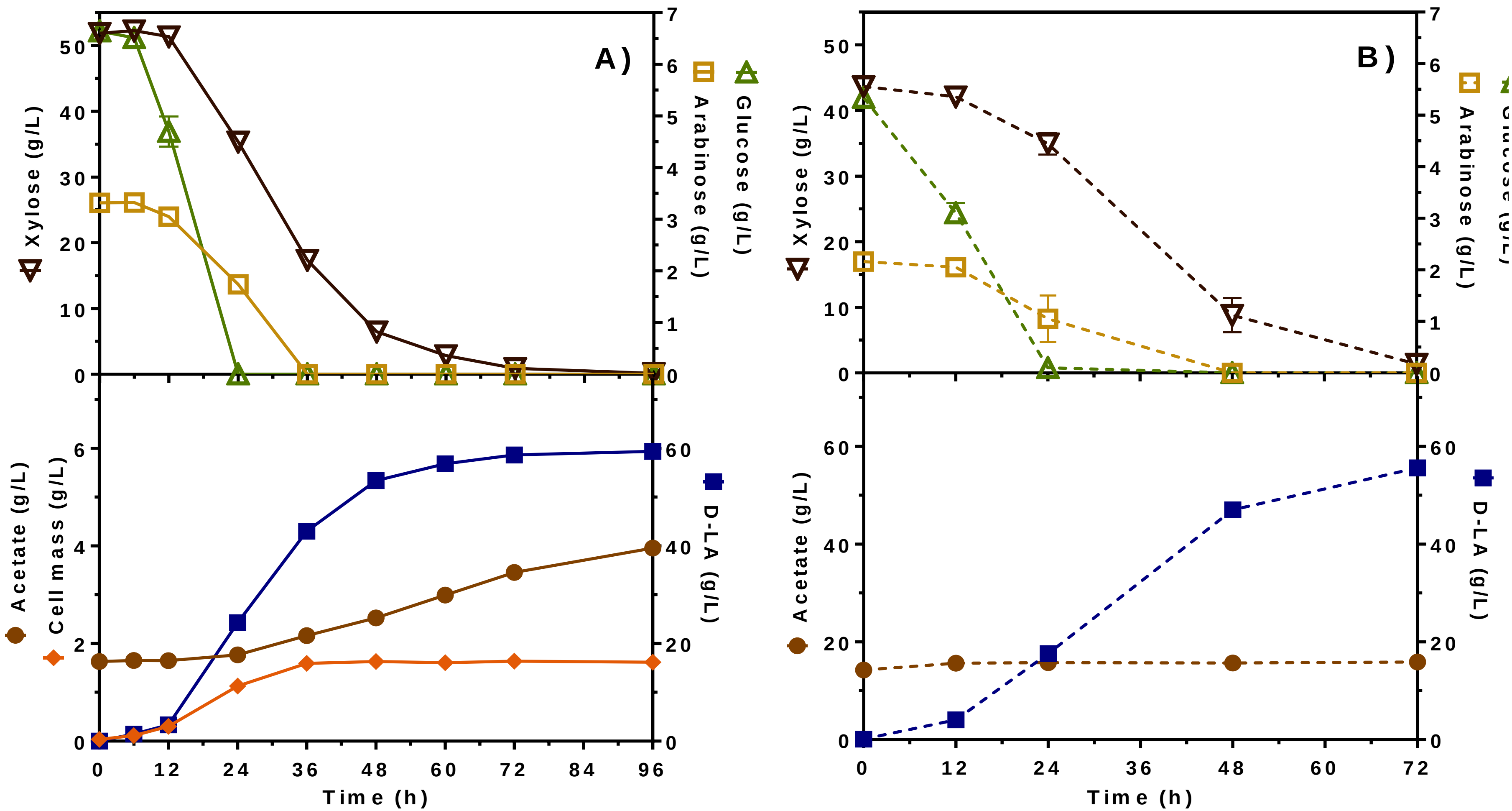


Utrilla et al. Met Eng. 2012

***gatC* S184L Point Mutation Serine → Leucine**

Position 184 of GatC Protein

A) Simulated hydrolysate B) SC-Bagasse hydrolysate



$Y_{D\text{-}LA}$ ($g_{D\text{-}LA}/g_{Sugars}$) = 0.94 ± 0.048
$Q_{D\text{-}LA}$ ($g_{D\text{-}LA}$/L h) = 1.11 ± 0.035

$Y_{D\text{-}LA}$ ($g_{D\text{-}LA}/g_{Sugars}$) = 1.11 ± 0.030
$Q_{D\text{-}LA}$ ($g_{D\text{-}LA}$/L h) = 0.98 ± 0.095

Utrilla et. al. Bioresource Technol. 2016

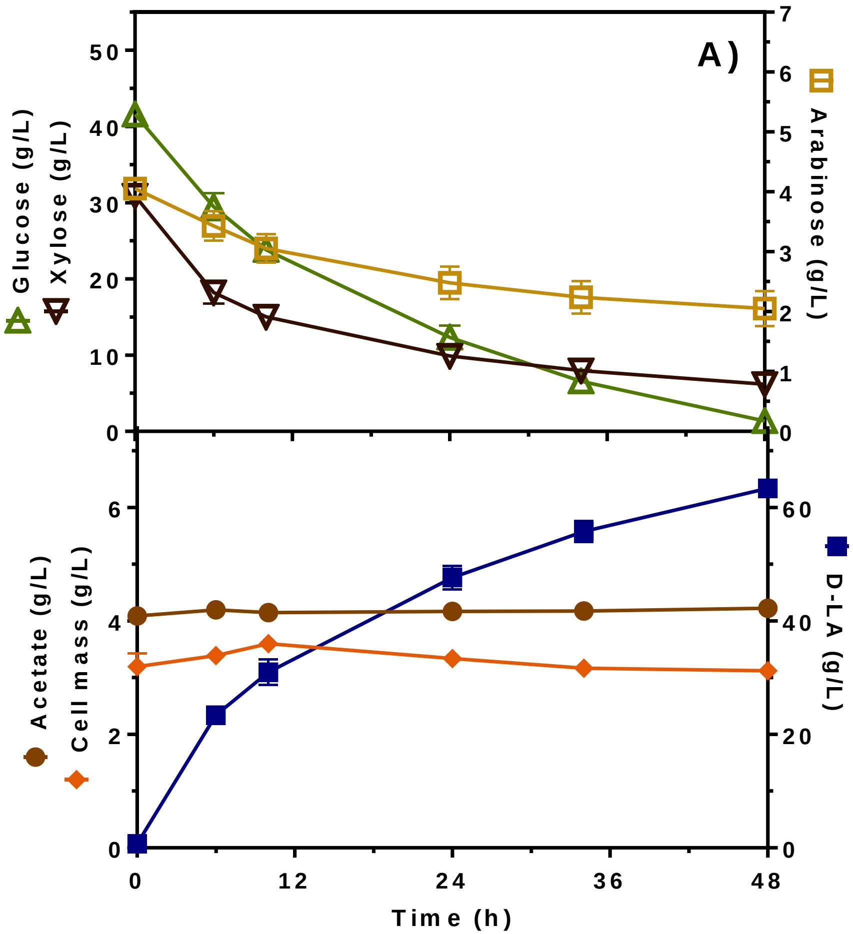
Strain JU15

Stover from White Corn

Sequential: Thermochemical Hydrolysis, Enzymatic Saccharification and Fermentation, without detoxification

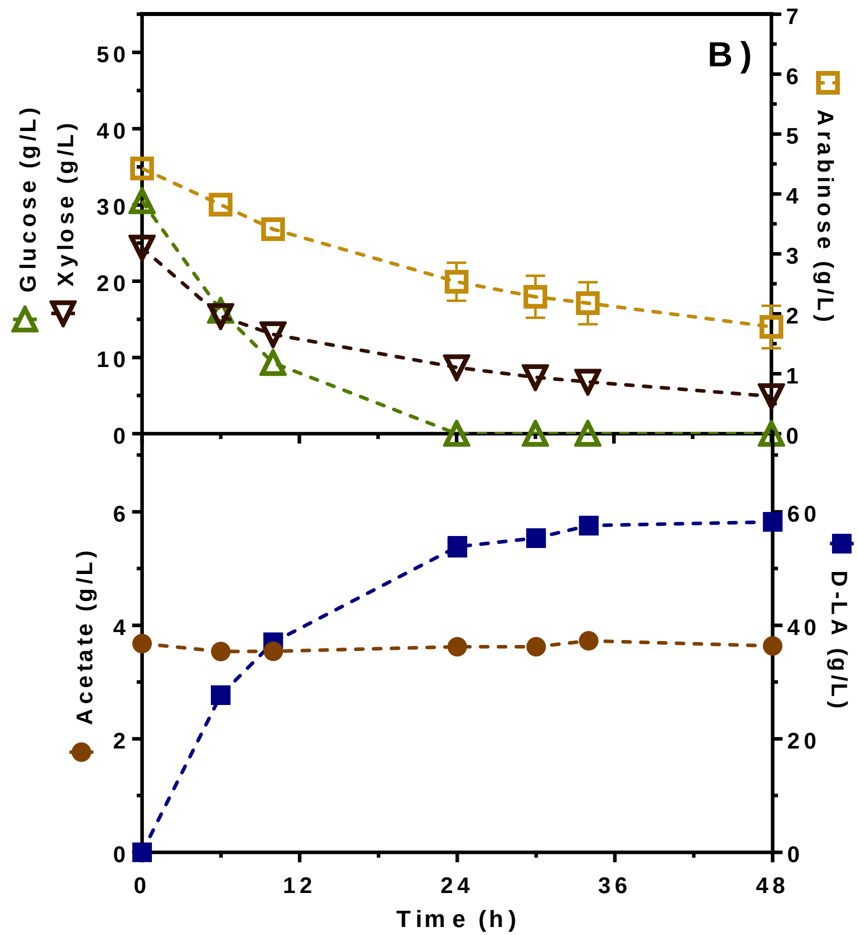
Moss-Acosta, 2012

A) Simulated hydrolysate B) Corn Stover hydrolysate



$Y_{D\text{-}LA}$ ($g_{D\text{-}LA}/g_{Sugars}$) = 0.95 ± 0.010

$Q_{D\text{-}LA}$ ($g_{D\text{-}LA}$/L h) = 1.32 ± 0.025



$Y_{D\text{-}LA}$ ($g_{D\text{-}LA}/g_{Sugars}$) = 1.11 ± 0.064

$Q_{D\text{-}LA}$ ($g_{D\text{-}LA}$/L h) = 1.21 ± 0.050

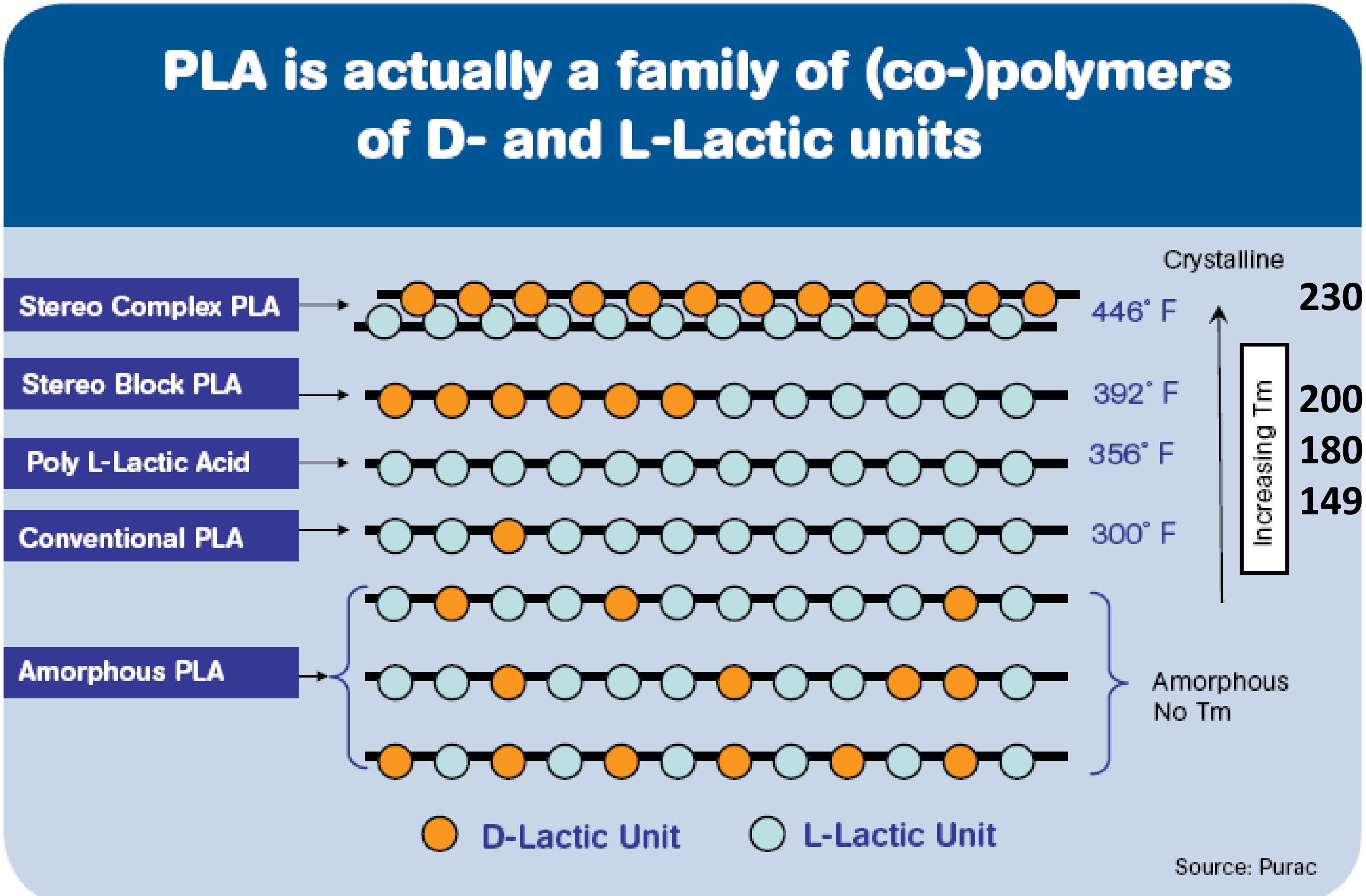
AV03: JU15 Δ*poxB*, Δ*ackA-pta*, Δ*mgsA*

Simultaneous sugar consumption

Y > 1 !!!

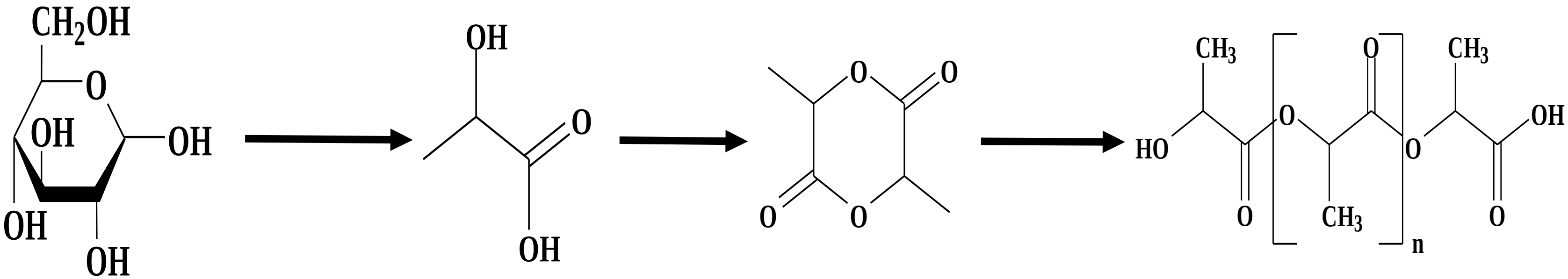
Utrilla et al. Bioresource Technol. 2016

PLA: PLLA sc-PLA



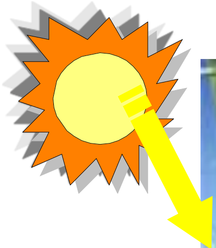
Purac's newly available D-lactide monomer is the "secret" ingredient in some high-heat PLA copolymers in development. Shown here (top to bottom): D/L lactide structures of stereocomplex (sc) PLA, stereo-block-copolymer PLA, poly-L-lactide homopolymer, standard PLA, and amorphous PLA.

L - Lactato Ópticamente Puro



Glucosa **Láctico** **Dímero** **PLLA**

2nd Generation Bio-Plastics: Small Scale BioRef.



Artificial CO_2 cycle

CO_2

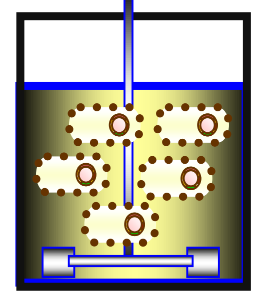
The Sun

1 kg of Sugar Yield 1 kg of Lactic Acid
> 1 USD / kg; PLLA: > 4 USA dol/kg

Lactate

Xylose, Cellobiose Glucose, etc. Cellulose, Hemicellulose

Hydrolysis



Lignocellulose - Biomass

Agricultural Residues

Sugar Cane Bagasse

Fermentation

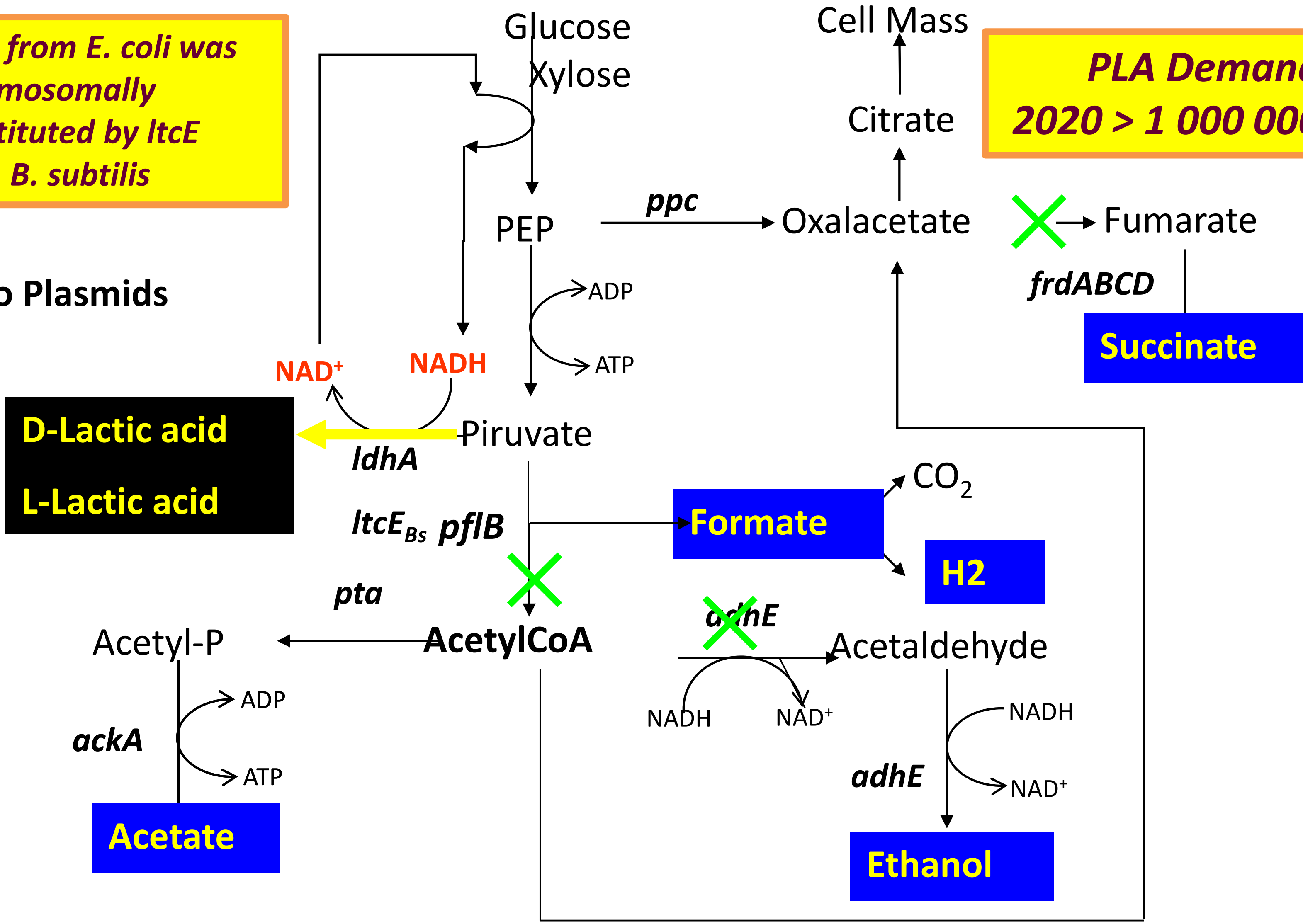
Purpose: Design microorganism and process to transform Lignocellulose (cellulose & hemicellulose: pentoses, hexoses, disaccharides) to optically pure lactates (D&L): Biopolymer Precursors

Lactic acid (D and L) production with Metabolic Engineered *E. coli* strains

ldhA from E. coli was chromosomally substituted by ltcE from B. subtilis

PLA Demand 2020 > 1 000 000 Ton

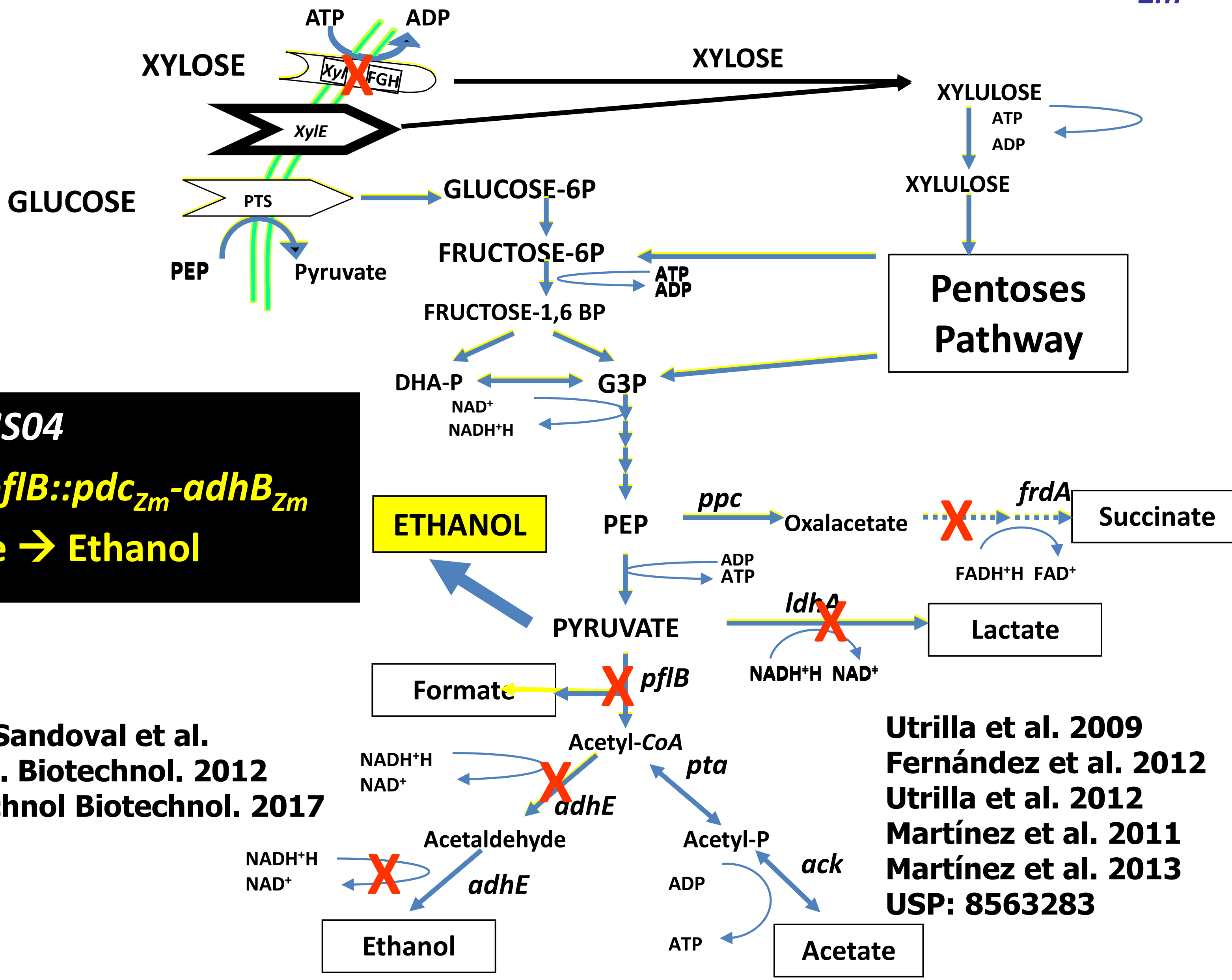
No Plasmids



Utrill Carreri et al., 2009; Leal Reyes 2010; Martinez et al. WO 2011 PCT 016706 A2

Ethanologenic *E. coli* strain to use pentose-hexose mixtures

MG1655: Δ*pflB*, Δ*adhE*, Δ*frdA*, Δ*xylFGH*, Δ*ldh*, *PpflB::pdc-adh*$_{Zm}$



- *Strain MS04*
- JU15: *PpflB::pdc*$_{Zm}$*-adhB*$_{Zm}$
- Pyruvate → Ethanol

Fernández-Sandoval et al.
App. Microb. Biotechnol. 2012
J. Chem Technol Biotechnol. 2017

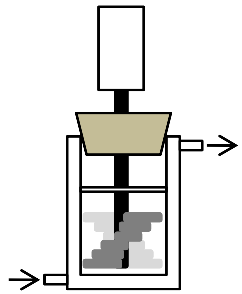
Utrilla et al. 2009
Fernández et al. 2012
Utrilla et al. 2012
Martínez et al. 2011
Martínez et al. 2013
USP: 8563283

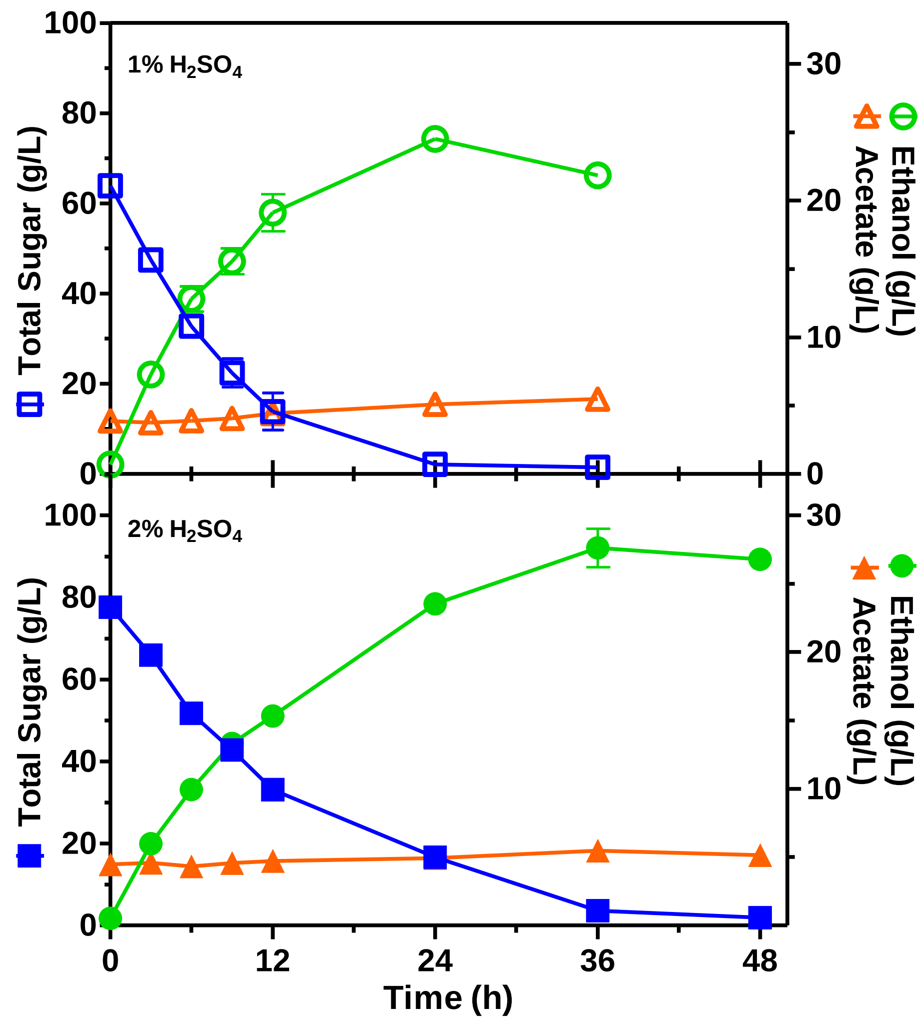
Stover from White Corn: Sequential: Thermochemical Hydrolysis, Enzymatic Saccharification and Fermentation

1 kg of Sugar Yields 0.51 kg of EtOH
~ 0.5 USD / L

Non-aerated Cultures with Ethanologenic *E. coli* MS04, 3.7 g/L, 0.2 L, 37°C, pH 7, 100 rpm. No salts were added. No detox.

Vargas-Tah et al., Bioresource Technol. 2015

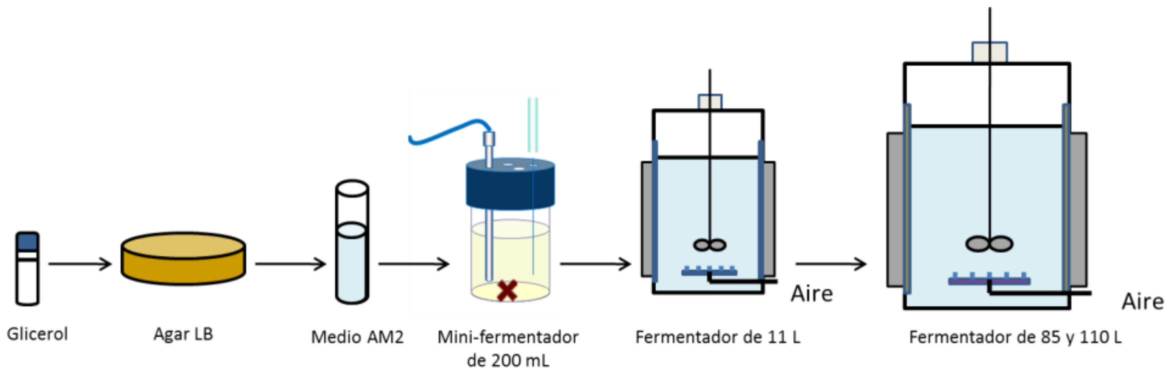




Comment
Small Scale Biorefinery: Ethanol

All sugars are fermented to ethanol by ethanologenic *E. coli* MS04 in 36 h

Lactic acid, Ethanol Scale-up



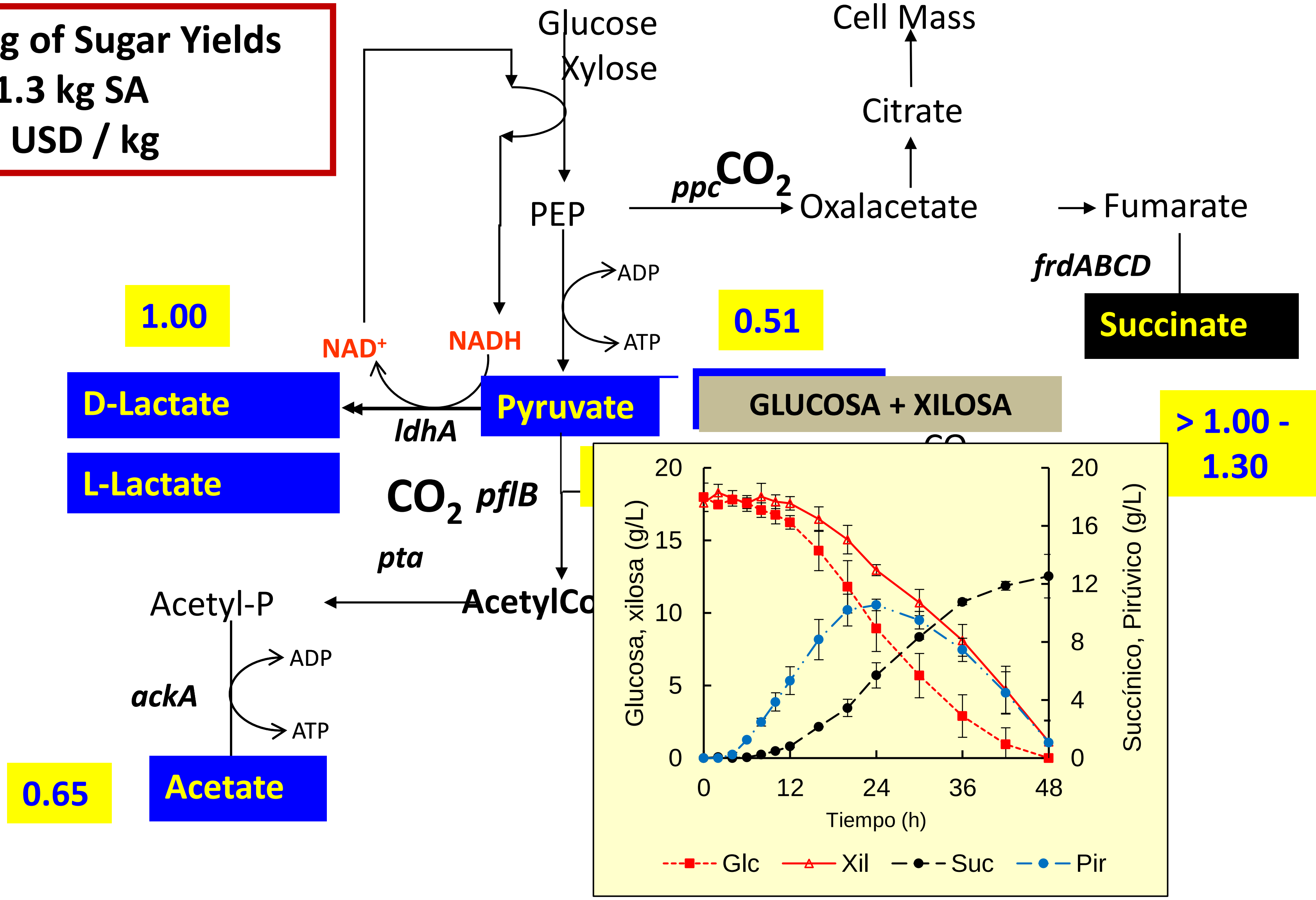
k_La 7.2 h^{-1}
Corn stover hydrolysates
0.2 ➔ 400 L

Yield: ~ 95%
Productivity
1.1 (– 0.5) g/L/h

Fernández-Sandoval et al. J. Chem Technol Biotechnol. 2017
Sierra-Ibarra 2017, To be submitted

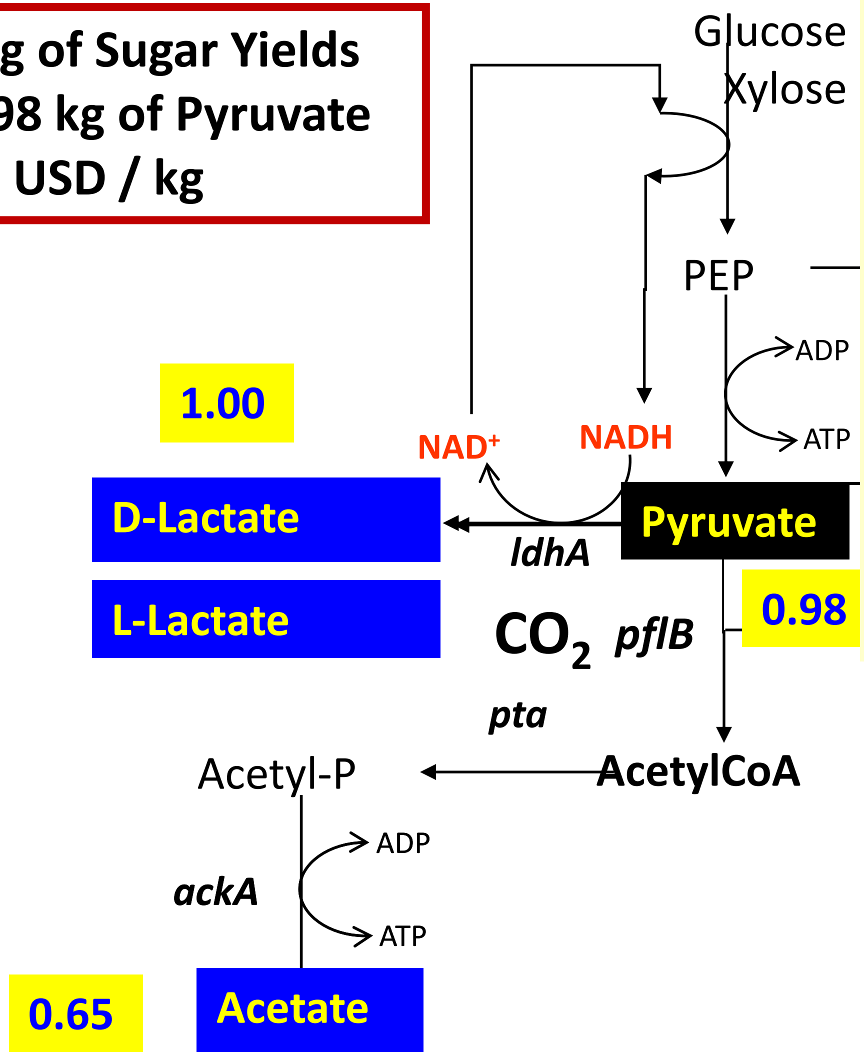
Succinic acid: Strain JU15 ΔldhA frdA Ptrc pck Δppc

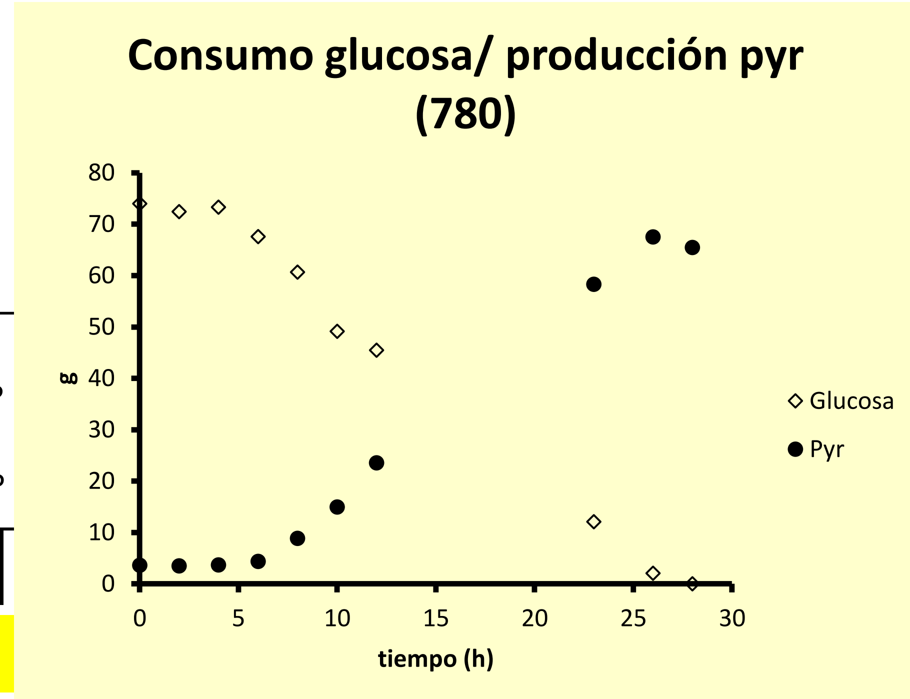
1 kg of Sugar Yields
1-1.3 kg SA
> 3 USD / kg

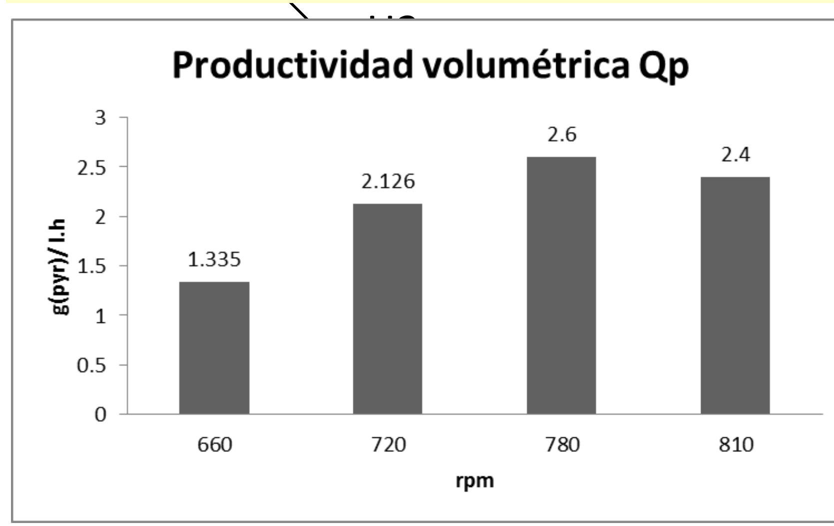


Pyruvic acid: Strain JU15 Δ*ldhA*

1 kg of Sugar Yields
0.98 kg of Pyruvate
> 1 USD / kg

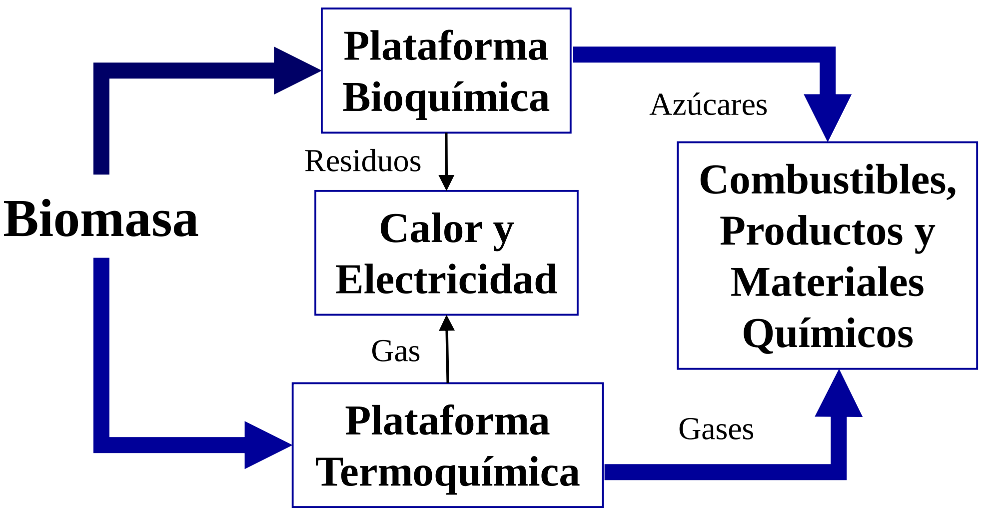






Biorefinery

Escherichia coli as Microbial Cell Factory for the (Small Scale) Biorefinery Concept



- **Etanol**
- Butanol
- Propanol
- ~~Metano~~
- Bio-Diesel
- Bio-Gasolina
- Isopentenol
- Bio-Queroseno
- Bio-Hidrógeno
- ~~Bio-Electricidad~~
- **1,3 Propanodiol**
- **PHB**
- Plásticos
- Polímeros
- Solventes
- Fenólicos
- ~~Resinas (furfural)~~
- Ac. grasos
- Ac. orgánicos
- Pigmentos
- ~~Detergentes~~

Who is *Escherichia coli*?
What does *E. coli* do for humans?

- Approximately 33% of the therapeutic proteins for human use are currently produced with *E. coli* in industrial fermenters.
- Human growth hormones; interferons; interleukins; erythropoietin; among others
- L-fenilalanine, PHB, and Propanediol, among others
- Easy to manipulate & cultivate

E. coli: Bacteria

Sanchez-Garcia et al. MCF 2016;15:33

Macromolecular composition of *N. oleoabundans* cells from batch and fed-batch cultures

Culture mode	Proteins (g_{PROT}/g_{DCW}*100)		Carbohydrates (g_{CARB}/g_{DCW}*100)		Lipids (g_{LIP}/g_{DCW}*100)	
	S	M	S	M	S	M
Batch C/N = 17	41.2 (±0.4)	**43.7 (±1.9)**	31.7 (±1.3)	30.9 (±3.5)	24.8 (±0.3)	24.0 (±0.9)
Batch C/N = 278	42.9 (±1.7)	14.4 (±1.2)	32.1 (±0.4)	33.3 (±0.5)	23.3 (±1.2)	**51.7 (±1.7)**
Fed-Batch	40.7 (±1.4)	11.6 (±0.9)	27.5 (±2.0)	**54.2 (±0.1)**	27.5 (±1.9)	33.7 (±0.6)

S: at the start of the culture
M: at the time of maximum cell mass produced

Combustibles Fósiles
Necesidad de E. Renovables
Biocombustibles

Combustibles Fósiles
Y Biocombustibles
Actuales

Fósiles

Petróleo
Gasolina
Diesel
Turbosina

1ra
Generación

Almidón
Sacarosa
Bio-Etanol

Aceites de Plantas
Bio-Diesel

Lignocelulosa
Bio-Etanol
Bio-Butanol

Oleaginosas no comestibles
Biodiesel
Bioturbosina

2da
Generación

CO_2
Algas y Cianobacterias
H_2 Fotobiológico
Bio-Diesel
Bio-Petróleo
Bio-Turbosina

3ra
Generación ++++
Biorefinería
Bioplásticos
Biosolventes
Proteínas Alimento
Ácidos grasos
Aceite comestible
Etc.

Biocombustibles – Biorrefinerías
Mediano y Largo Plazo

Islas y Martínez 2010

Gracias Preguntas

- **CONACyT**
- **UNAM PAPIIT – DGAPA**