Fuel ethanol production from corn stover with ethanologenic bacteria

Produktion von Ethanol-Kraftstoff aus Maisstroh mit ethanbildenden Bakterien

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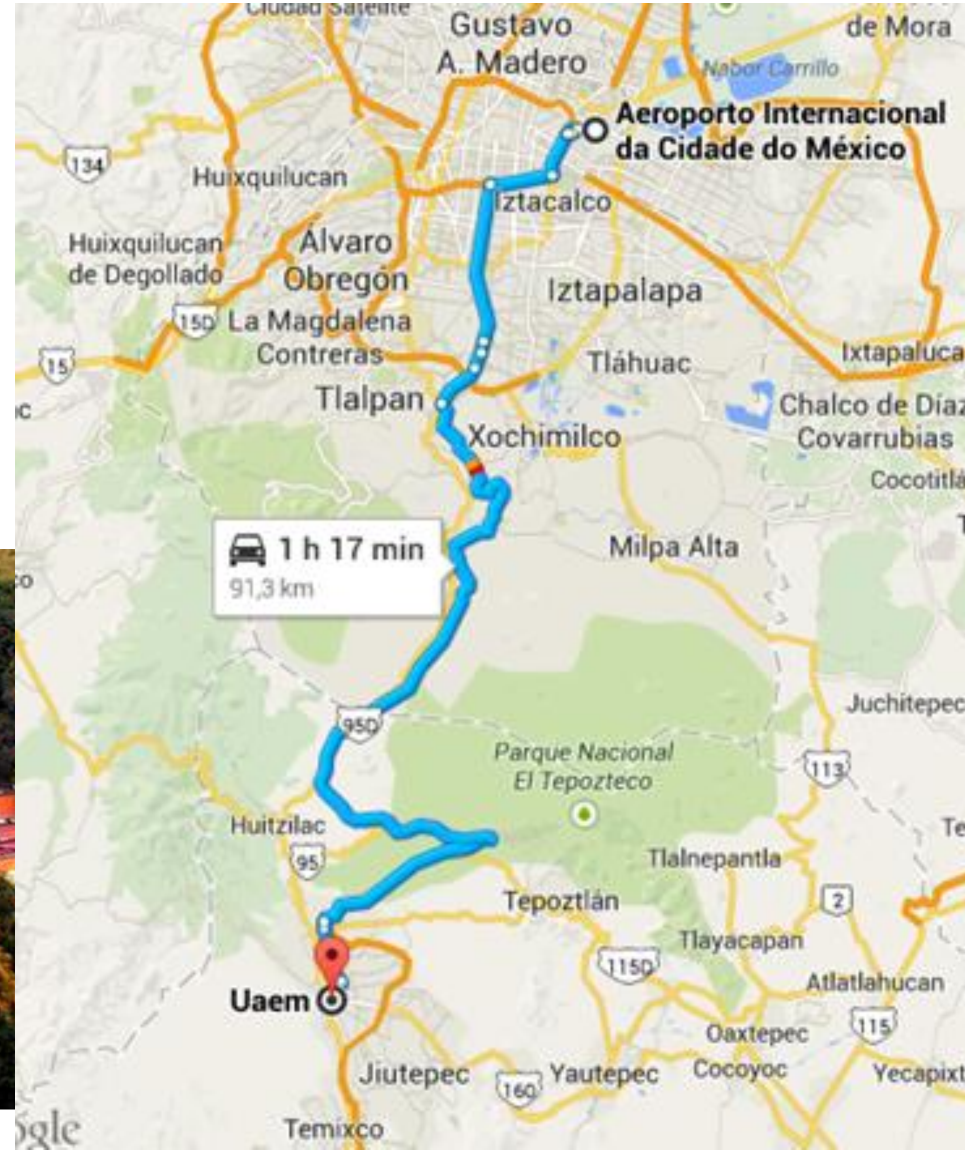
Where we are:



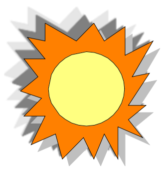
Campus Morelos - Universidad Nacional Autónoma de México

www.ibt.unam.mx

What we do:

- Metabolic engineering, synthetic biology and bioprocess development with *Escherichia coli* (starting now with *S. cerevisiae*) for biofuels (fuel ethanol, 2,3 butanediol and long chain alcohols), mono and dicarboxylic acids (D & L-lactate, pyruvate, butyrate and R-3-hydroxybutyrate) as biopolymer precursors) production.
- Physiological studies with oleaginous microalgae under heterotrophic conditions.

Generation of Ethanol (Agro-Fuels) and (Agro-) Chemicals from Lignocellulose 2nd Generation Ethanol



The Sun

Artificial CO_2 cycle

CO_2

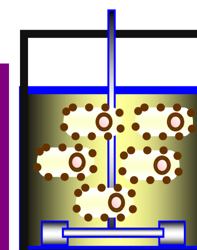
Lignocellulose – Biomass,
Agricultural Residues, Bagasses and Stovers, Energy Crops

1 kg of Sugar
Yields 0.51 kg of Ethanol

Chemicals
Ethanol (Fuel)

Hydrolysis

Sugars + Acetic & Glucuronic Acids Furans



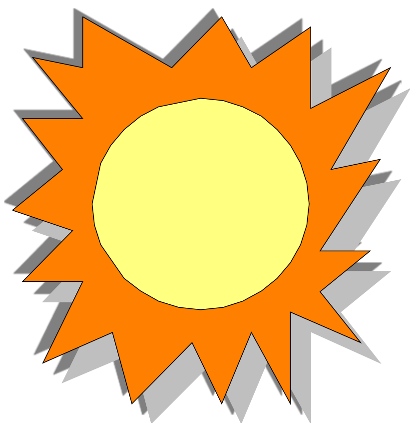
Fermentation

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

Carreón Rodríguez et al., 2009

Sun → Biomass → Bio-Fuels. Fermentation Products

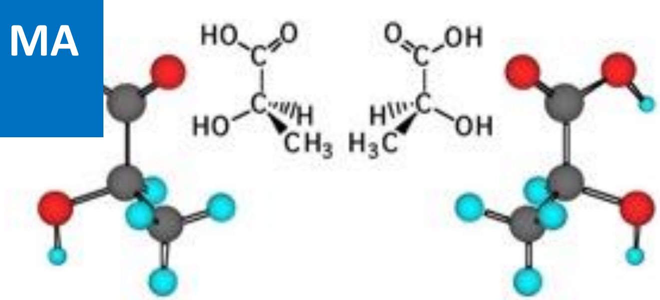
Bio-Refinery → Bio-Plastics, Bio-Polymers, Bio-Resins, Chemicals



!~ 1 Year!

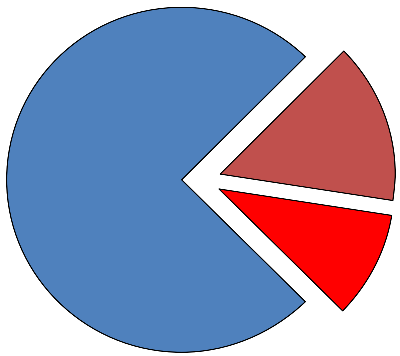
Chemicals

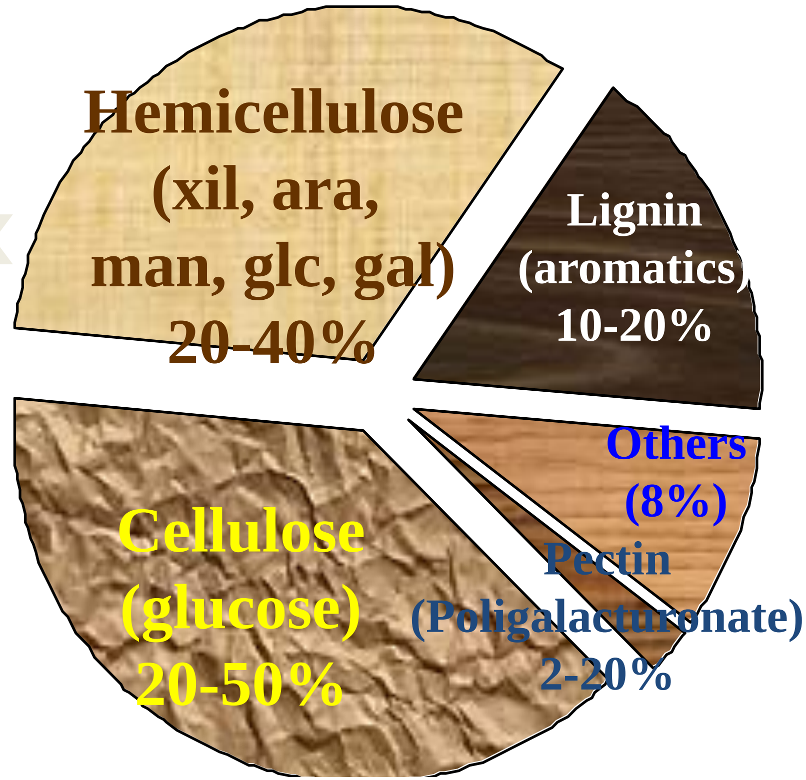
BIODEGRADABLE
AMIGABLE CON EL MA
RENOVABLE



Martínez 2009

Lignocellulose





Hemicellulose:
Thermochemical Hydrolysis
Low or high pH, T > 100°C

Cellulose:
Enzymatic Hydrolysis
pH 4.5-5, 50 °C

Pentoses + Hexoses (Xil + Ara) +
(Gluc+Man+Gal)
<u>+ Acetate</u> + Glucuronic Acid
+ Toxins

Ingram et al., 1999

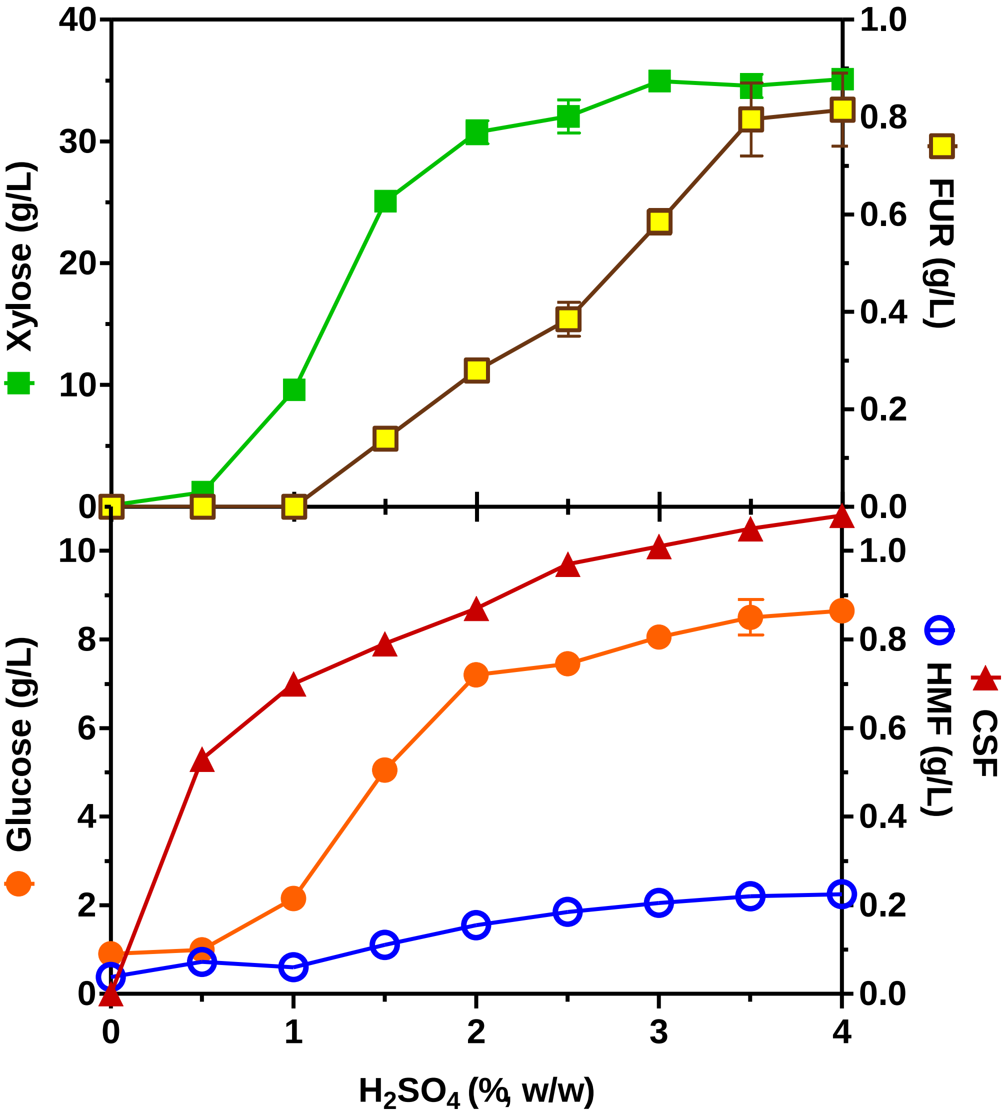
Why corn stover?

- Corn is produced worldwide
- Most of the stover is left in the field (in LA) or burned before new harvest.
- 1.9 Ton of corn stover are generated from 1 ton of corn produced
- 50% of the corn stover can be “harvested” to be used as feedstock

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

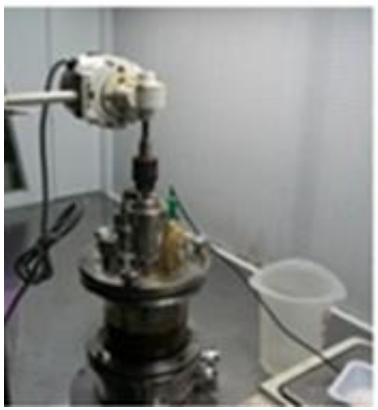
Diluted Acid Treatment
15% solids, 1-L Parr Reactor
Effect of H_2SO_4 concentration
8 min at 130°C

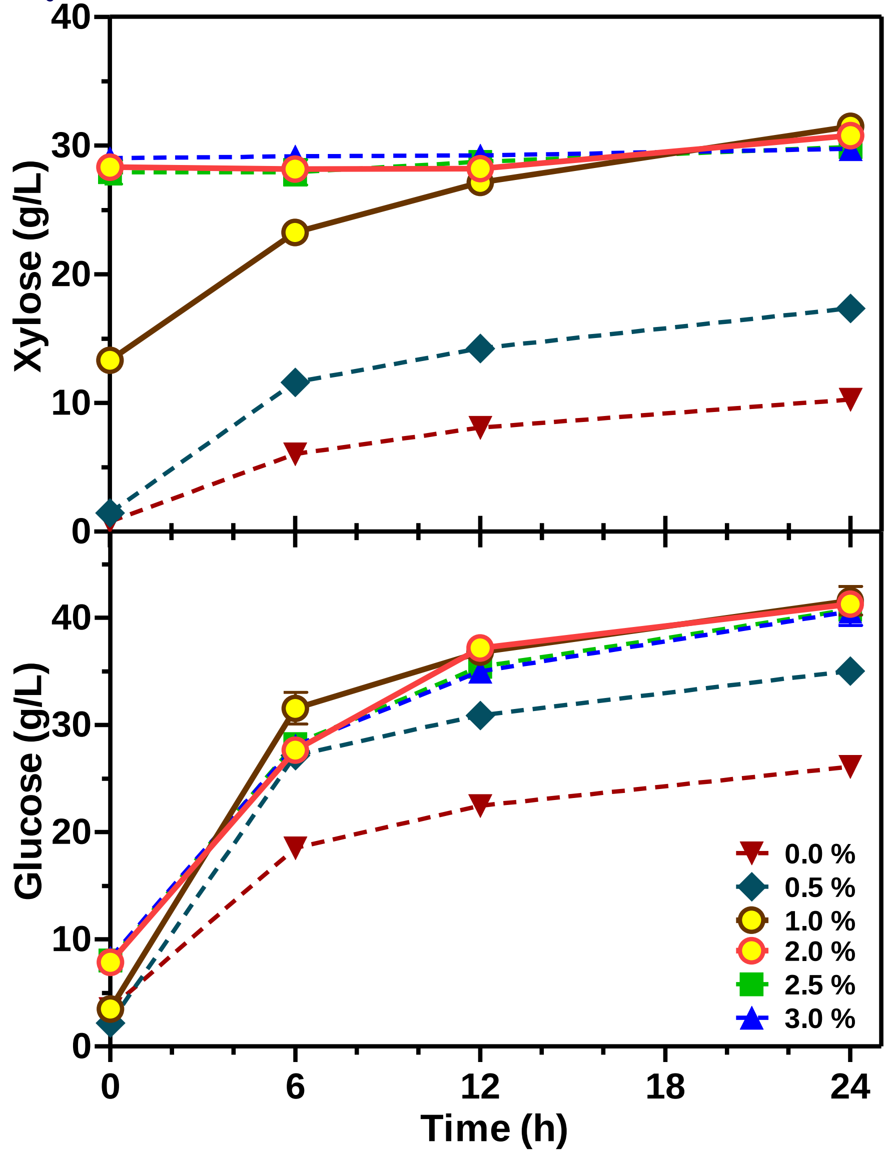
Above 2% H_2SO_4 most of the xylose is released, with low amounts of Furans



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

Diluted Acid Pretreated Slurries
Saccharification Peg Mixer Reactor
15 FPU/g Stover, 30 CBU/g Stover
50 °C pH = 5.0, 60 rpm

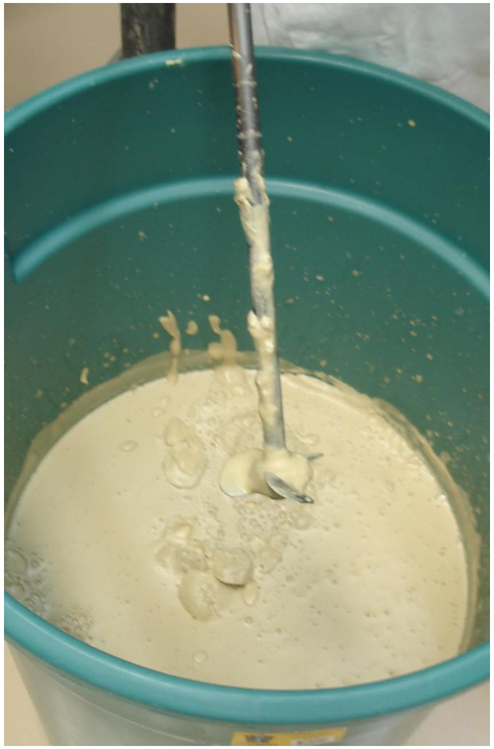




Most of the Xylose & Glucose are released from the pretreated corn stover above 1% H_2SO_4 in 12 – 24 h

Corn stover

- Corn stover contains (at least) 55% dw (w/w) of polymerized sugars (30% glucan, 20% xylan, 5% arabinan)
- If correctly processed (hydrolyzed) 550 kg of sugars per ton (dry weight basis), including maximum yields and loses.
- **Glucose, xylose and arabinose:**
- **Need to be fermented together**

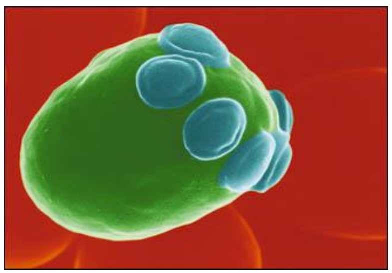


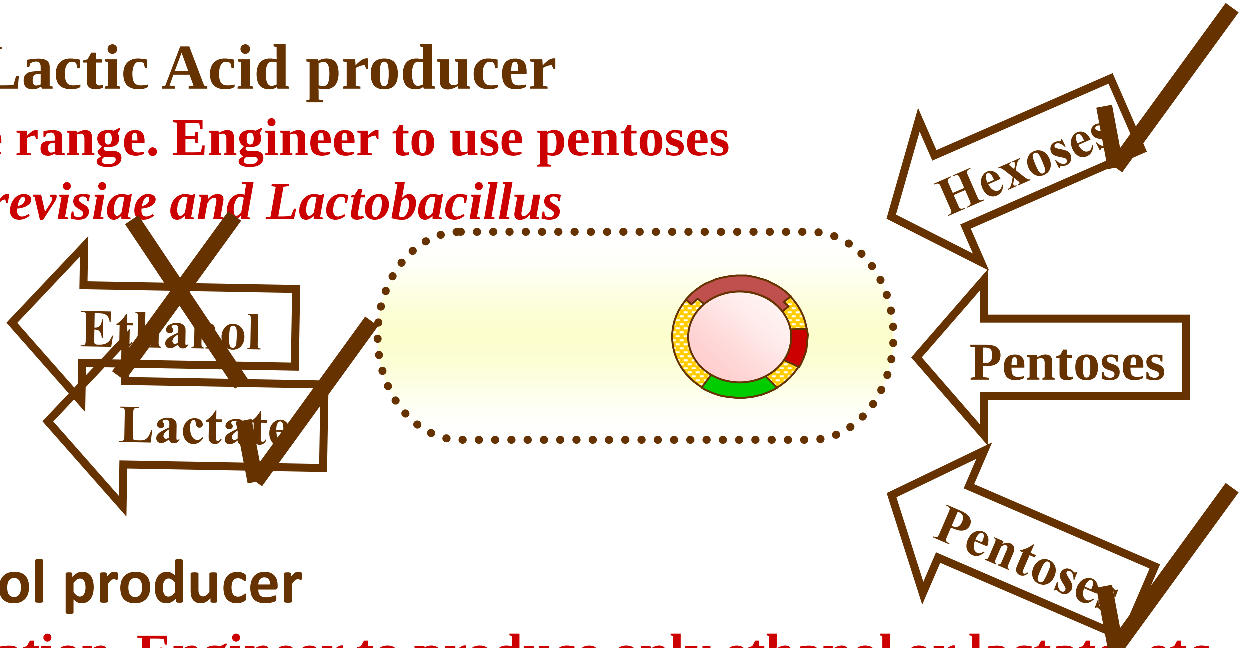
The BioTech. Challenge: Pentoses → Biochemicals
Metabolic Pathway Engineering

A: Ethanol or Lactic Acid producer

Increase substrate range. Engineer to use pentoses

Saccharomyces cerevisiae and Lactobacillus

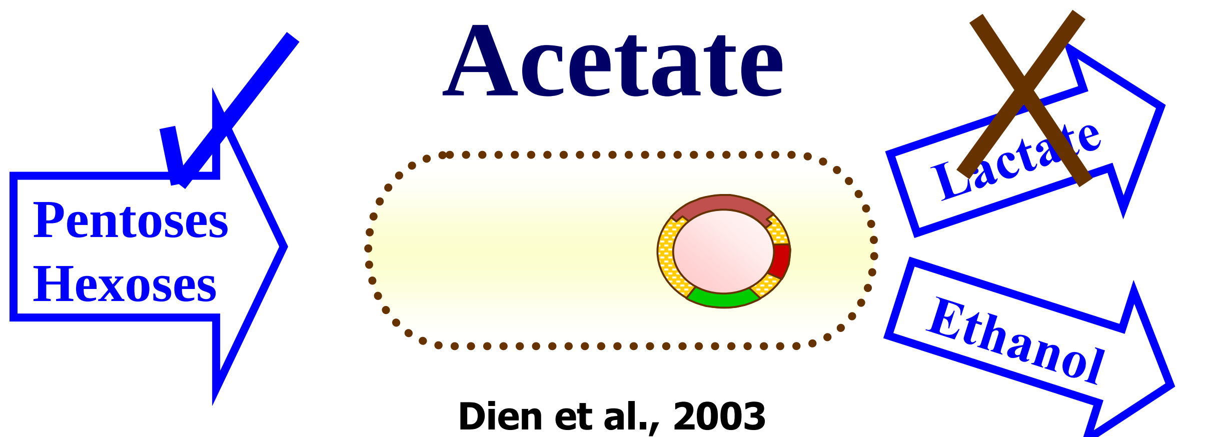




B: NO good Ethanol producer

Pathway complementation. Engineer to produce only ethanol or lactate, etc.

Escherichia coli



Dien et al., 2003

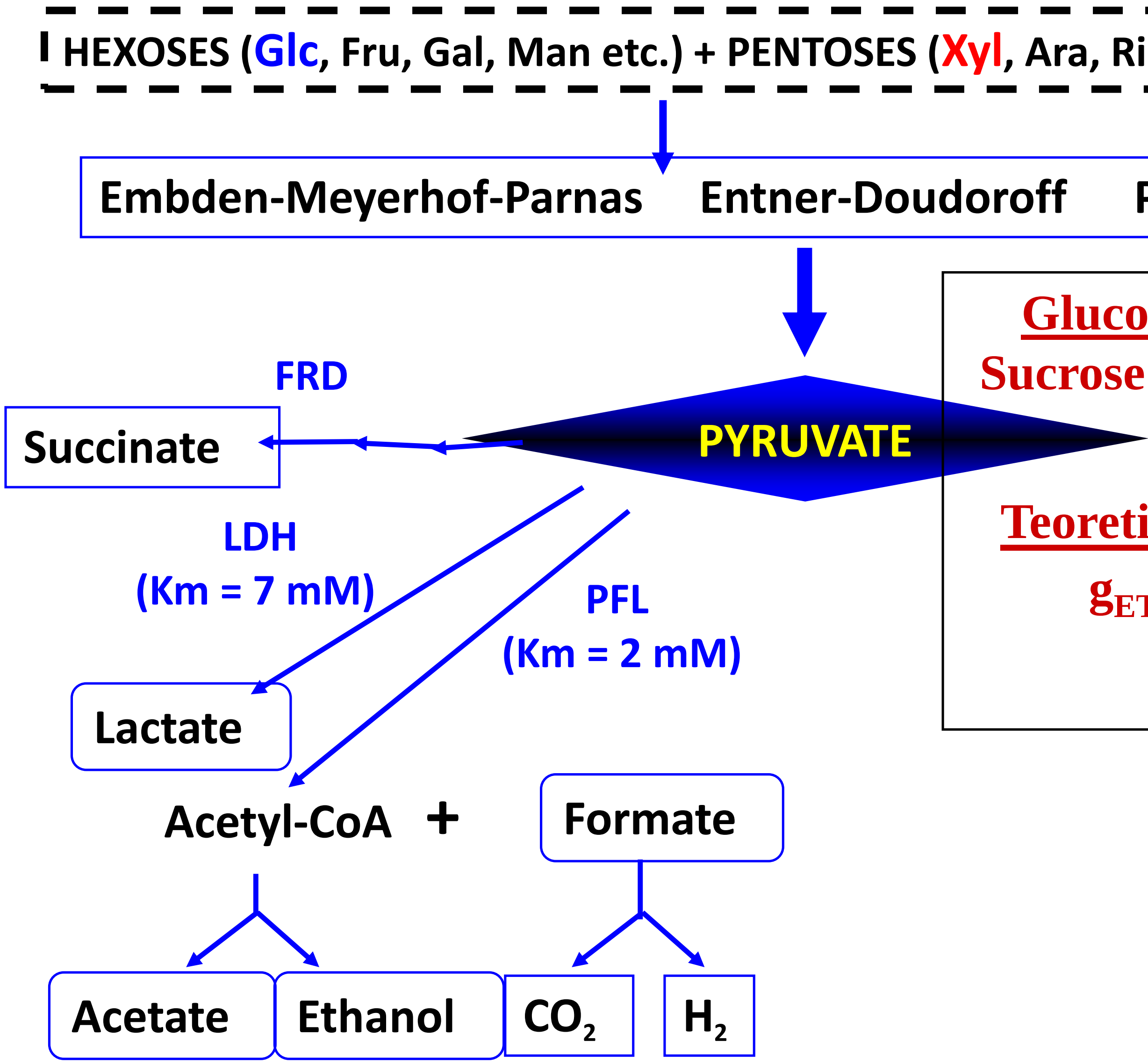
Escherichia coli? Products

E. coli
Bacteria

- Approximately 33% of the therapeutic proteins for human use are currently produced using *E. coli* using industrial fermenters.
- Human growth hormones; interferons; interleukins; erythropoietin; among others
- L-phenylalanine, PHB, and Propanediol, among others
- Recently (2016) 1,4 Butanodiol
- ME & SB *Escherichia coli* laboratory

Sanchez-Garcia et al. Microbial Cell Factories201615:33

Escherichia coli Fermentative Phatways



Glucose → 2 Ethanol + 2 CO_2

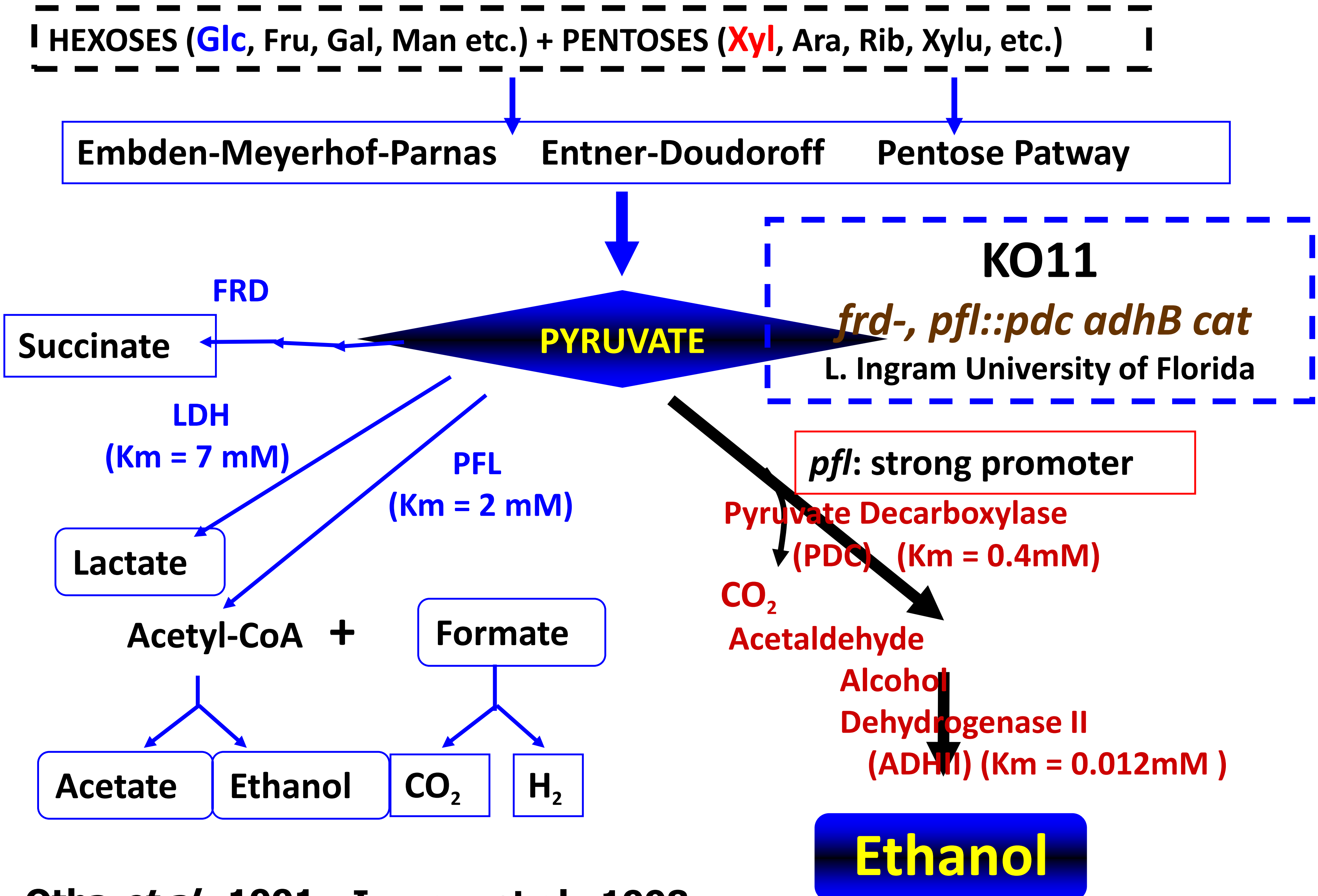
Sucrose → 4 Ethanol + 4 CO_2

Teoretical Yield: 0.51 g/g

$g_{ETHANOL} / g_{(GLC\ o\ SAC)}$

0.64 L_{EtOH}/kg

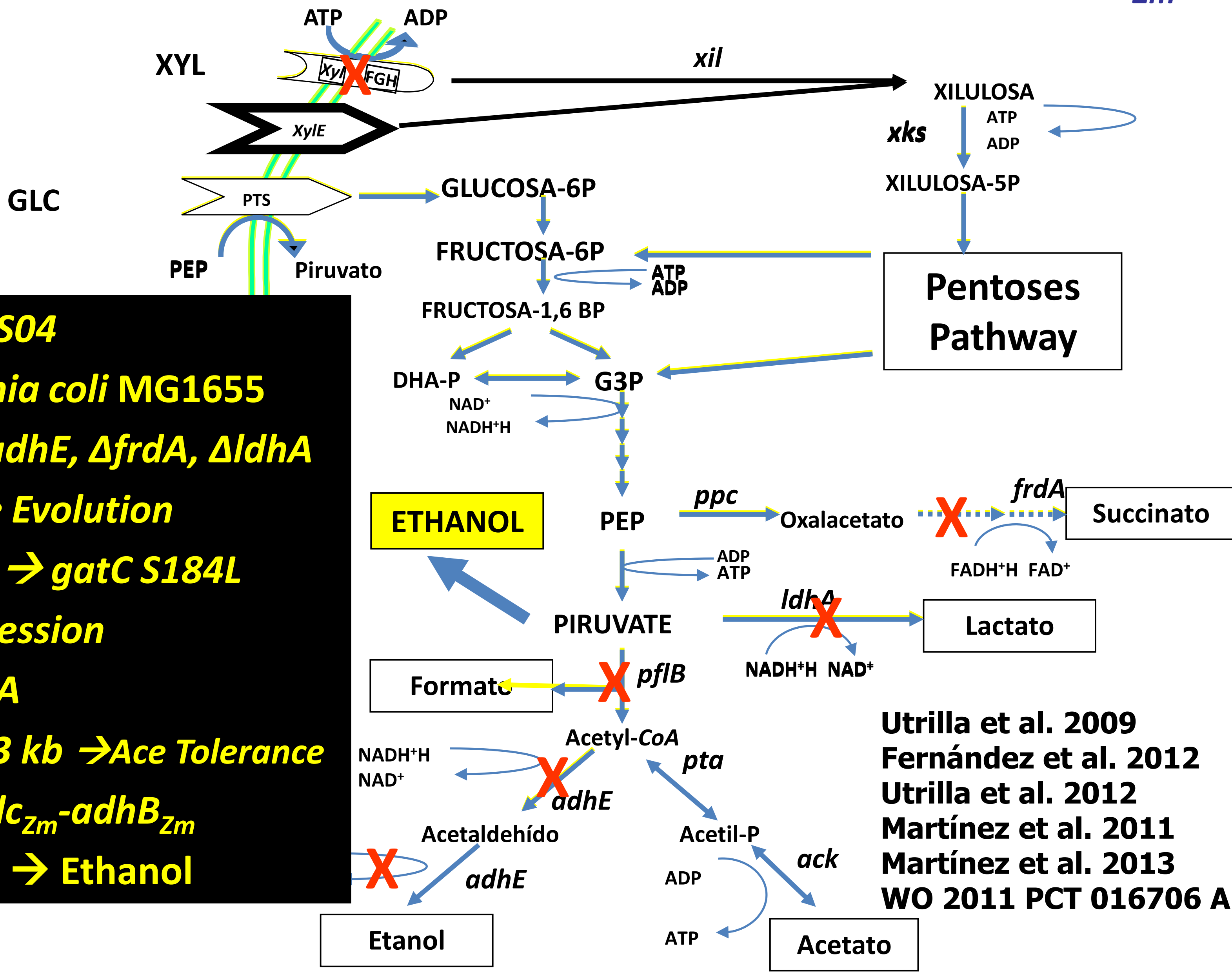
Ethanologenic *E. coli* 1st generation



Otha *et al.*, 1991 Ingram et al., 1998

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

MG1655: *ΔpflB, ΔadhE, ΔfrdA, ΔxylFGH, Δldh, PpflB::pdc-adh*$_{Zm}$

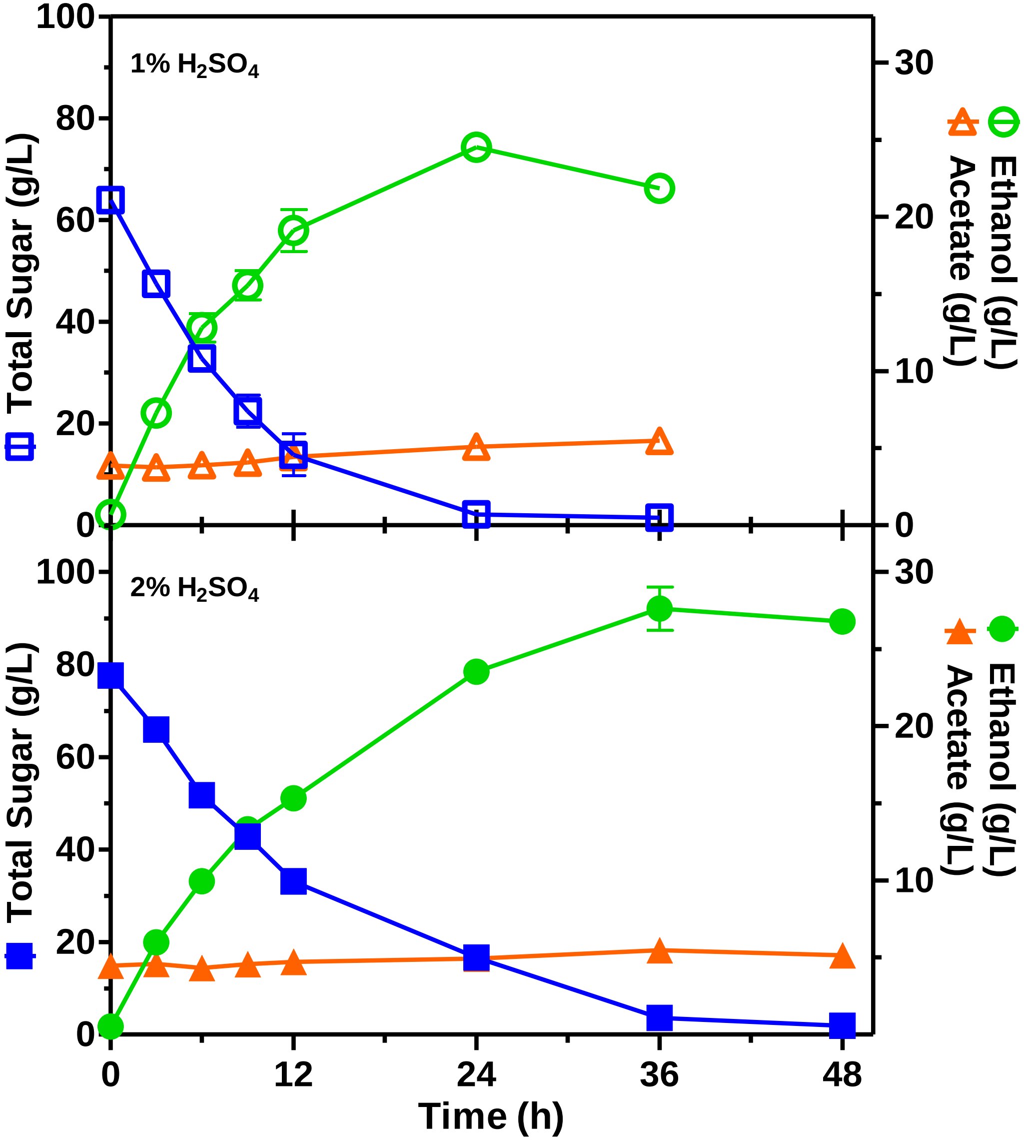


- *Strain MS04*
- *Escherichia coli* **MG1655**
- *ΔpflB, ΔadhE, ΔfrdA, ΔldhA*
- *Adaptive Evolution*
- *ΔxylFGH → gatC S184L*
- *Pdh expression*
- *ΔmidarpA*
- *Δreg 27.3 kb →Ace Tolerance*
- *PpflB::pdc*$_{Zm}$*-adhB*$_{Zm}$
- **Pyruvate → Ethanol**

Utrilla et al. 2009
Fernández et al. 2012
Utrilla et al. 2012
Martínez et al. 2011
Martínez et al. 2013
WO 2011 PCT 016706 A2

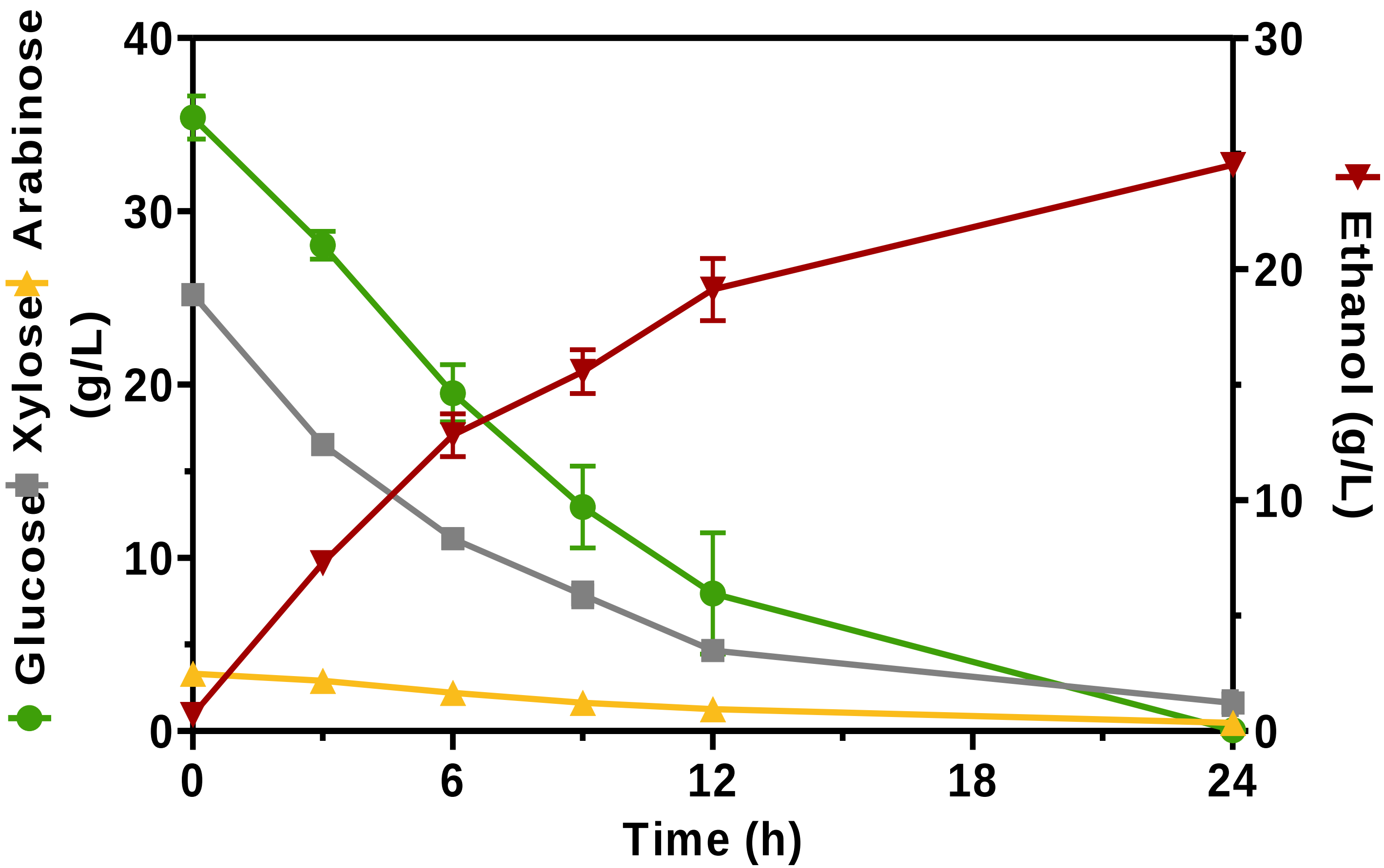
Corn Stover. Sequential: Thermochemical Hydrolysis, Enzymatic Saccharification and Fermentation

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

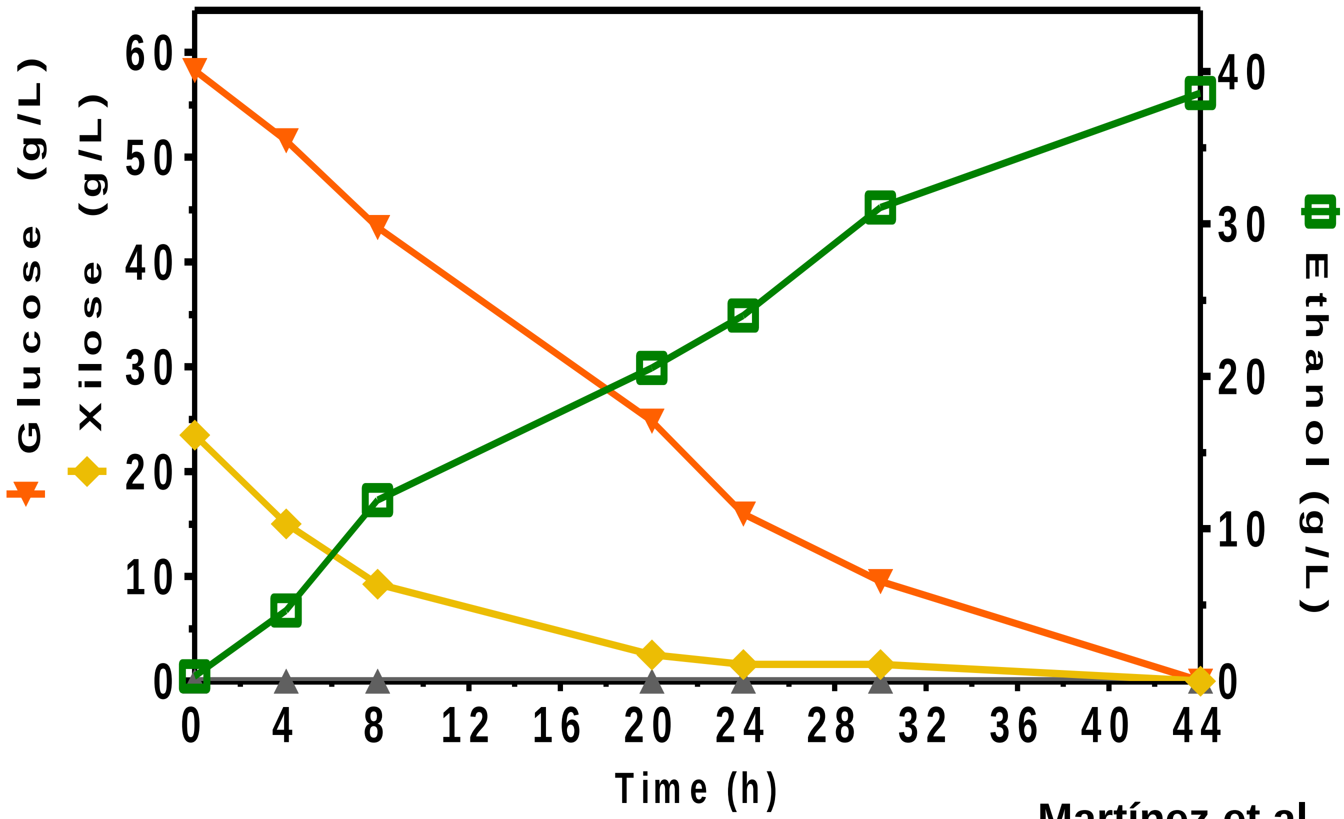


All sugars are fermented to ethanol by ethanologenic E. coli MS04 in 24 - 36 h

Fermentation 1 % H_2SO_4



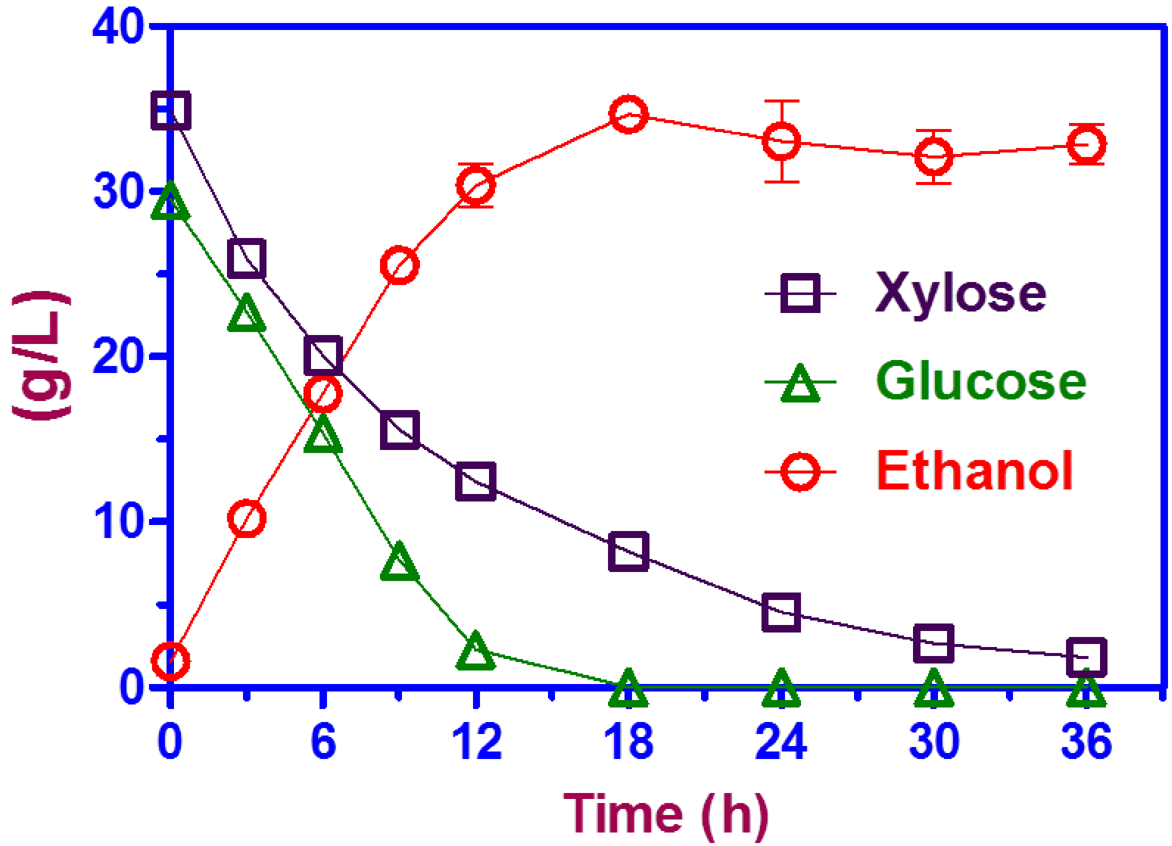
Ethanol Production in Laboratory-Mineral Medium: Xylose (24 g/L) - Glucose (58 g/L)



E. coli **MS04 (1.8 g/L)**
Yield >90%
~ 1 g/L/h
Acetic 4 g/L

Martínez et al., 2007
Orencio et al., 2008
Utrilla et al., 2009
Fernández et al., 2011
Martínez et al., 2012
Martínez et al., 2013

Corn cobs (& many lignocellulosic feedstocks)



- Fermentation kinetics of syrups from hydrolyzed corncobs with the ethanologenic *E. coli* strain MS04.

Fernández-Sandoval et al. 2012. Applied Microbiology Biotechnology. 15:855-866.

Fuel ethanol production from corn stover with ethanologenic bacteria

Process Integration: IBt - UNAM

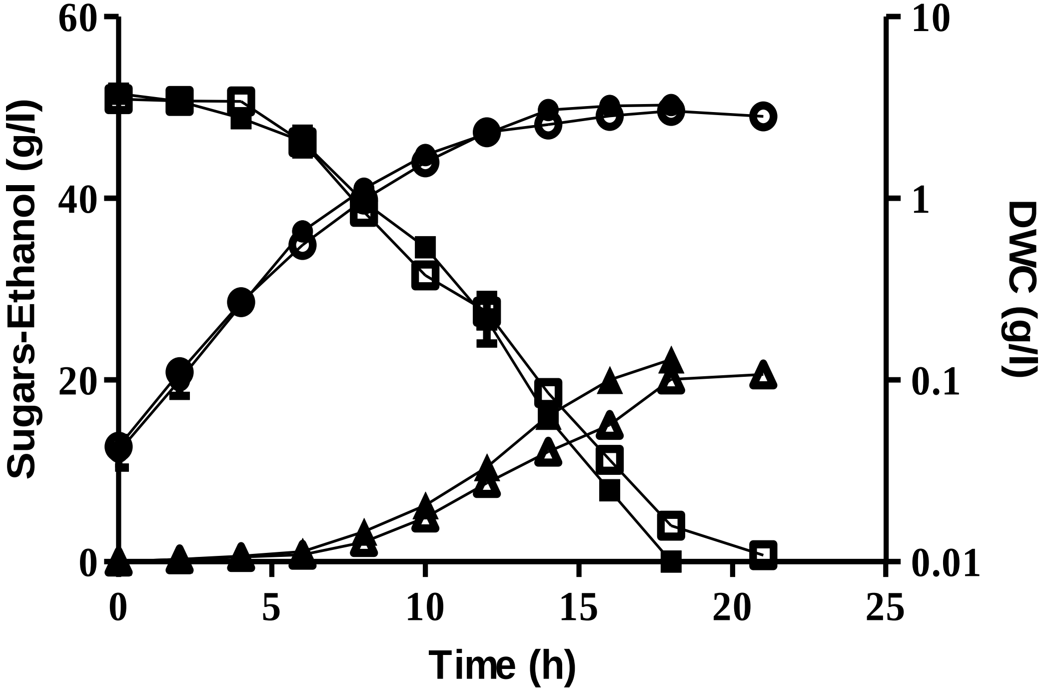
Lab scale: 270 L_{EtOH}/Ton

Goal: 320 L_{EtOH}/Ton

1 Ton Corn -> 1 Ton CS –

550 kg sugars – 320 L

Scale-up Ethanologenic

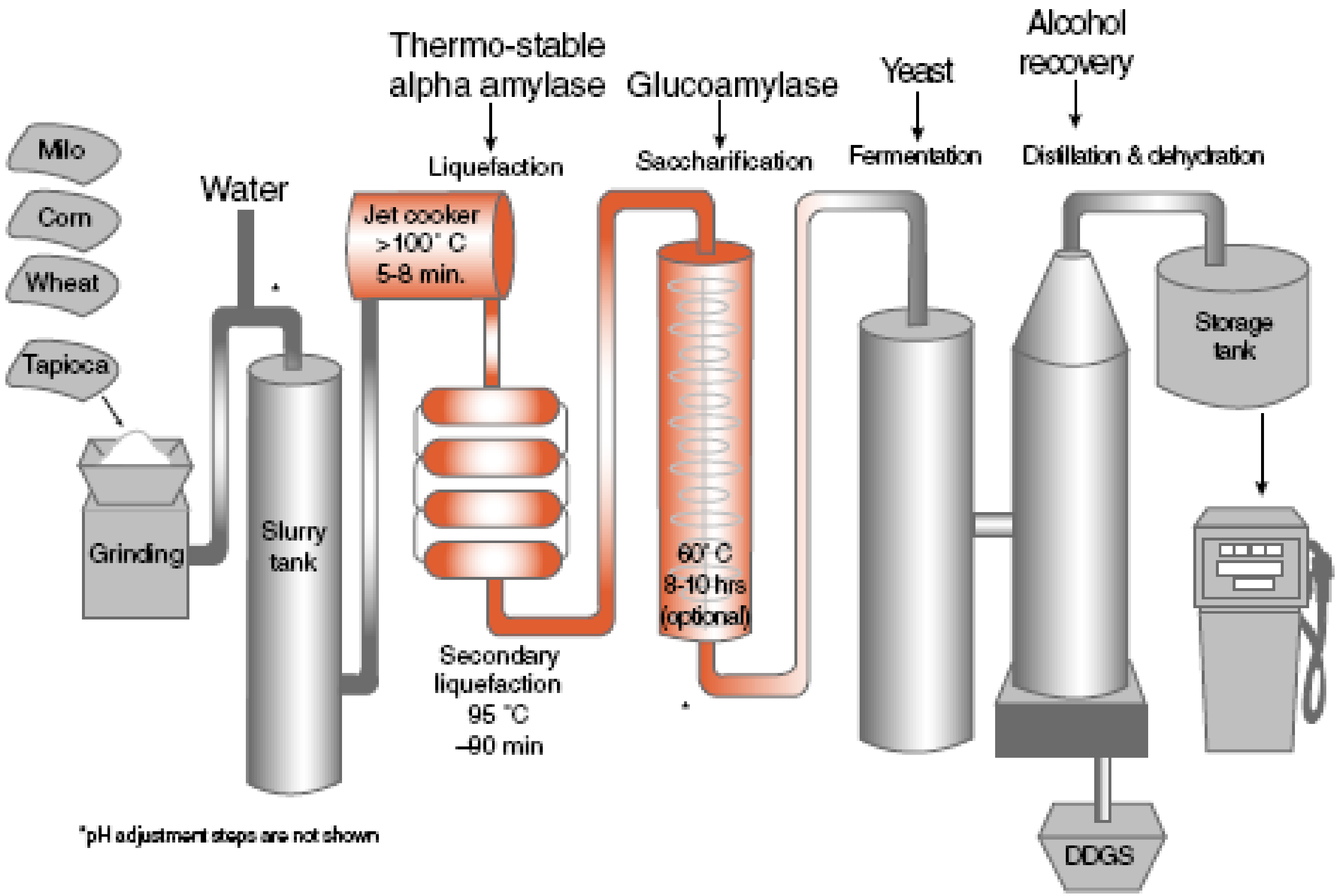


Xylose- Glucose
Scale-up
0.2 ➔ 10 L

Hydrolysates
Corn Stover
0.2 ➔ 400 L

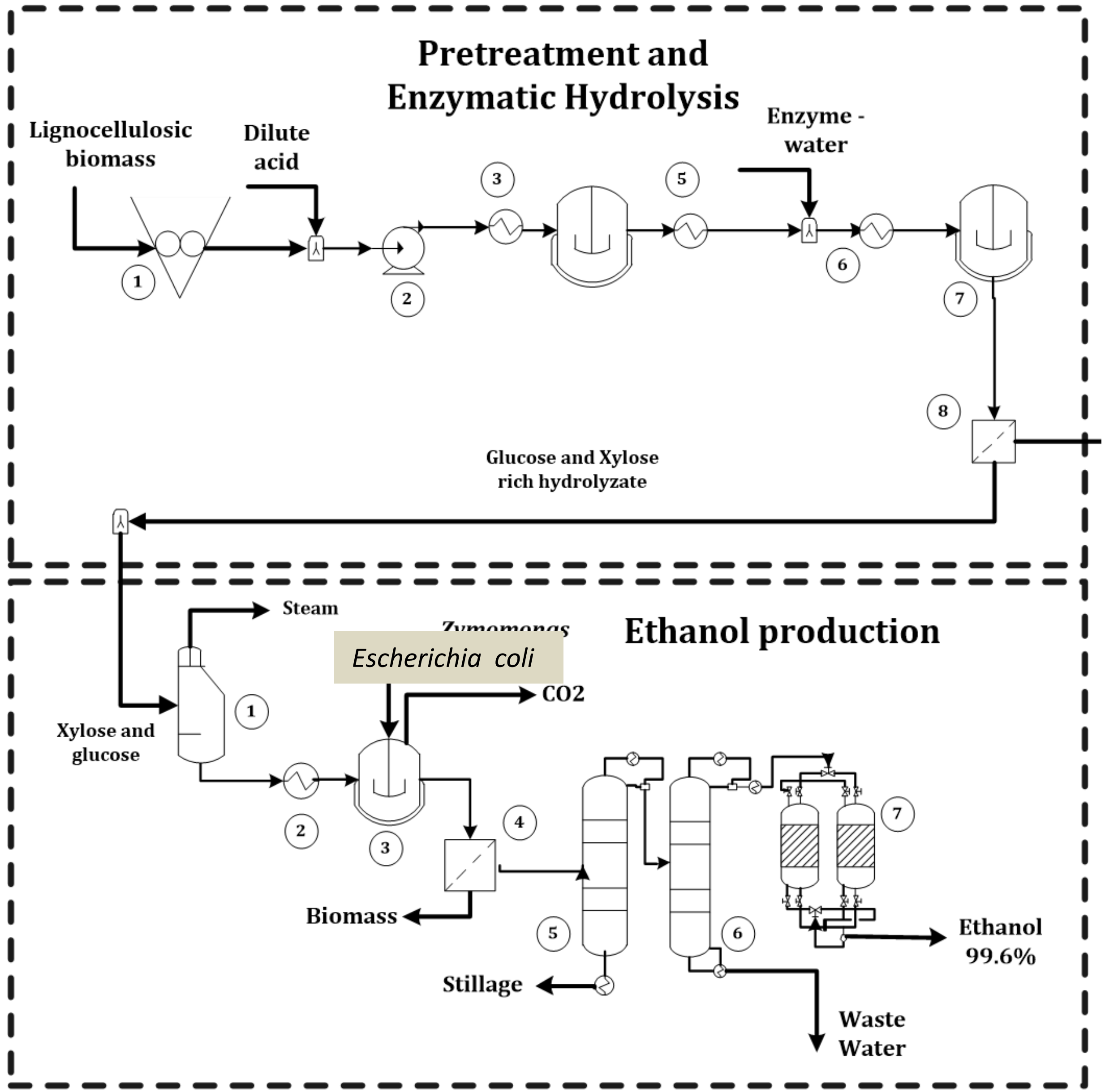
Yield: ~ 88-95%
of theoretical
Productivity
0.5-1.1 g/L/h

Needs to be transformed in a "Mature Technology"



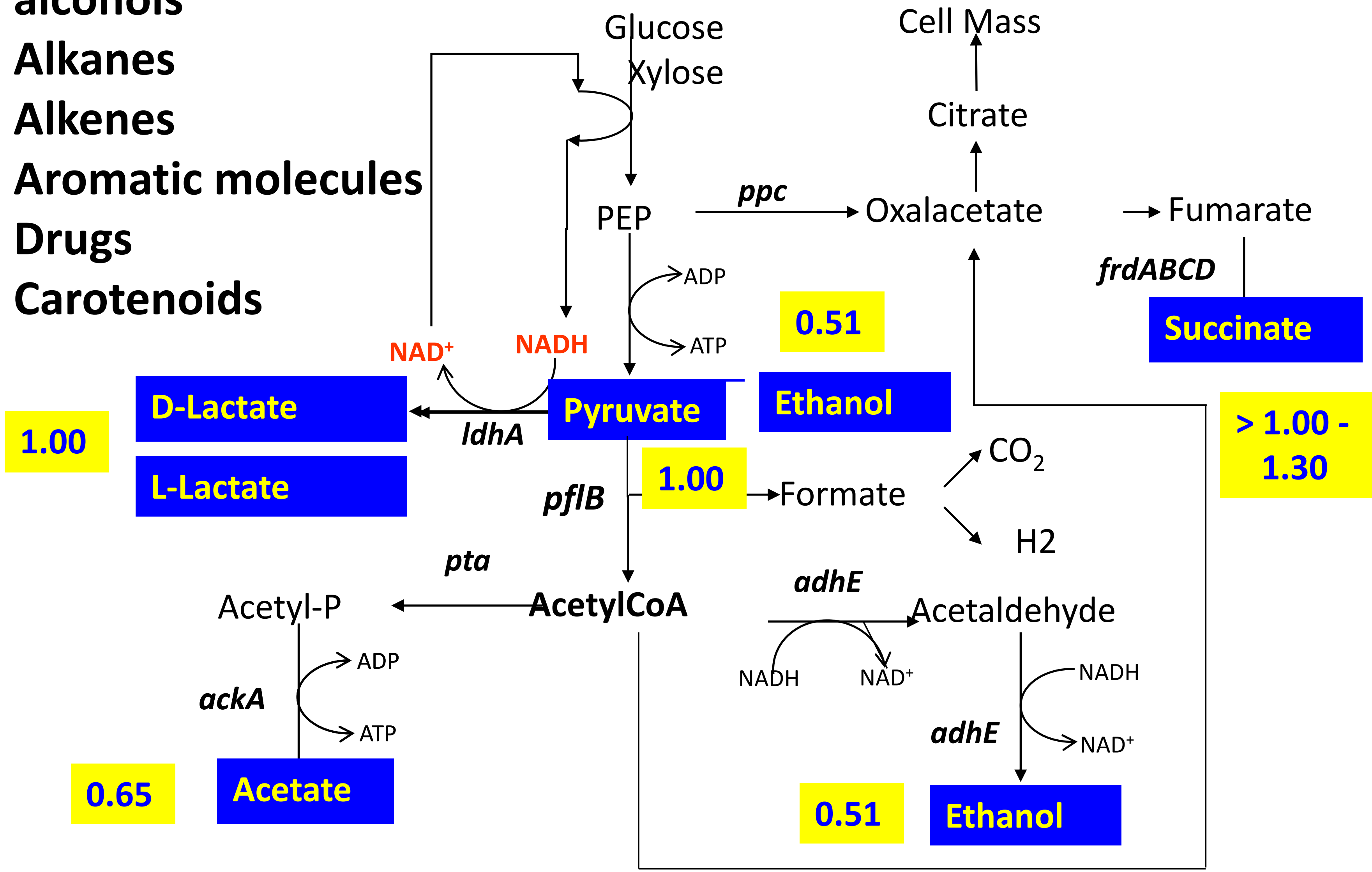
Process integration key factors for biorefineries:

Feedstock: **No wash**
Feedstock: **No dry**
Pretreatment: **No detoxification**
Saccharification: **No separation of C5 and C6 streams**
Fermentation: **All sugars, C5 and C5**
Multiple-Products



Fermentation Products Biochemical Platform

Fatty acids
Medium and long chain alcohols
Alkanes
Alkenes
Aromatic molecules
Drugs
Carotenoids



Thanks
Questions

CONACYT

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