Escherichia coli as Microbial Cell Factory for the Biorefinery Concept

Escherichia coli como Fábrica Celular Microbiana para el Concepto de Biorrefinería

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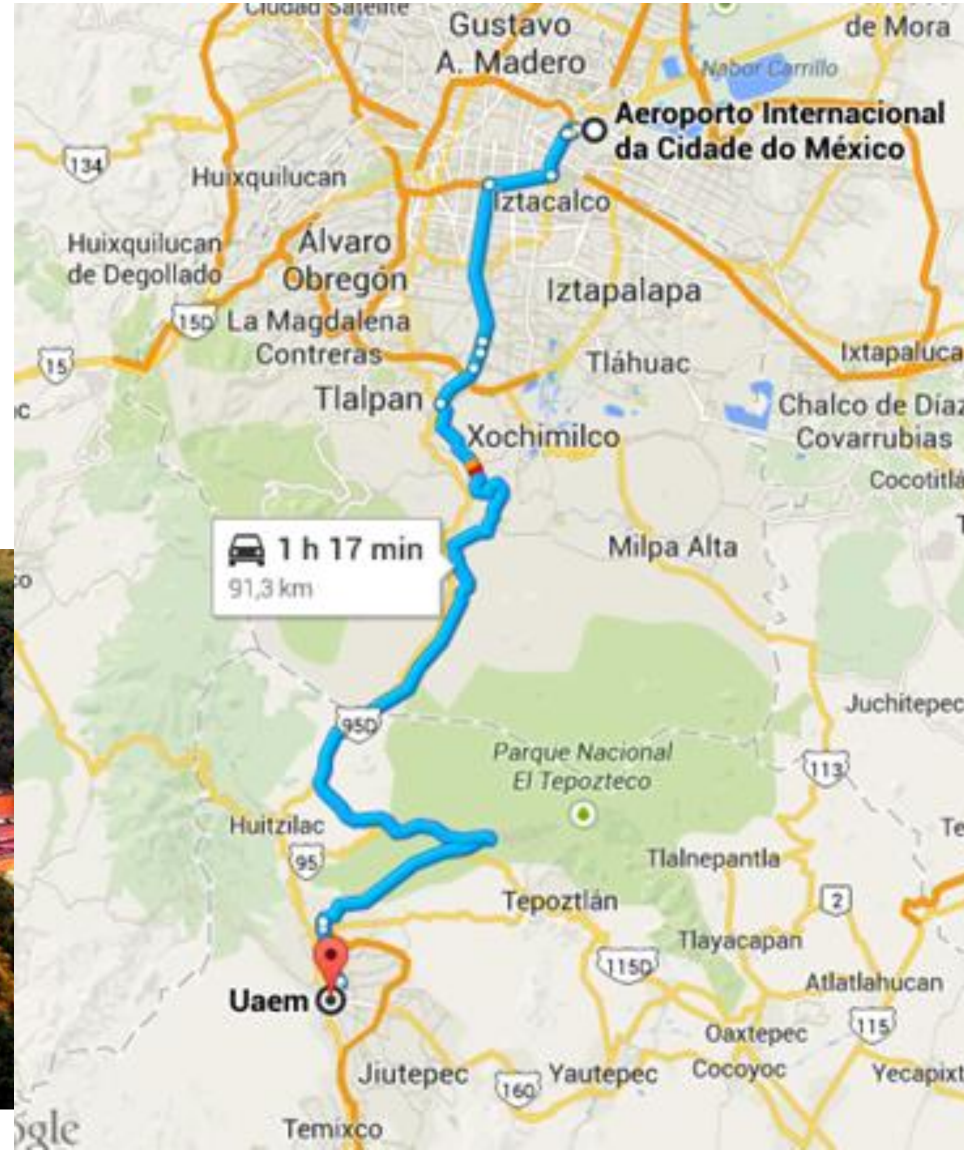
Where we are:



Campus Morelos - Universidad Nacional Autónoma de México

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Diversas areas agrupadas en 5 Departamentos

30 AÑOS
Instituto de Biotecnología

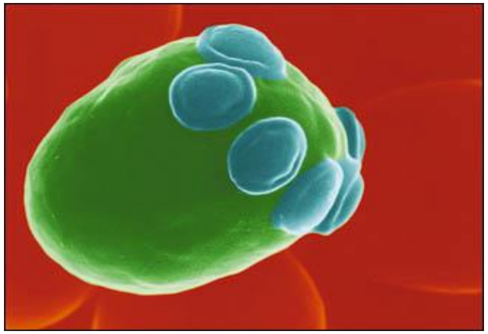
- Biología Molecular de Plantas
- Genética del Desarrollo y Fisiología Molecular
- Medicina Molecular y Bioprocesos
- Microbiología Molecular
- Ingeniería Celular y Biocatálisis

What we do:

- Alfredo Martinez: Fermentative Pathways, Biofuels and Biochemicals.
- Metabolic engineering, synthetic biology and bioprocess development with *Escherichia coli* for biofuels (fuel ethanol, butanol and long chain alcohols), lactate, butyrate and R-3-hydroxybutirate (biopolymer precursor) production.
- Physiological studies with oleaginous microalgae under heterotrophic conditions.

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

Saccharomyces cerevisiae
Yeast: Produce Ethanol



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-fenilalanina, PHB, and Propanediol, among others

Sanchez-Garcia et al. Microbial Cell Factories, 201615:33

(Future!) Global Challenge: New fuels & materials are needed to substitute fossil fuels and oil derivatives

Energías Alternas

Eólica
Hidráulica
Mareomotriz
Geotérmica
Nuclear
Solar:
Electricidad

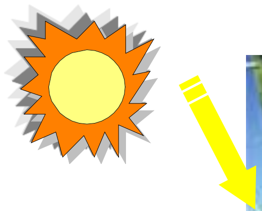
Se requieren Combustibles:
Sólidos
Gaseosos
Líquidos:

Bio-Etanol
Bio-Diesel
Bio-Turbosina
Bio-Gasolina

Bio-Plásticos
Bio-degradables
& Bio-Químicos, otros

Martínez 2009

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



The Sun

Artificial CO_2 cycle

CO_2

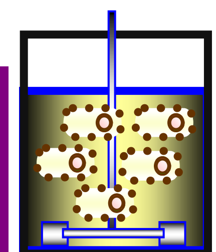
Chemicals
Ethanol (Fuel)

Lignocellulose - Biomass
Agricultural Residues
Bagasses and Stovers
Energy Crops

1 kg of Sugar
Yields 0.51 kg of Ethanol
0.5 USD / L

Hydrolysis

Sugars +
Acetic &
Glucuronic
Acids
Furans

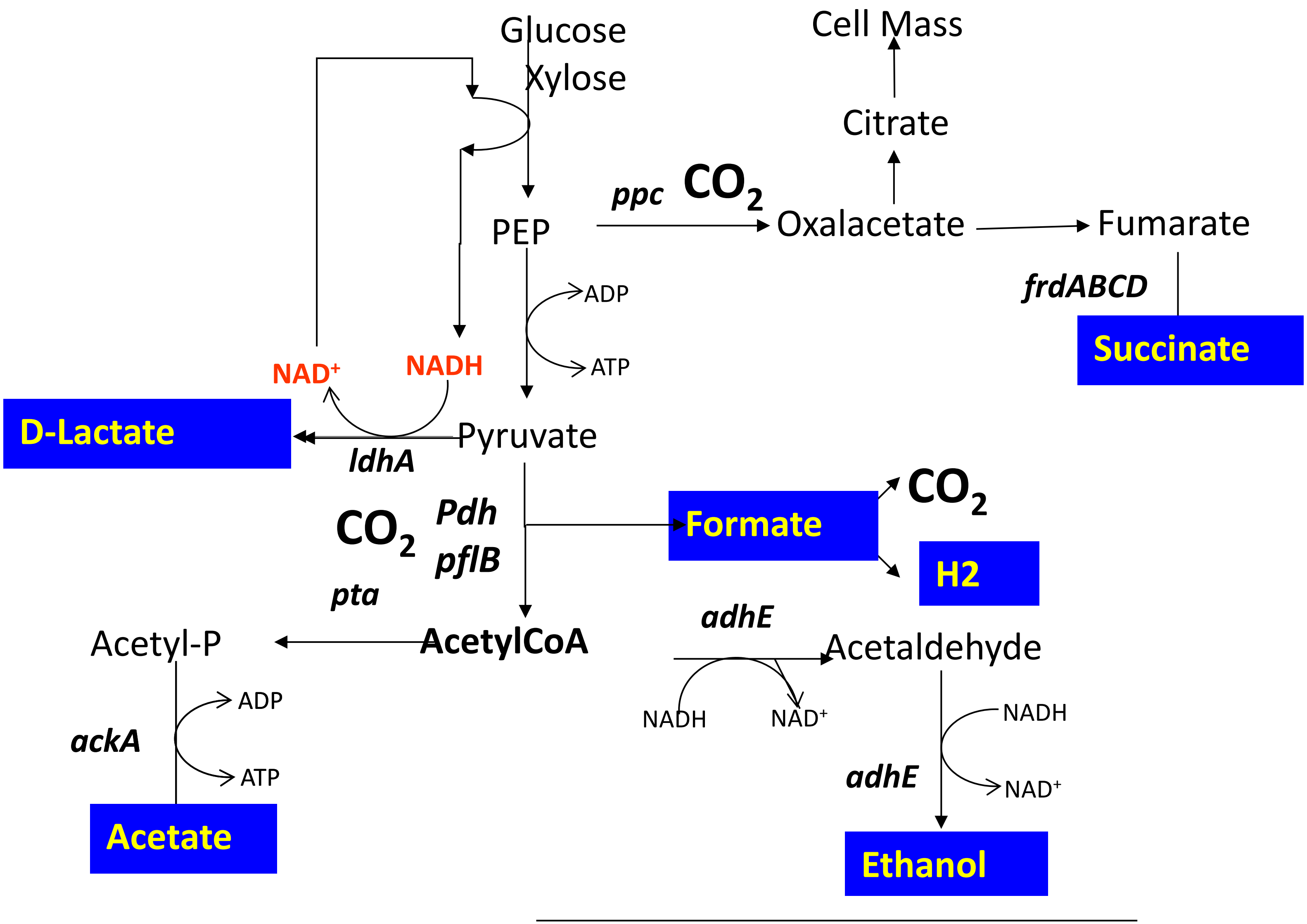


Fermentation

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

Fermentation Products *Escherichia coli*



Second Generation Bio-Plastics

The Sun

Artificial CO_2 cycle

CO_2

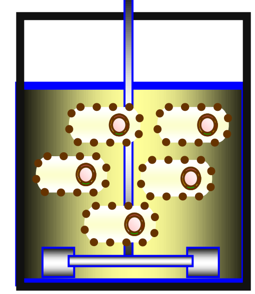
1 kg of Sugar Yield 1 kg of Lactic Acid
>1 USD / kg
PLLA: > 4 USA dol/kg

Lactate

Lignocellulose - Biomass
Agricultural Residues
Sugar Cane Bagasse

Xylose, Cellobiose Glucose, etc. Cellulose, Hemicellulose

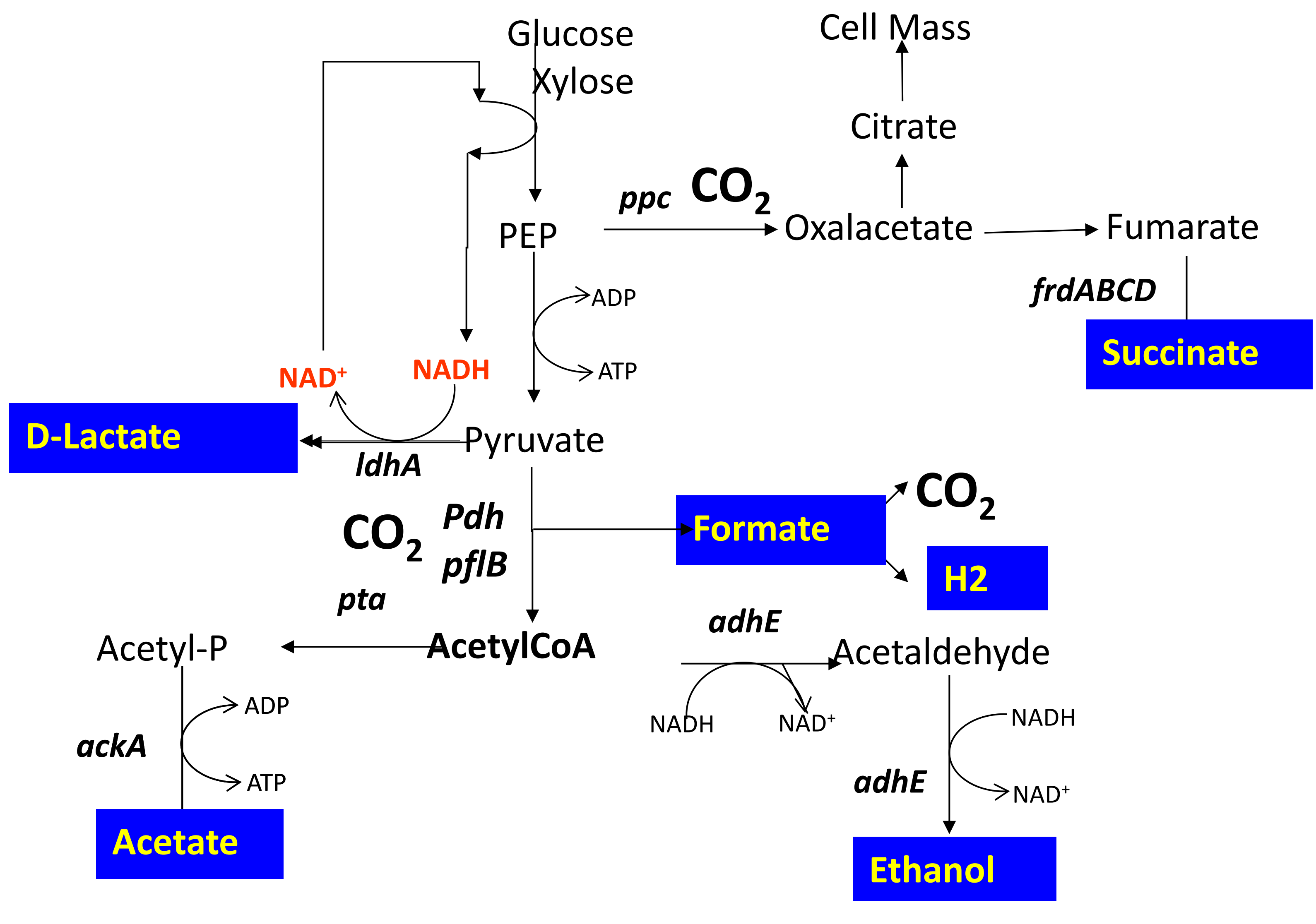
Hydrolysis



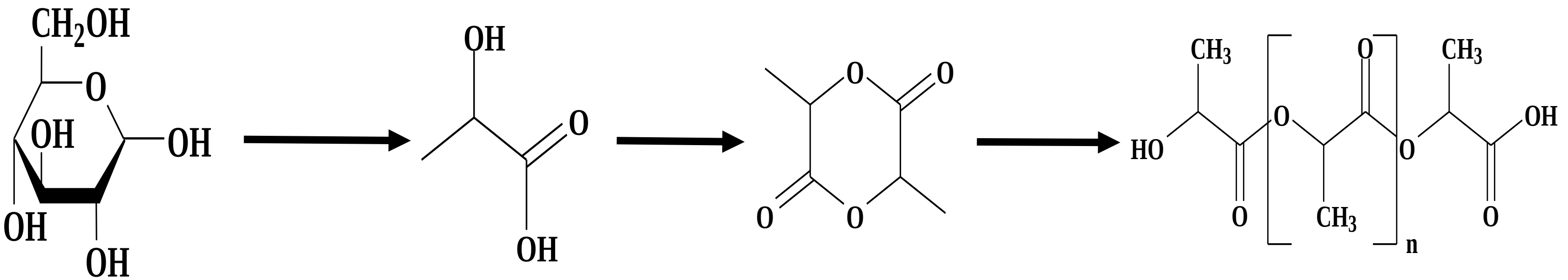
Fermentation

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

Fermentation Products *Escherichia coli*

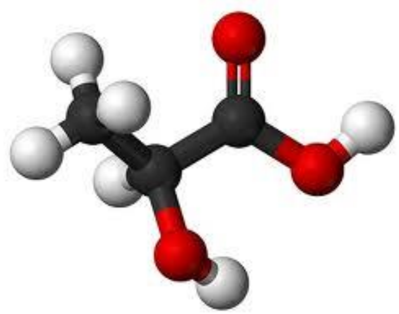


Lactic Acid → PLA, and other uses

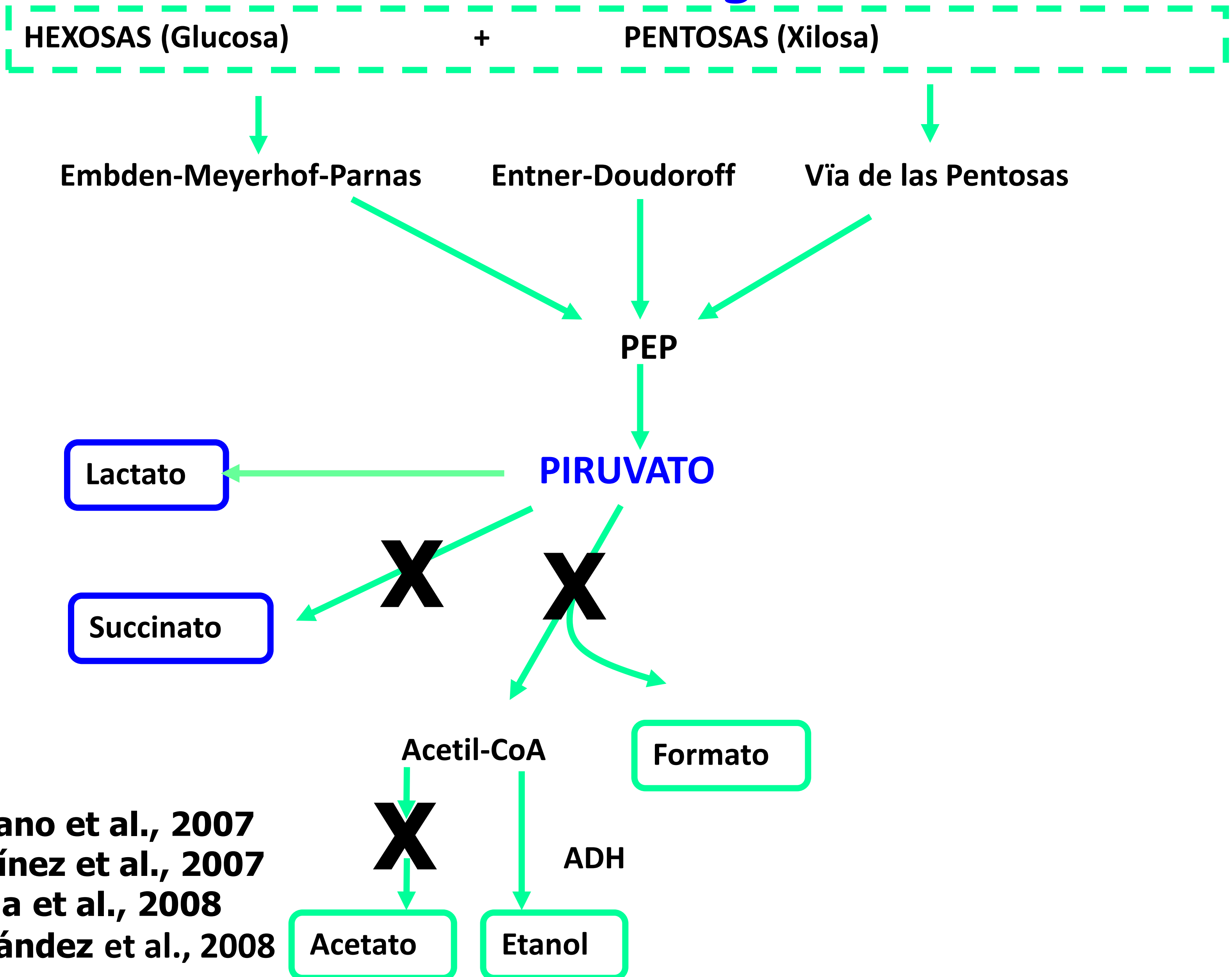


Glucosa | **L-Láctico Ópticamente puro** | **Dímero** | **PLA: Poly Lactic Acid**

L-Lactato
D-Lactato



E. coli Lactogénicas

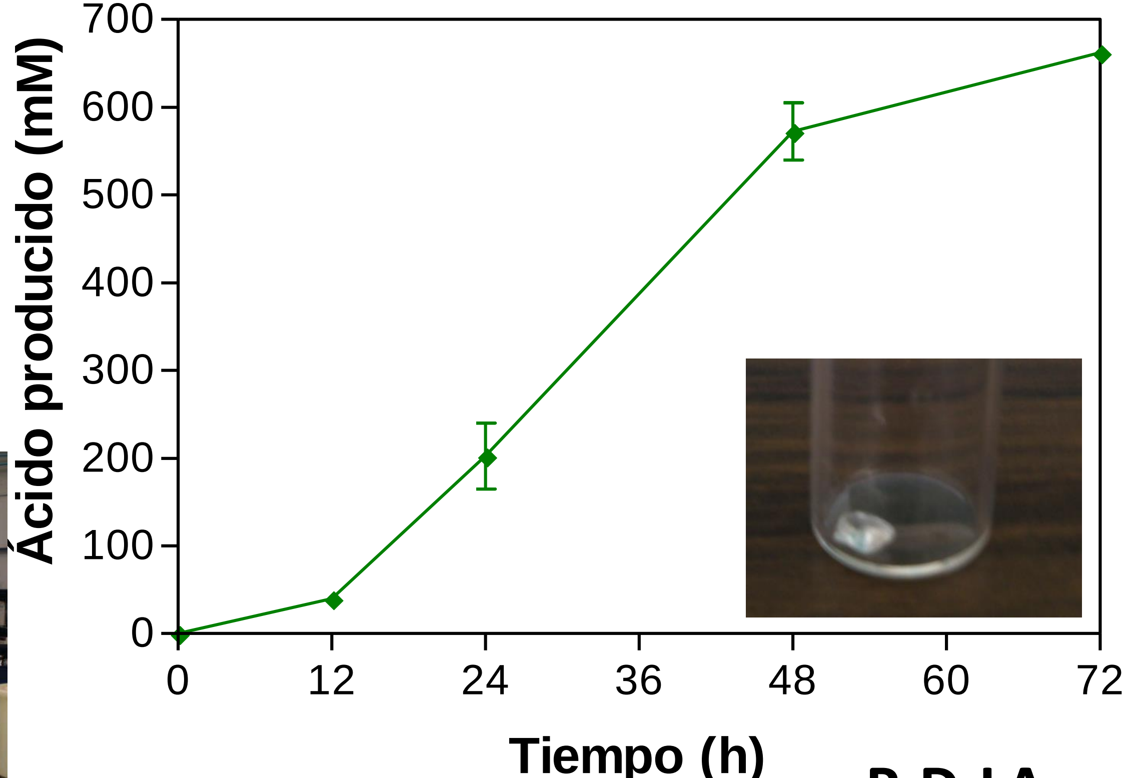


Yomano et al., 2007
Martínez et al., 2007
Utrilla et al., 2008
Fernández et al., 2008
Orencio et al., 2008

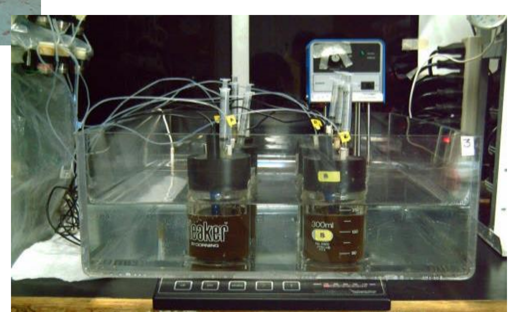
Sugar Cane Bagasse Hydrolysate

60 g/L total sugars, acetic acid
Aprox. 1 g/L Cells

Hydrolysate +
0.9 g/L $(NH_4)_2HPO_4$ – $NH_4H_2PO_4$
1 mM Betaine
0.1 g/L Citric acid

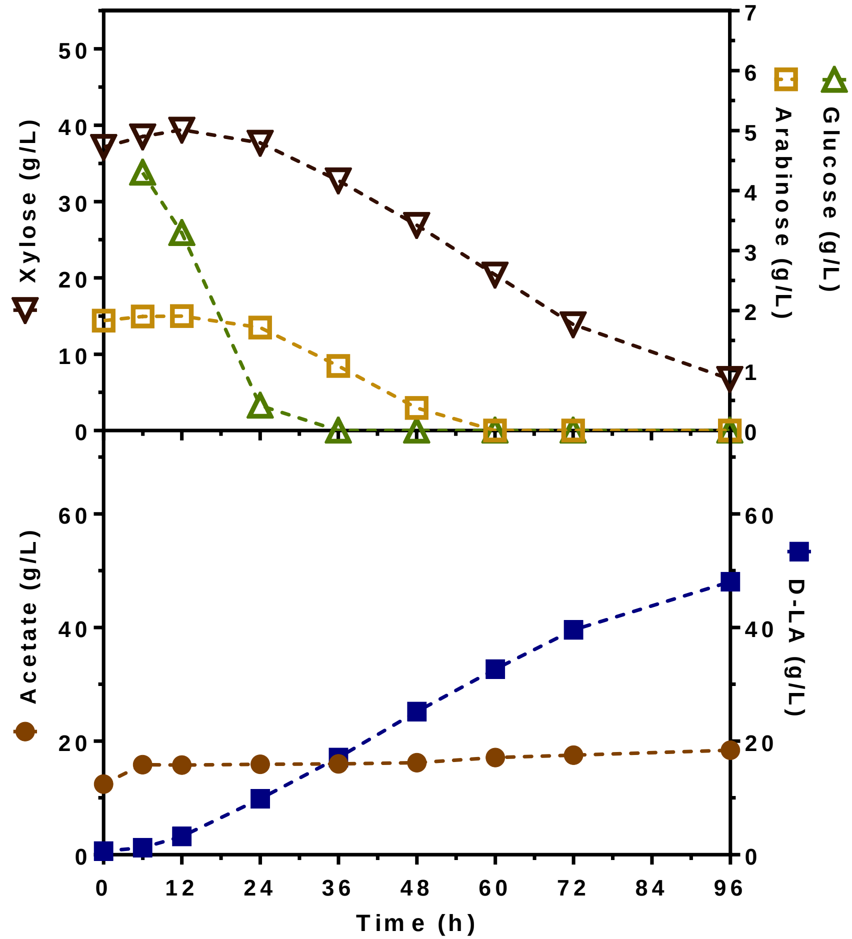


P-D-LA



D-Lactate
Berenice Trujillo 2008

Sugar Cane Bagasse Hydrolysate
JU15 Sequential sugar consumption



$Y_{D\text{-}LA}$ ($g_{D\text{-}LA}/g_{Sugars}$) = 1.30 ± 0.009

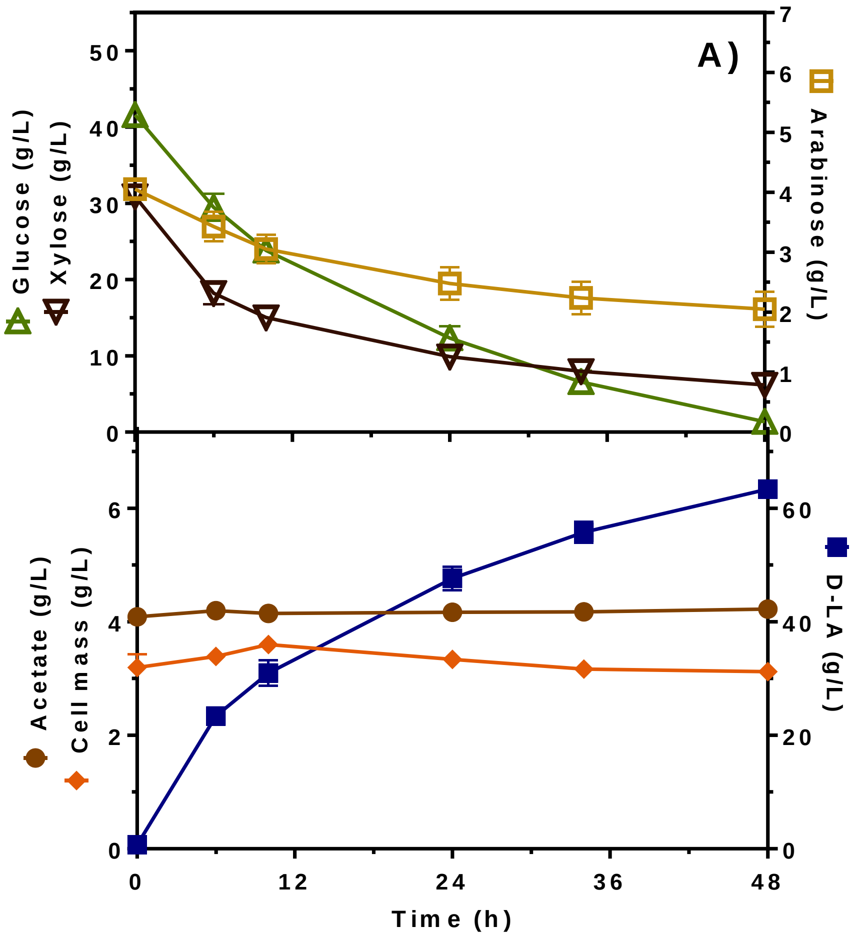
$Q_{D\text{-}LA}$ ($g_{D\text{-}LA}$/L h) = 0.51 ± 0.011

Utrilla – Vargas-Tah et al. 2016

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

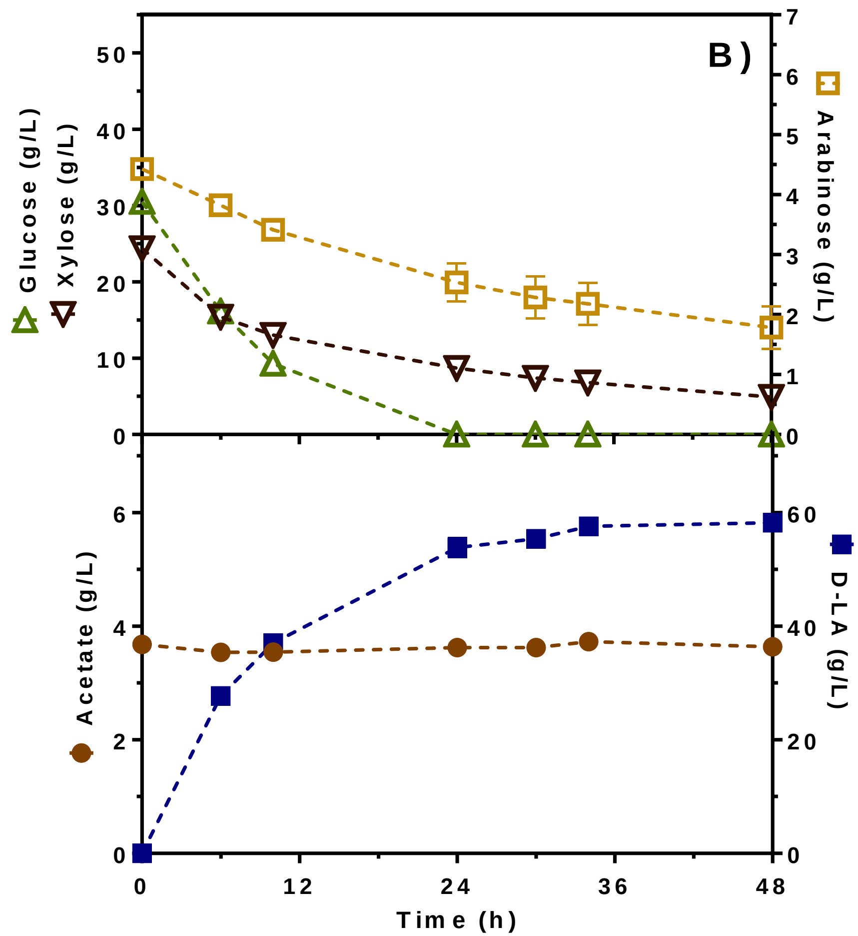
Agave Bagasse
Ethanol and Lactic Acid

Corn Stover Hydrolysate
AV03: Simultaneous sugar consumption



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

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



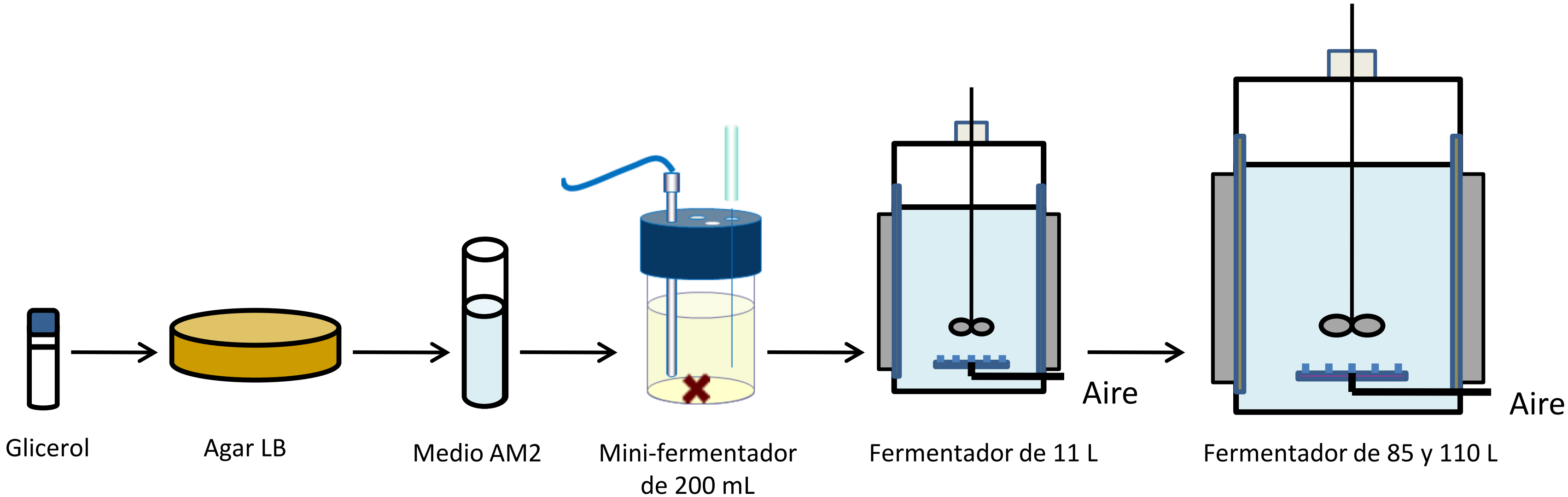
$Y_{D\text{-}LA}$ ($g_{D\text{-}LA}/g_{Sugars}$) = 1.11 ± 0.064

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

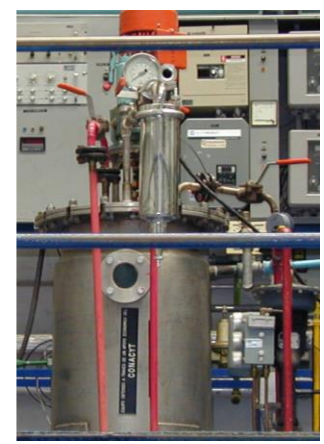
Utrilla – Vargas-Tah et al. 2016

Y > 1 !!!

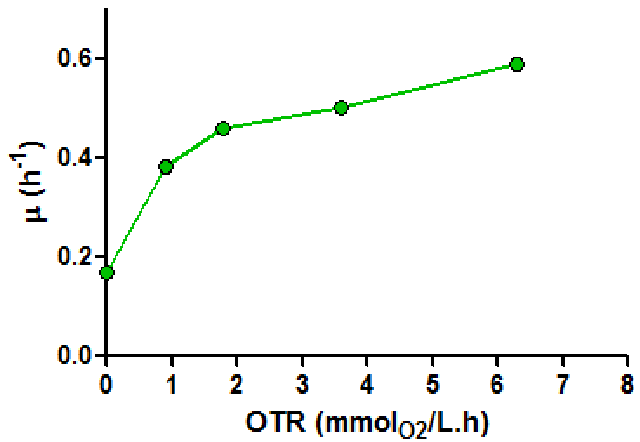
Scale-Up

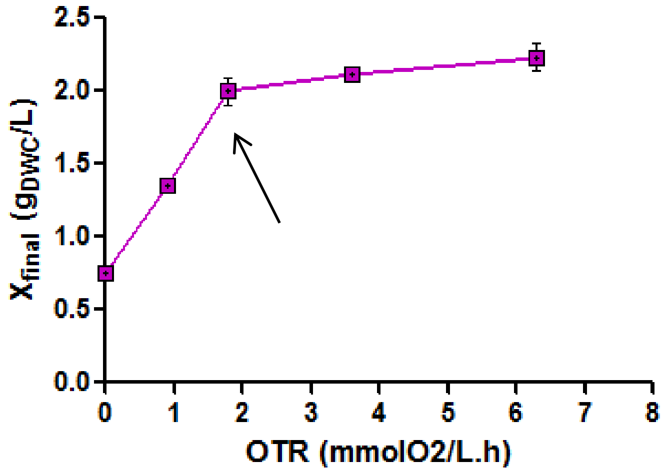


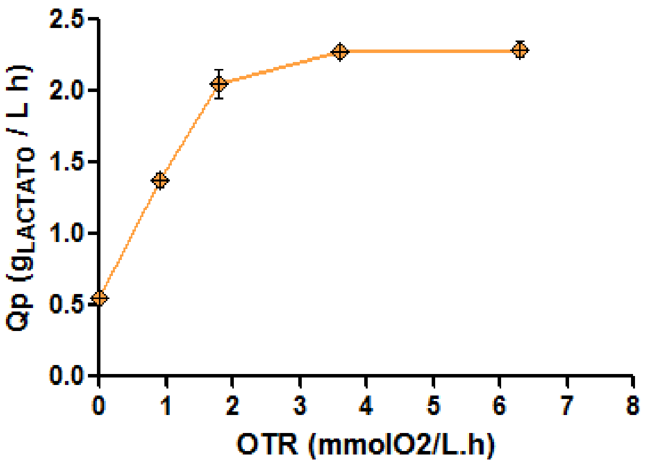
Glicerol
Agar LB
Medio AM2
Mini-fermentador de 200 mL
Fermentador de 11 L
Aire
Fermentador de 85 y 110 L
Aire



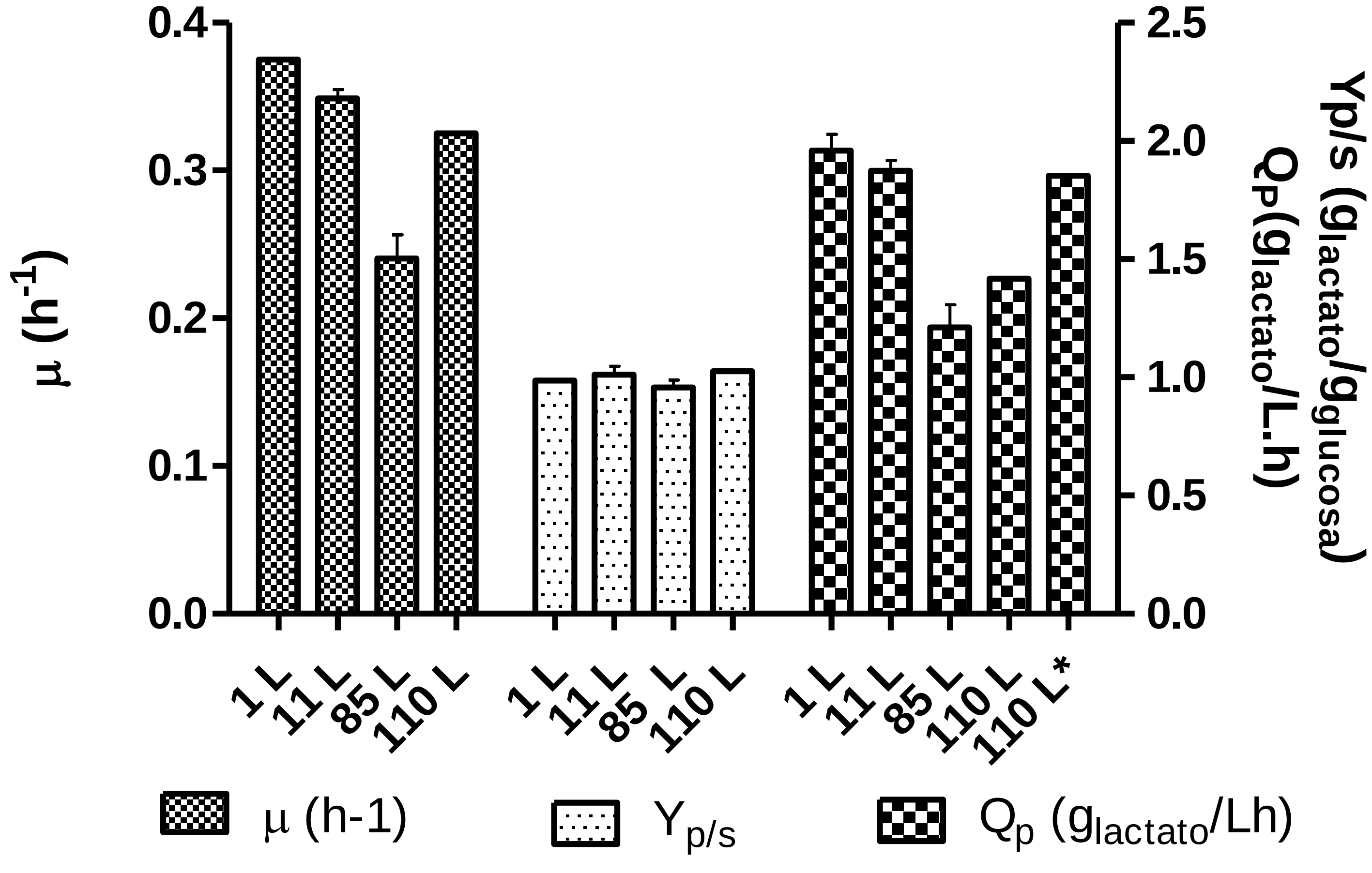
Base case Study - 1 Liter







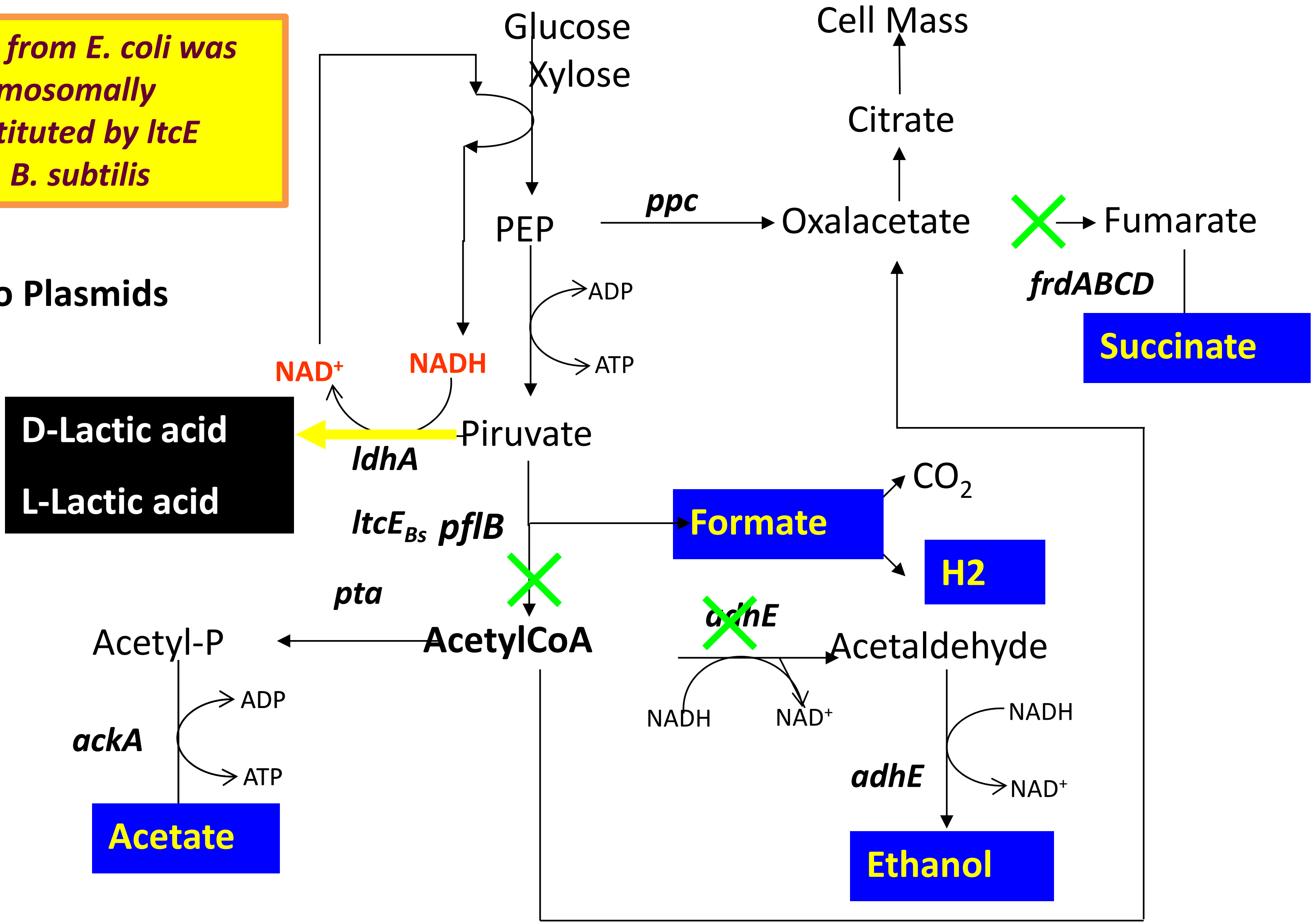
Scale-Up: 1 → 110 Liters



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

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

No Plasmids



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

Lactato
Como materia prima para la Industria Química

Polipropileno: 80 millones ton (2014)

Mercado PLA

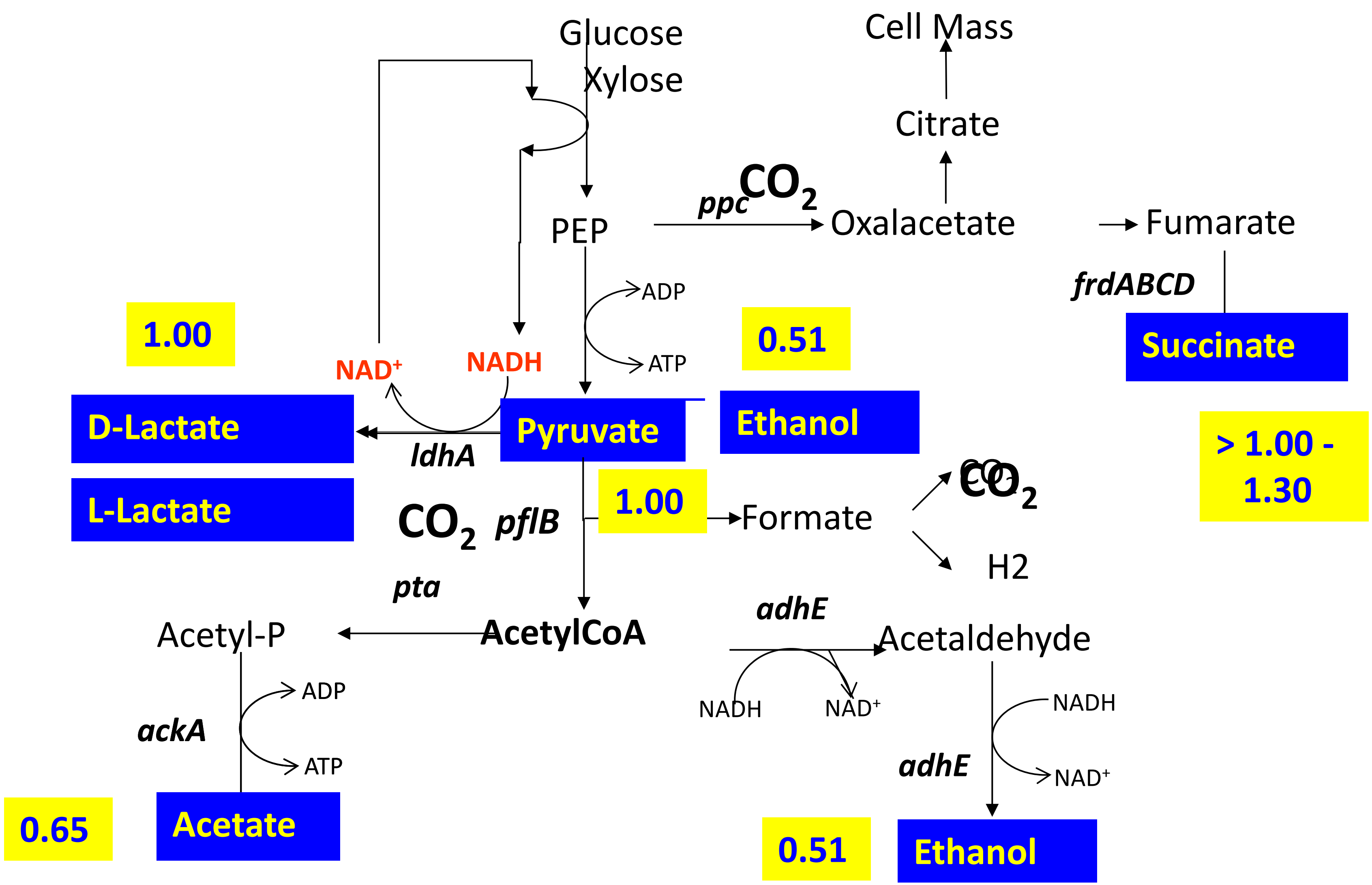
2005 → 220 000 ton

2010 → 500 000 ton

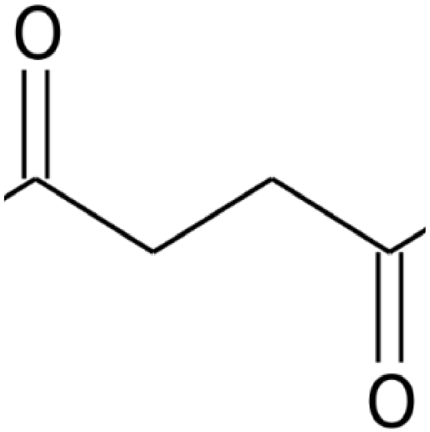
2020 → >1000 000 ton

Chemical Market Report 2001

Fermentation Products *Escherichia coli:* Biochemical Platform: Homo-Fermentative



Ácido Succínico



El ácido succínico → polímeros, surfactantes, solventes, detergentes, saborizantes y fragancias

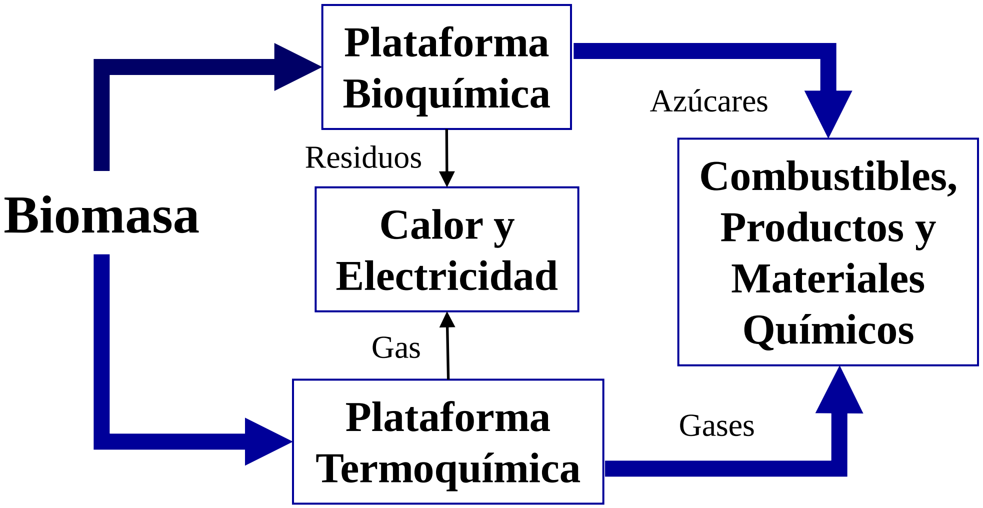
Mercado global potencial de $15 billones de dólares.

Bio-Refinería

Integra los procesos de **conversión de biomasa** para obtener **combustibles, electricidad y productos químicos** (plásticos, resinas, intermediarios, etc.).

Base de una nueva industria cimentada en **materiales** biológicos **biodegradables, renovables, sustentables, menos contaminantes.**

Ácido Succínico
Ácido Láctico
Propanodiol
PHB



Con *E. coli* se ha investigado la producción de

- Etanol
- Butanol
- Propanol
- ~~Metano~~
- Bio-Diesel
- Bio-Gasolina
- Isopentenol
- Bio-Queroseno
- ~~Bio-Electricidad~~

- Plásticos
- Polímeros
- Solventes
- Fenólicos
- ~~Resinas (furfural)~~
- Ac. grasos
- Ac. orgánicos
- Pigmentos
- ~~Detergentes~~

Gracias

- **CONACyT**
- **ERAnet-LAC 2015-2018**

BIOECONOMÍA ARGENTINA 2016 | LA BIOECONOMÍA Y EL TERRITORIO INTELIGENTE

SMIBIO

23 DE NOVIEMBRE 2016

Taller Internacional
Biorrefinerías de Pequeña Escala
SMIBIO WORKSHOP