Biorrefinerías
Aprovechamiento de biomasa vegetal usando microorganismos como biofabricas

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IICA. CoMABio. SADER.
CONVERSATORIO SOBRE BIOECONOMÍA: UNA PERSPECTIVA DE AGRICULTURA SUSTENTABLE Y GIRA TECNOLÓGICA.
Cuernavaca, Mor. 22/Ago/2019

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Ingeniería Metabólica y Biología Sintética de Microorganismos

- Ingeniería metabólica y de bioprocesos, biología sintética y evolución adaptativa de ***Escherichia coli*** (a partir de 2018 con ***Saccharomyces cerevisiae***), para la obtención de productos que sustituyan derivados del petróleo: biocombustibles (etanol carburante, alcoholes de cadena larga, 2,3 butanodiol) y precursores de biopolímeros biodegradables (D y L – lactatos, piruvato, butirato, succinato, R-3-HB).
- Estudios fisiológicos para la producción de biomasa con microalgas oleaginosas.

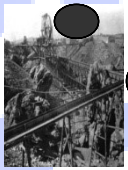
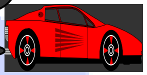
Global Challenge: New fuels & materials are needed to substitute fossil fuels and oil derivatives

Oil

- ✓Depletion (Mexico)
- ✓Price Variability
- ✓Contamination-Spills
- ✓Emissions
- ✓Economic National Support

CO_2 Accumulation
Climatic Change
Global Warming



Oil, natural gas and carbon: Industrial activities, electricity generation, transport, etc.

Non-biodegradable
Product accumulation

Alternative E
Wind
Hydro
Waves
Geothermic
Nuclear
Solar:
Electricity

Liquid Bio-fuels For Transportation
Fuel Ethanol
Biodiesel

Biodegradable Bioplastics & Chemicals

Martínez 2009

Biorefineries: Biofuels and Chemicals from Lignocellulose

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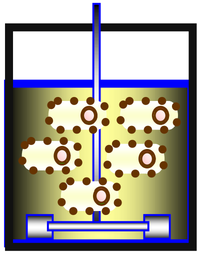
Artificial CO_2 cycle

The Sun

CO_2

Xylose
Glucose
Cellobiose
Mannose
Galactose

Chemicals
BioFuels



Agricultural Residues
Bagasses and Stovers

Sugars

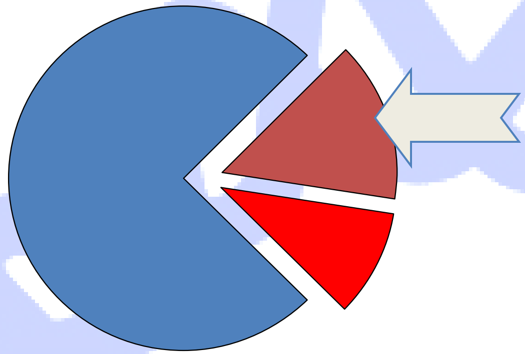
Hydrolysis

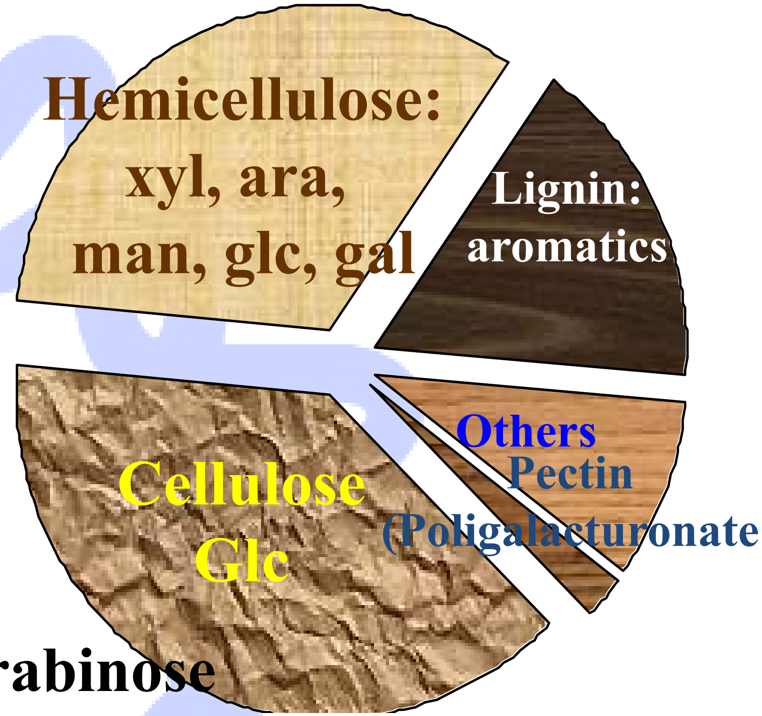
Fermentation

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

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





Lignocellulose Hydrolysates
Contain high amount of
Pentoses, mainly Xylose & some Arabinose
Hexoses, mainly glucose
and Glucuronate, Acetate and Furans

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

Ingram et al., 1999, 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!

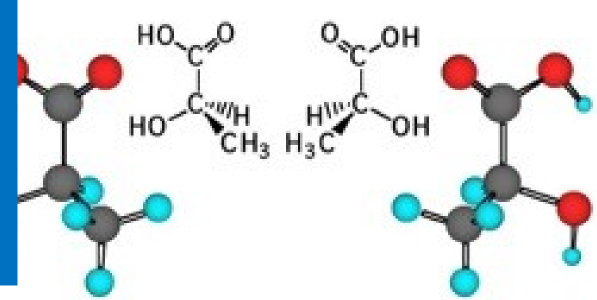
vs Petróleo

Costos

Bio-Plásticos Son 3 R

Bio-Etanol

BIODEGRADABLE
AMIGABLE CON EL MA
RENOVABLE
H_2O



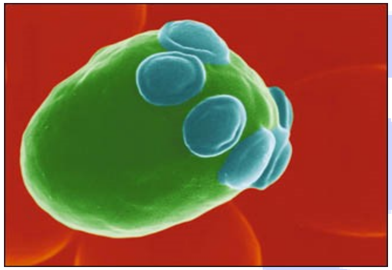
Martínez 2009

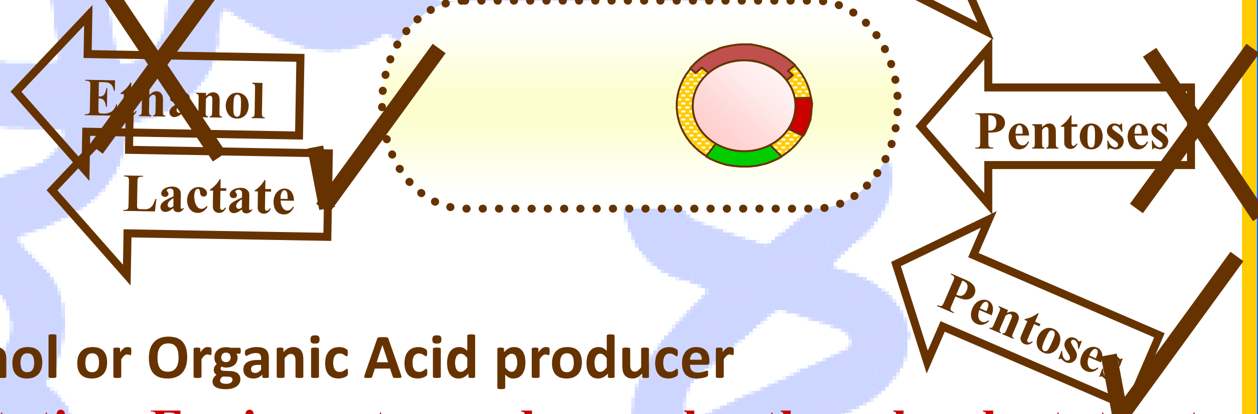
Metabolic Pathway Engineering: XYLOSE

A: Ethanol or Organic Acid producer

Increase substrate range. Engineer to use pentoses

Saccharomyces cerevisiae and Lactobacillus

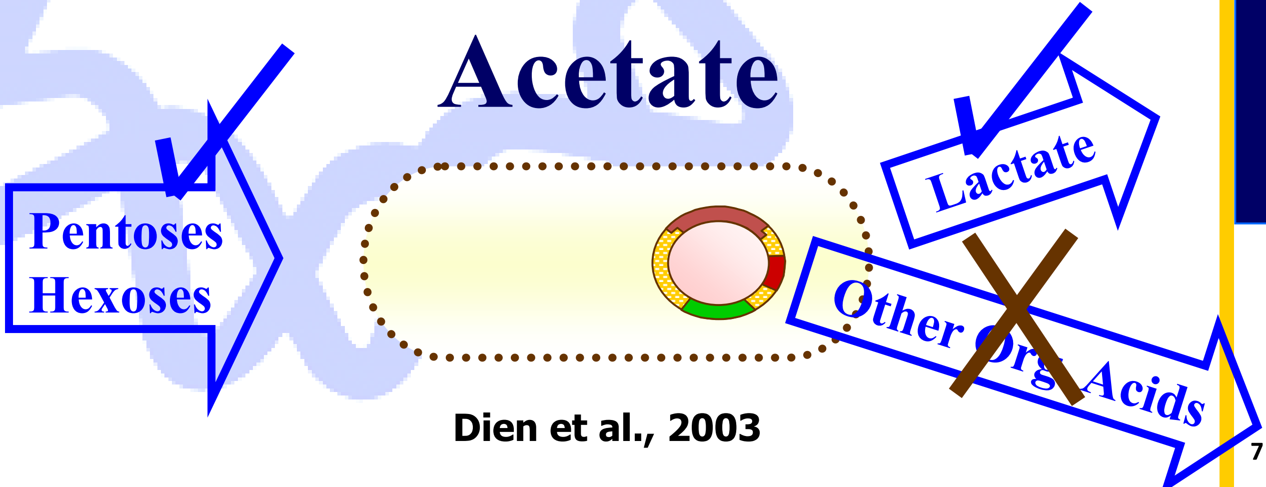




B: NO good Ethanol or Organic Acid producer

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

Escherichia coli



Dien et al., 2003

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

E. coli: **Bacteria**

- **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 1,3-Propanediol and 1,4-BDO**
- **Easy to manipulate & cultivate**

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

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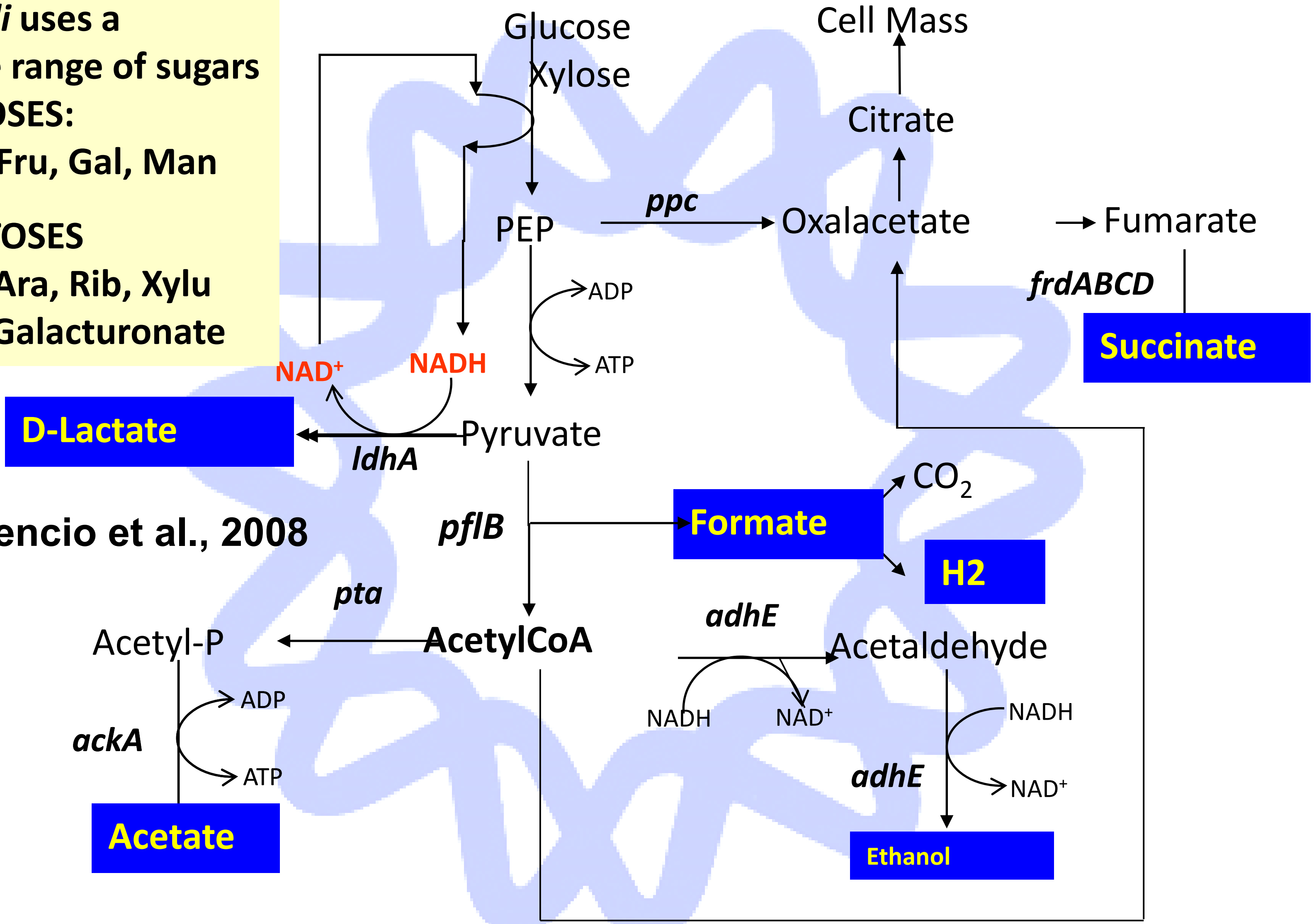
Fermentation Products *Escherichia coli*

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

Sugar Cane Bagasse Hemicellulosic Hydrolysate

60 g/L total sugars, acetic acid

Aprox. 1 g/L Cells

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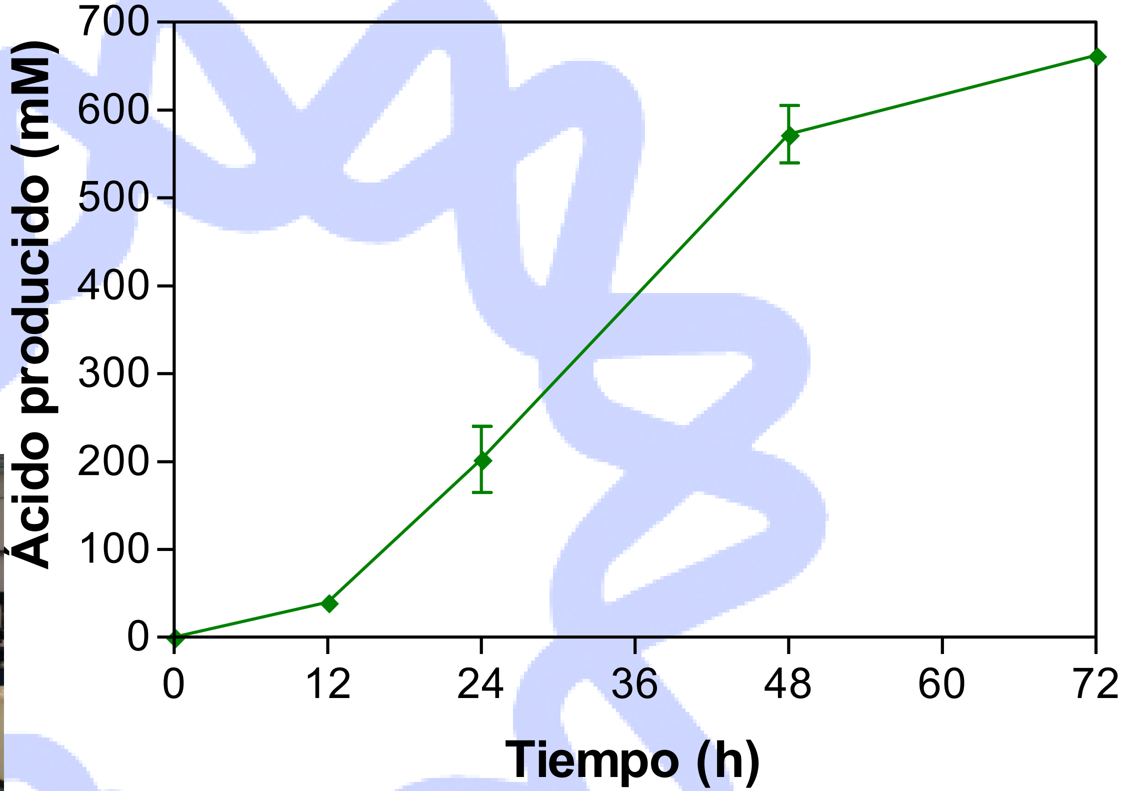
Hydrolysate +
0.9 g/L $(NH_4)_2HPO_4$ – $NH_4H_2PO_4$
1 mM Betaine
0.1 g/L Citric acid



Utrilla et al., Bioresorce Technol. 2017

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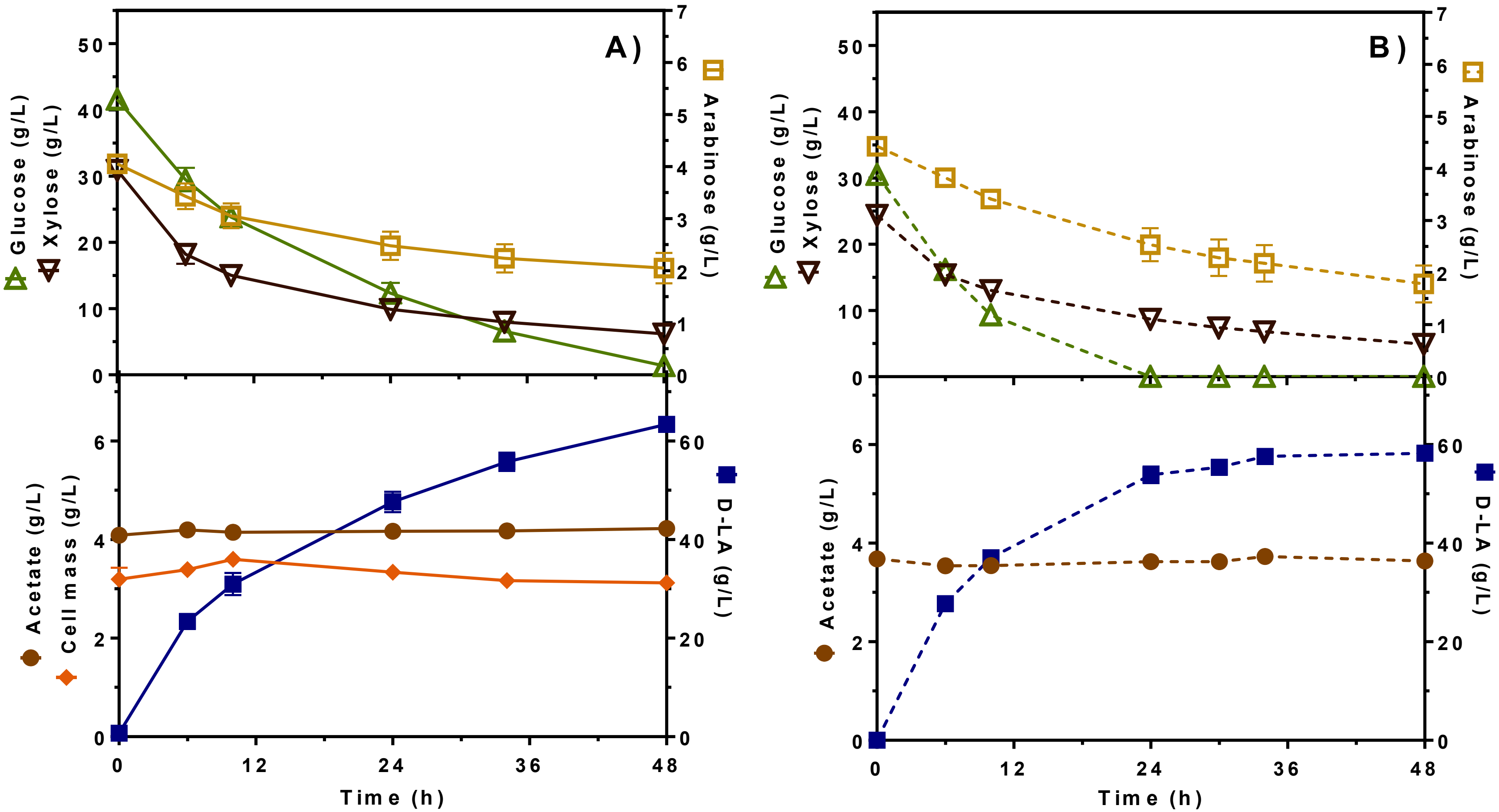
Stover from White Corn

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

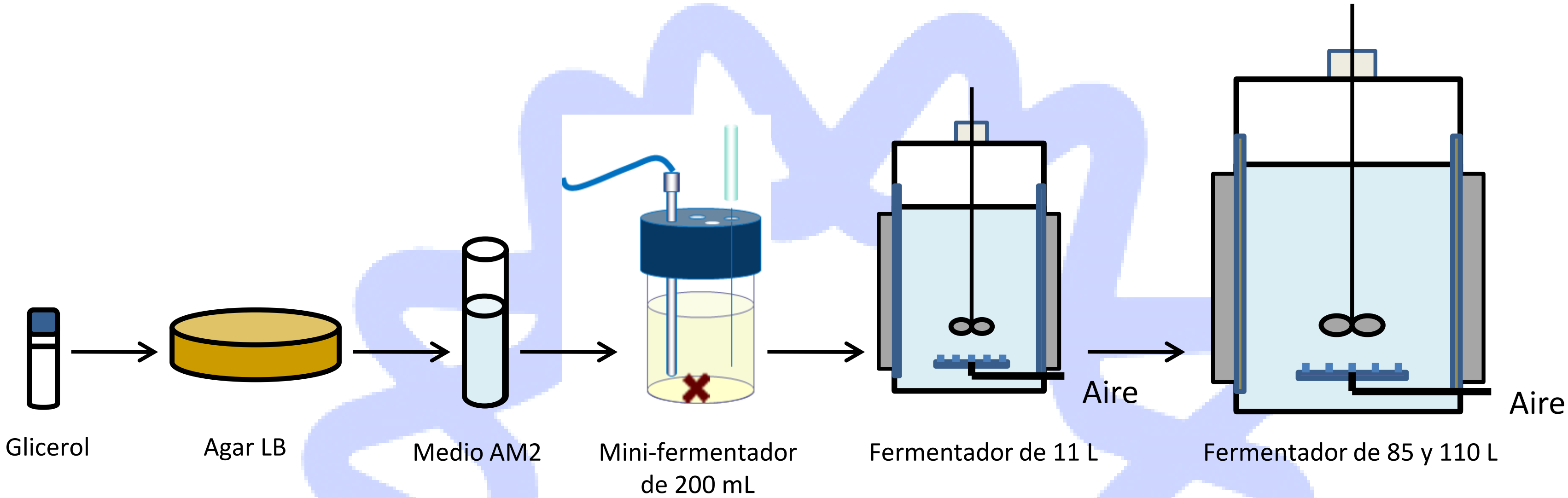
AV03: JU15 Δ*poxB*, Δ*ackA-pta*, Δ*mgsA*

Simultaneous sugar consumption

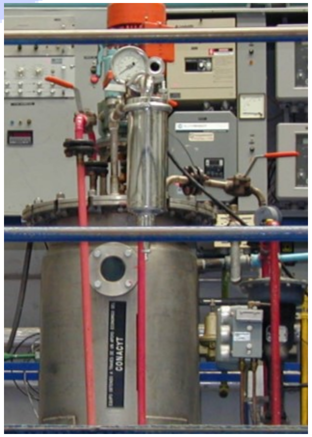
Y > 1 !!!

Utrilla et al. Bioresource Technol. 2016

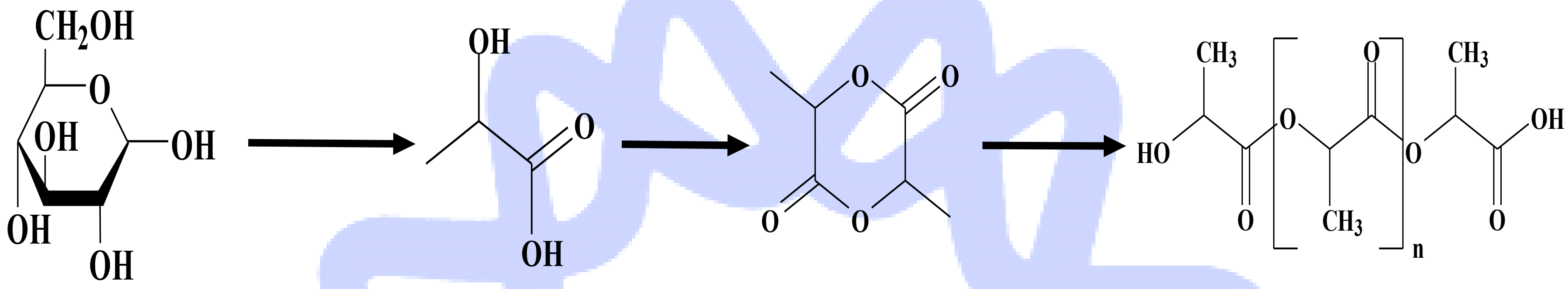
Laboratory Scale-Up: 1, 11, 130 L



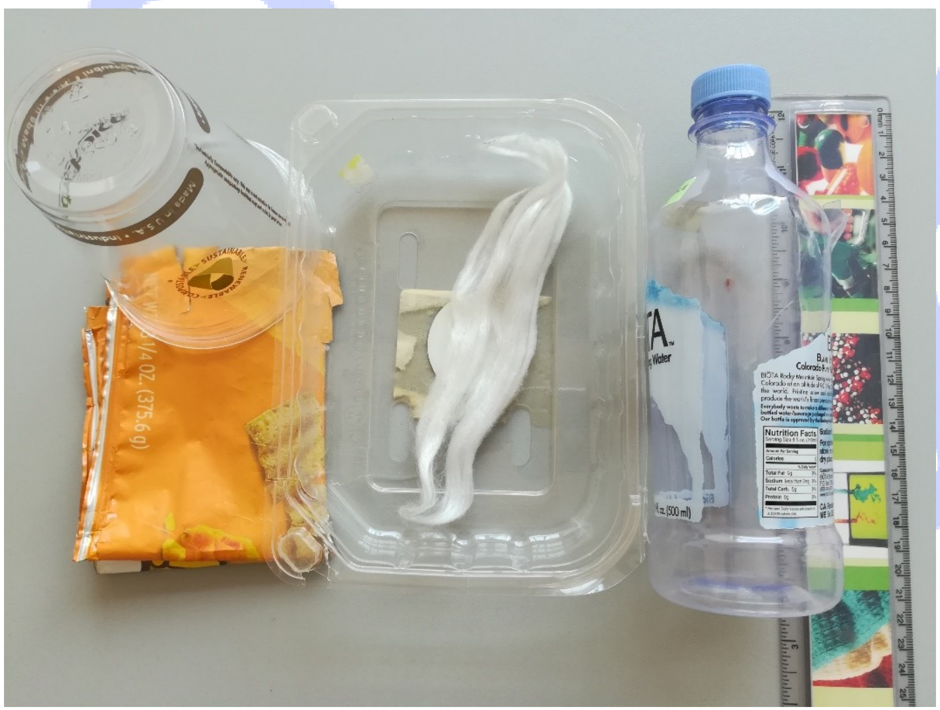
Sierra et al., Submitted. March 2019



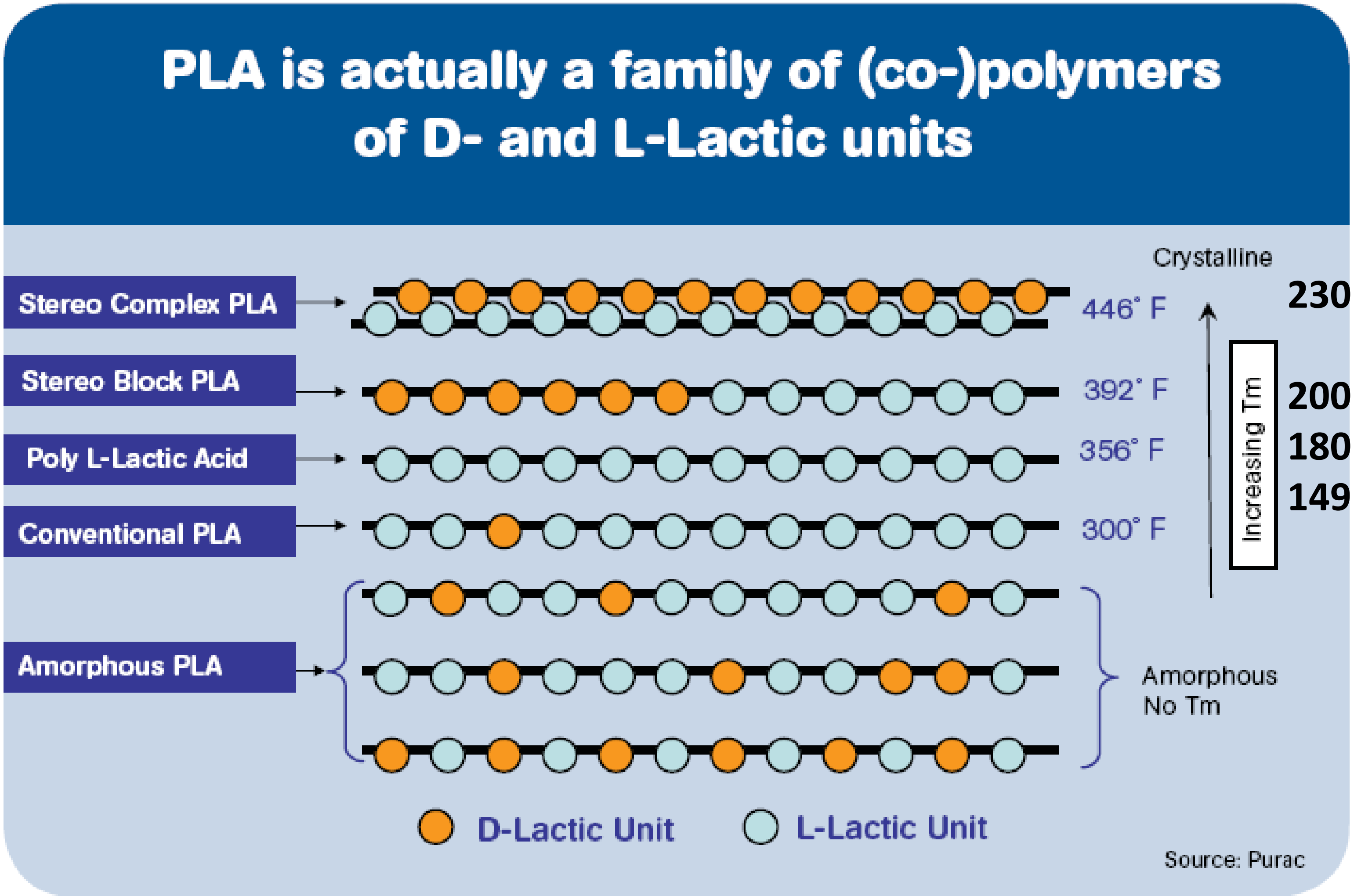
L - Lactato Ópticamente Puro



Glucosa Láctico ¡¡¡¡¡Dímero!!!!! ¡¡¡¡¡PLLA!!!!!



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.

Second Generation Bio-Plastics

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Artificial CO_2 cycle

CO_2

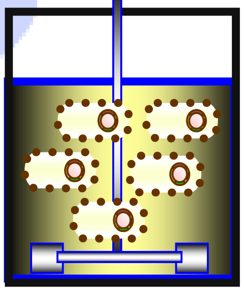
The Sun

1 kg of Sugar Yield 1 kg of Lactic Acid

- **1 USD / kg; PLLA: > 4 USA dol/kg**
- **Demand 2020 > 1000,000 ton**

Lactate

Xylose, Cellobiose Glucose, etc. Cellulose, Hemicellulose



Lignocellulose - Biomass

Agricultural Residues

Sugar Cane Bagasse

Hydrolysis

Fermentation

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

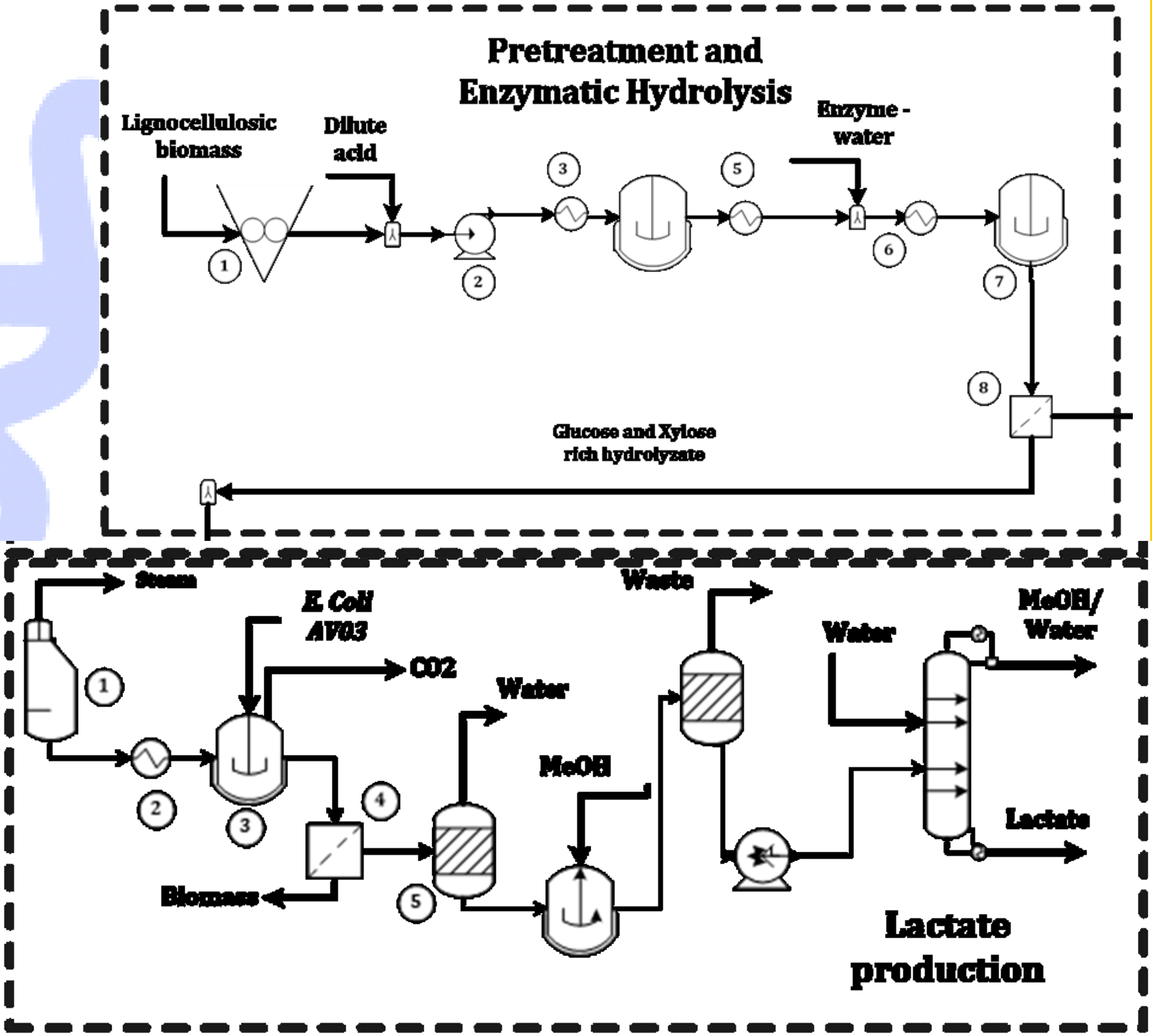
LA production from corn stover with ethanologenic bacteria

Process Integration

Lab scale: ~ 520 kg_{LA}/Ton

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



Economic Analysis

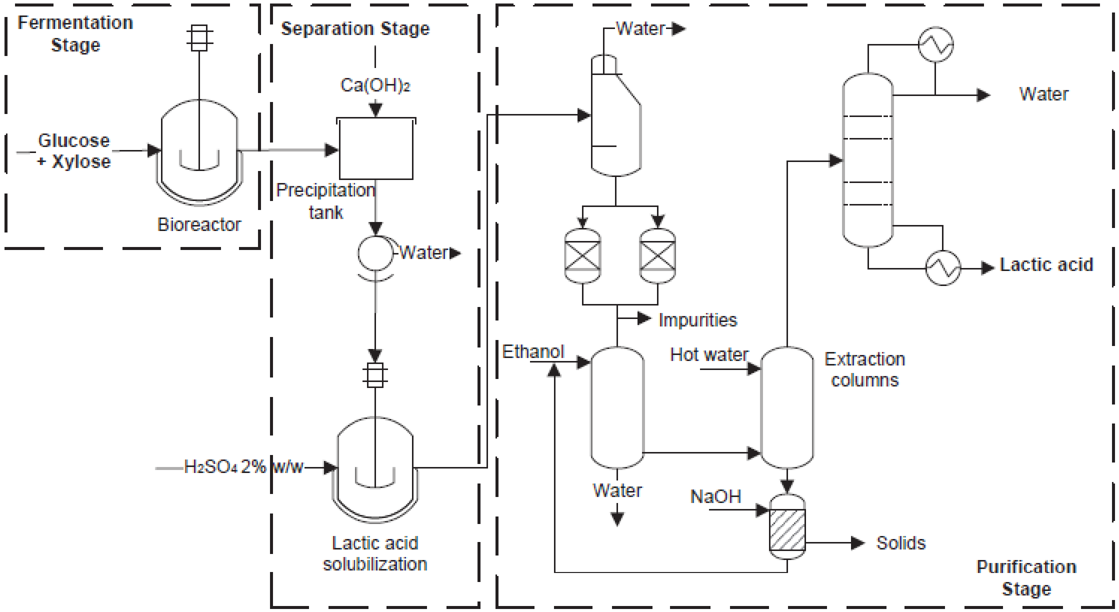


Fig. 1. The lactic acid production process.

Parra, Martinez, Cardona. Biores. Technol. 2019, 273:86-92.

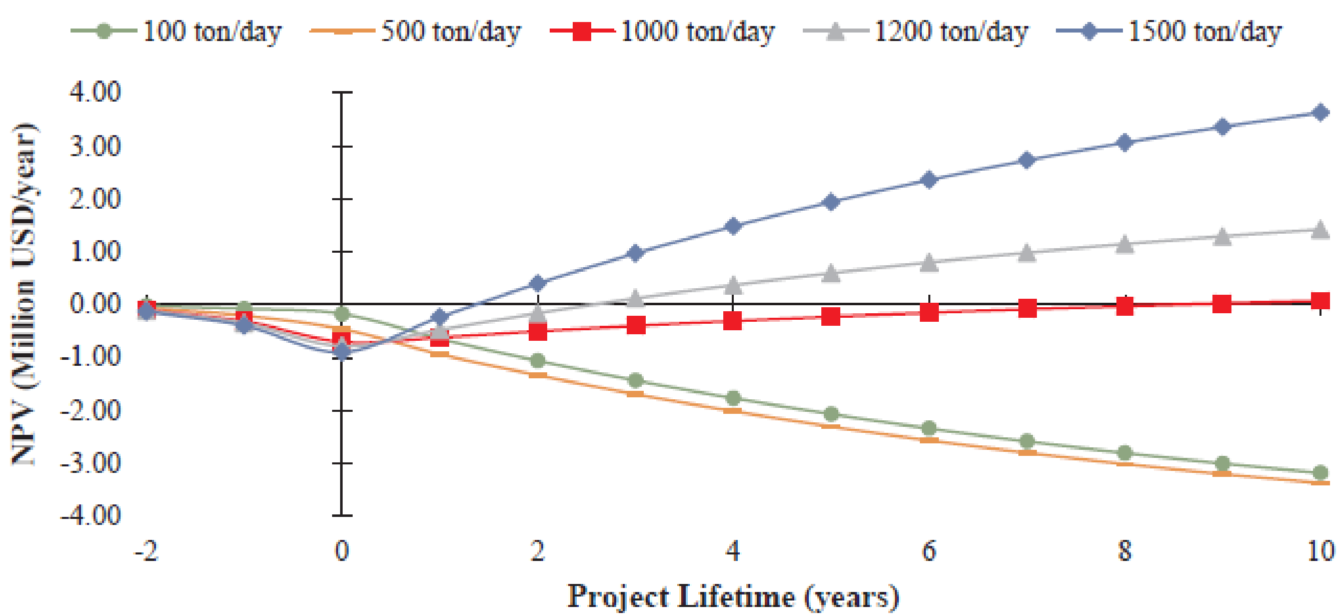


Fig. 4. NPV over project lifetime at different scales.

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

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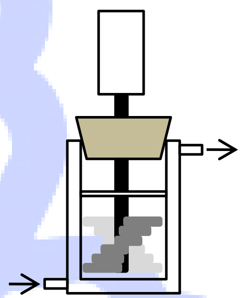
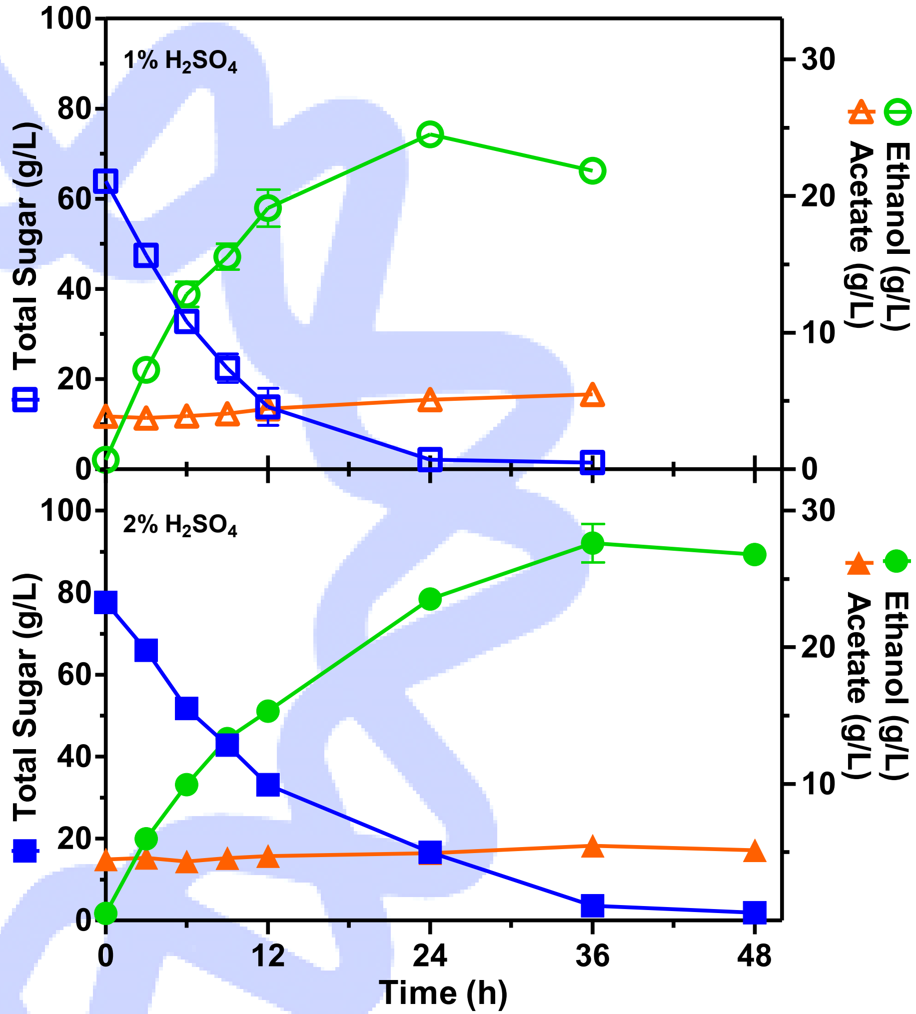
1 kg of Sugar Yields 0.51 kg of EtOH ~ 0.5 USD / L

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

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Bagazo de Agave

Adición de Ácido

Tratamiento Termoquímico

Celulosa Pretratada

Etanol Carburante

Integración del Proceso en el IBt - UNAM

330 L_{EtOH}/Ton Bagazo de Agave Seco

Jarabe de Pentosas

Hidrólisis Enzimática

Fermentación

Destilación

Secado

L. Caspeta Guadarrama 2014

Lactic acid & Ethanol: Scale-up

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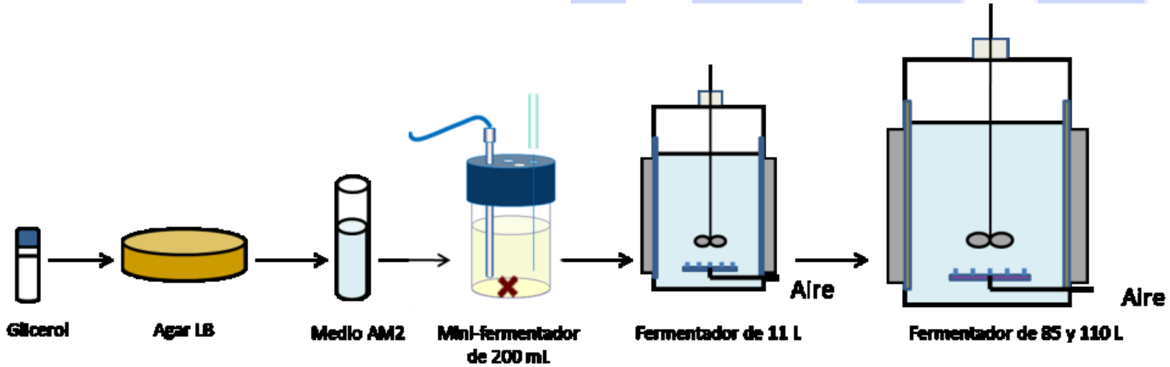
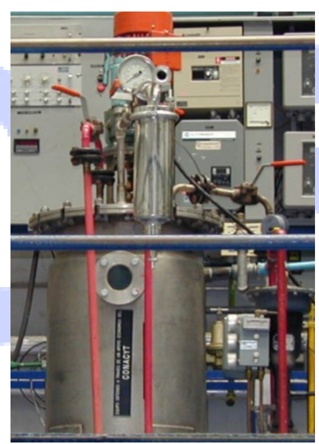
k_La Scale-up
Corn stover hydrolysates
0.2 → 400 L

Yield: > 83%
Productivity >1 (0.5) g/L/h



Fernández-Sandoval et al. J. Chem Technol Biotechnol. 2017



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Economic Analysis

Parra, Martinez, Cardona. BEJ 2018, 140:123–129

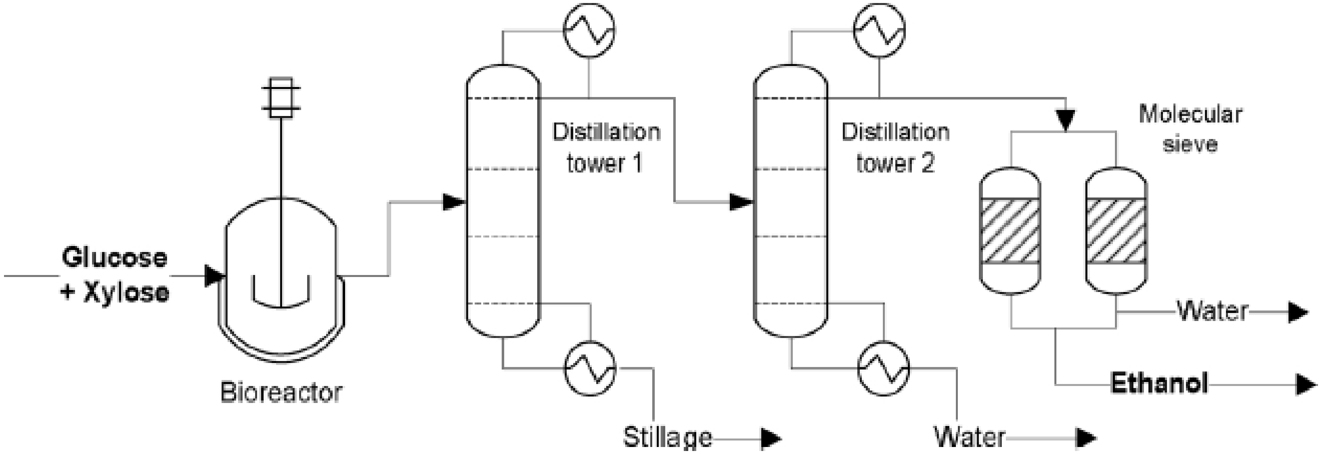

Fig. 1. The production process of ethanol.

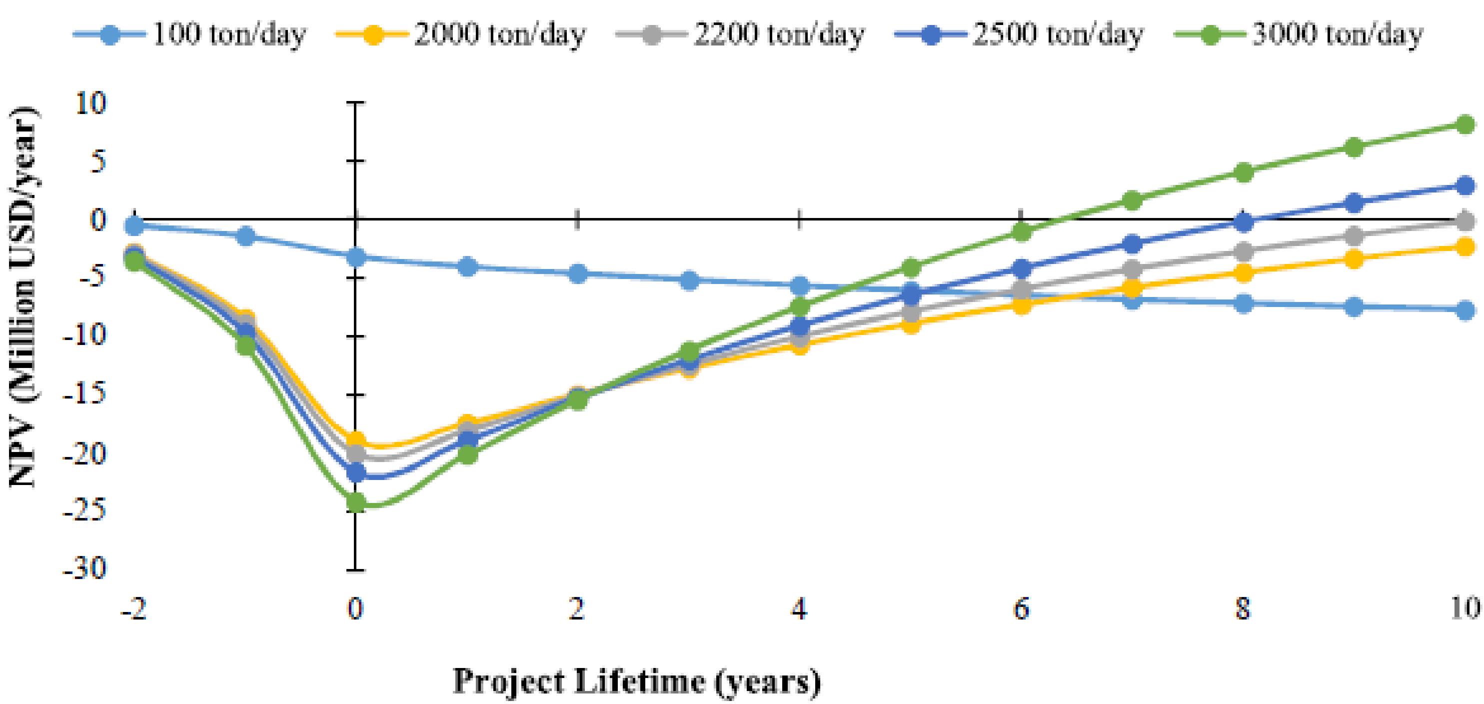

Fig. 7. Net present value of the process at different scales.

Pyruvic acid: Strain JU15 Δ*ldhA*: *MS01*

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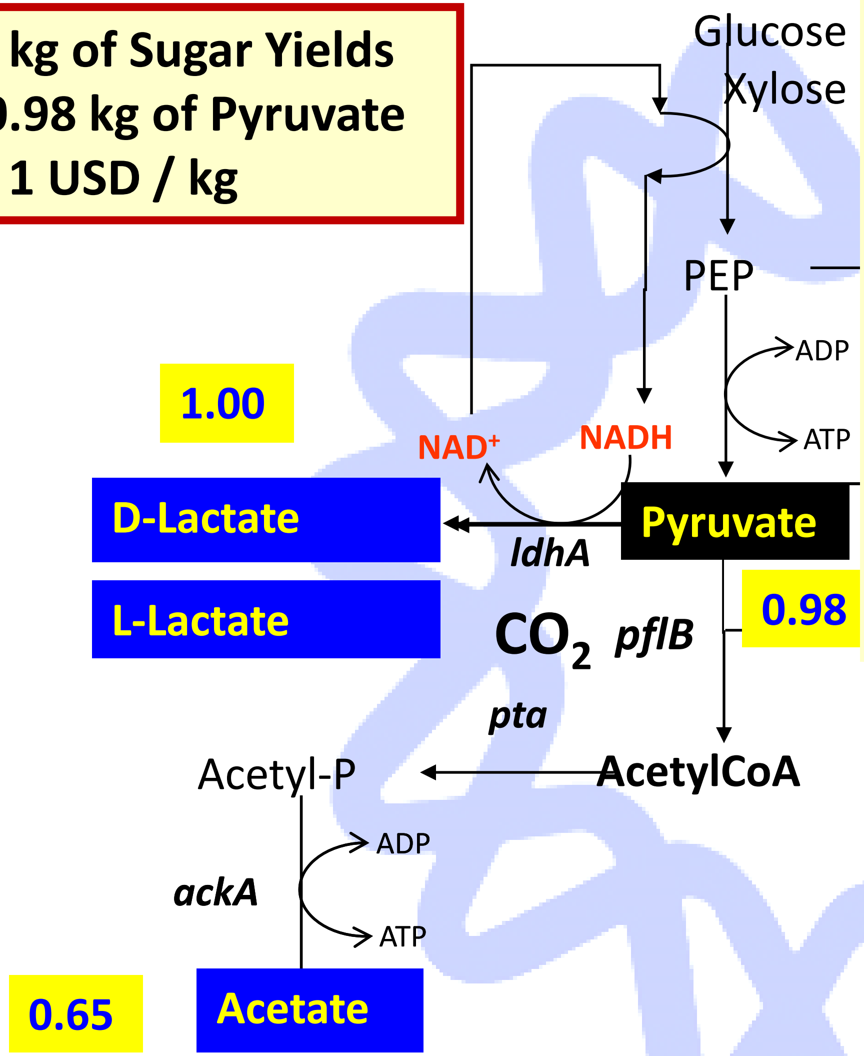
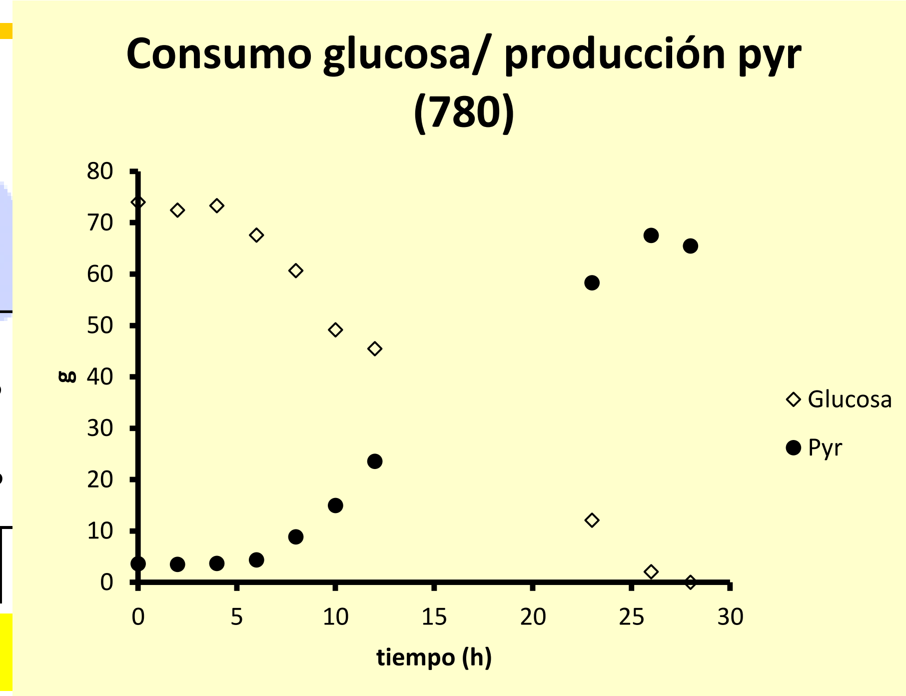
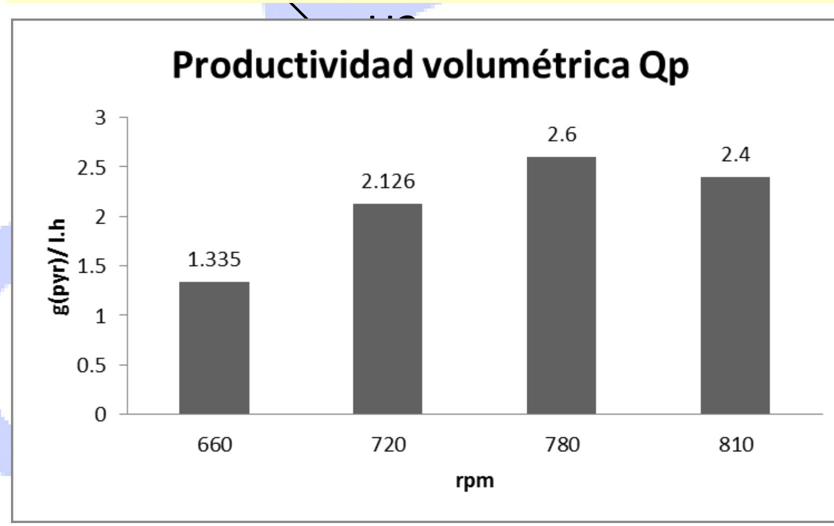
1 kg of Sugar Yields
0.98 kg of Pyruvate
> 1 USD / kg







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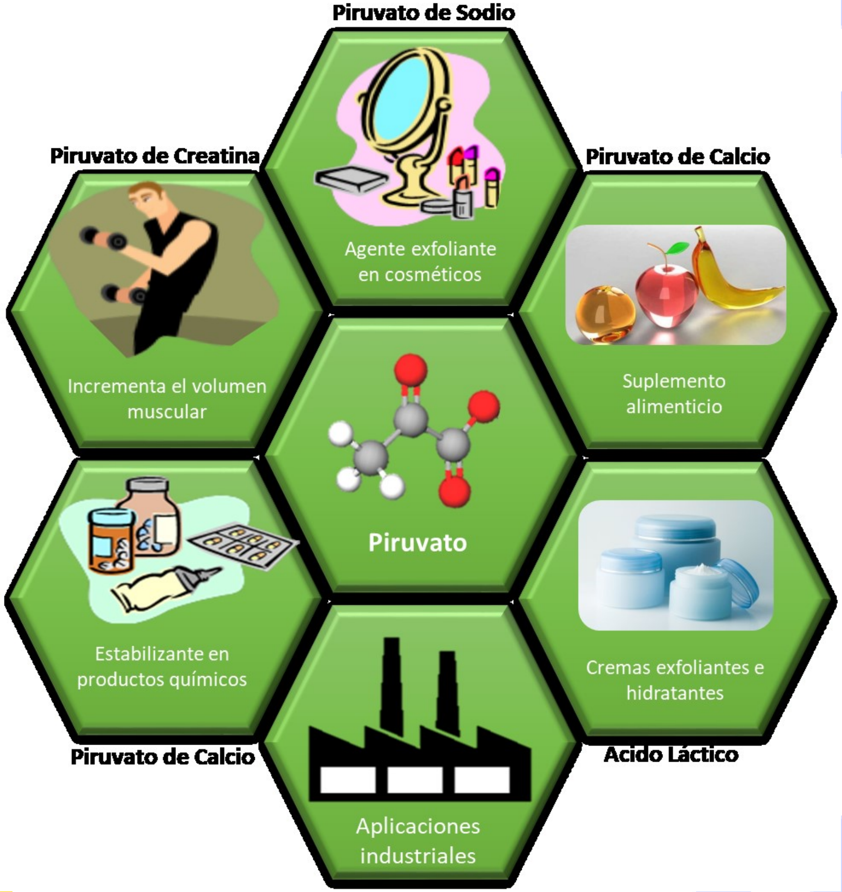
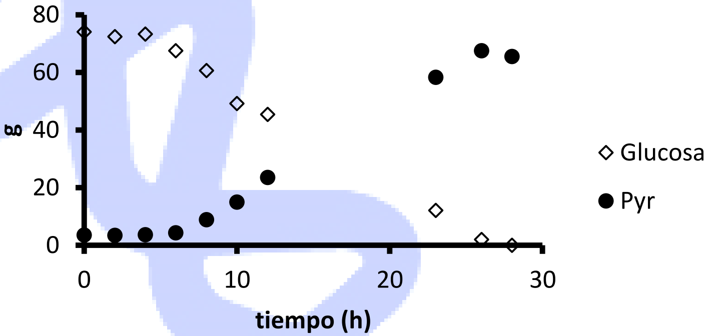
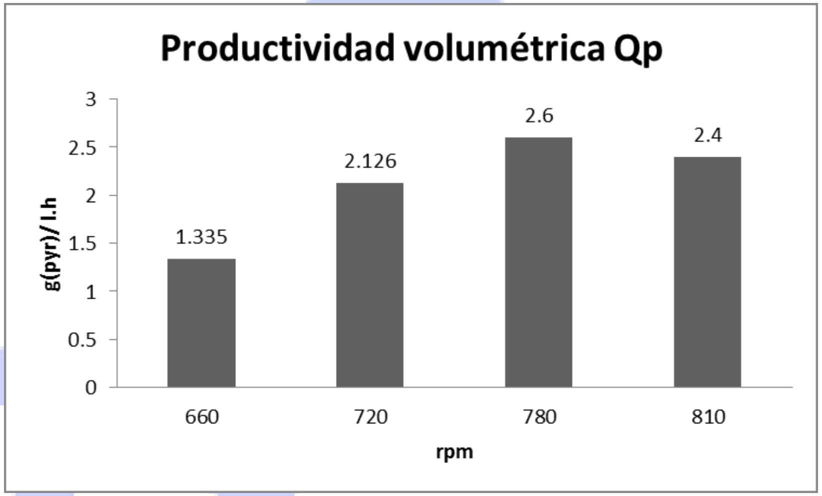
Pyruvic acid. Strain JU15 *ΔldhA*

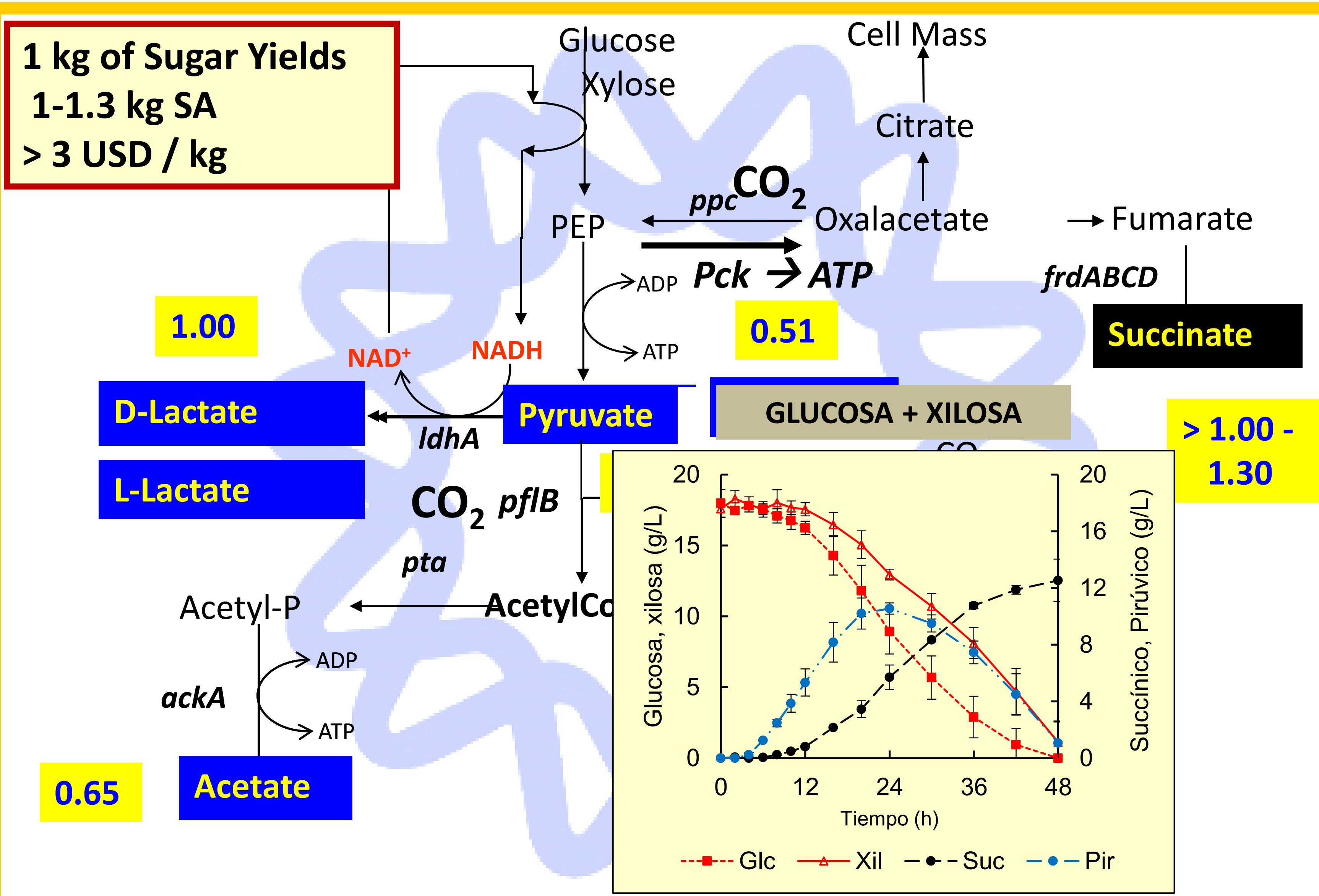


Consumo glucosa/ producción pyr (780)

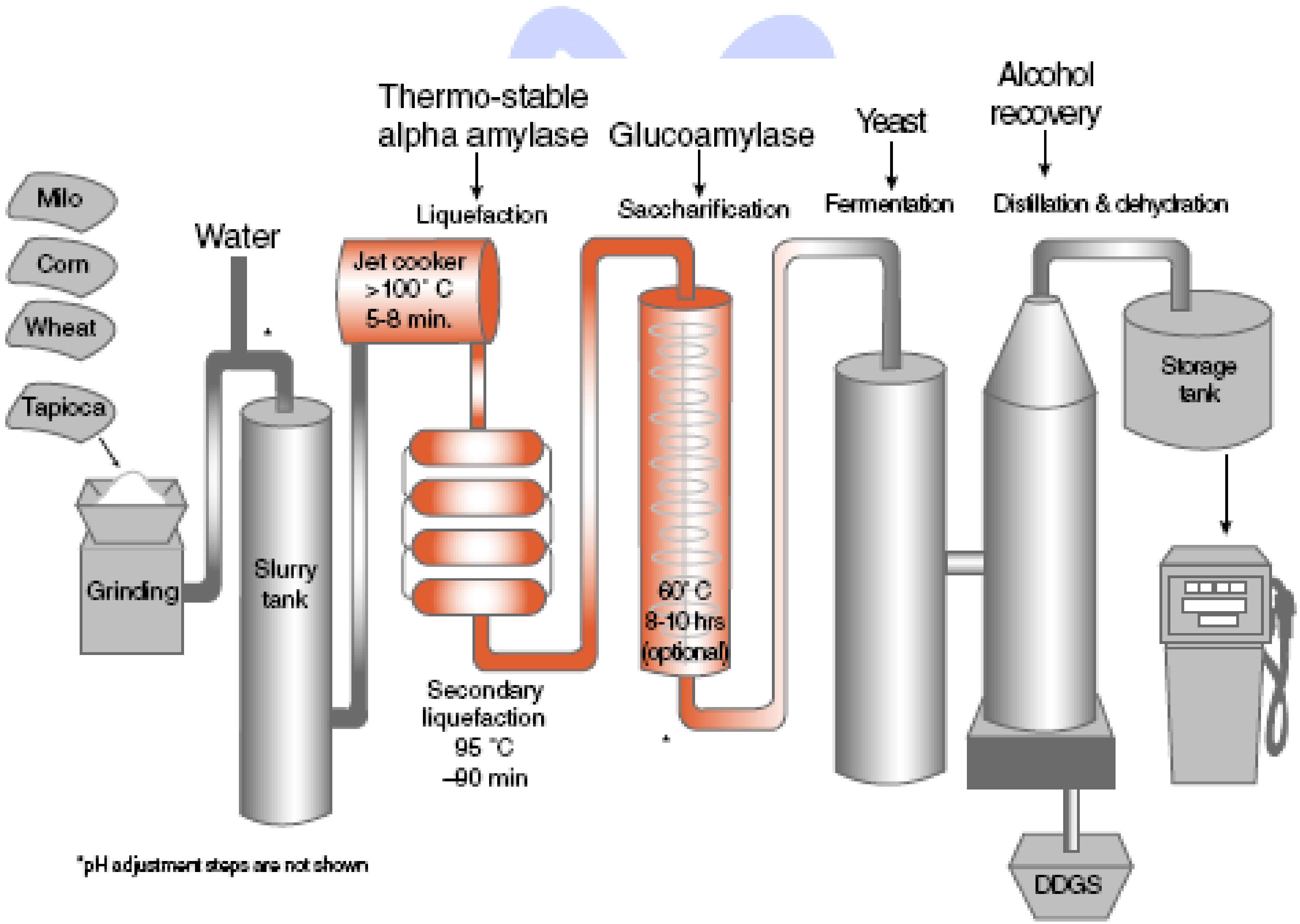




Succinic acid: Strain JU15 ΔldhA frdA Ptrc pck Δppc



Proceso maduro para la producción de etanol a partir de granos



Bio-Refinería: Caña de Azúcar

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