Fuel Ethanol from Sugar Cane Bagasse

Alfredo Martínez Jiménez

Dpto. Ingeniería Celular y Biocatálisis
Instituto de Biotecnología – UNAM

alfredo@ibt.unam.mx
http://pbr322.ibt.unam.mx/~alfredo/

Competence Platform on Energy Crop and Agroforestry Systems for Arid And Semi-Arid Ecosystems

Puente de Ixtla, Morelos.
Wednesday 4, 2009

Outline of the Presentation

- Motivation and the Mexican perspective
- Lignocellulose – Biomass
- 2ng Generation Fuel Ethanol
- Carbon metabolism studies in ethanologenic *Escherichia coli*
- Biomass Sources and Potential
- Final Remarks
- Acknowledgements
- Questions

New fuels are needed to substitute oil derivatives and fossil fuels

Oil

- ✓Depletion (Mexico ~2020)
- ✓Price Increase
- ✓Contamination-Spills
- ✓Emissions
- ✓Economic National Support

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

Alternative Renewable E

Wind
Hydro
Waves
Geothermic

Solar

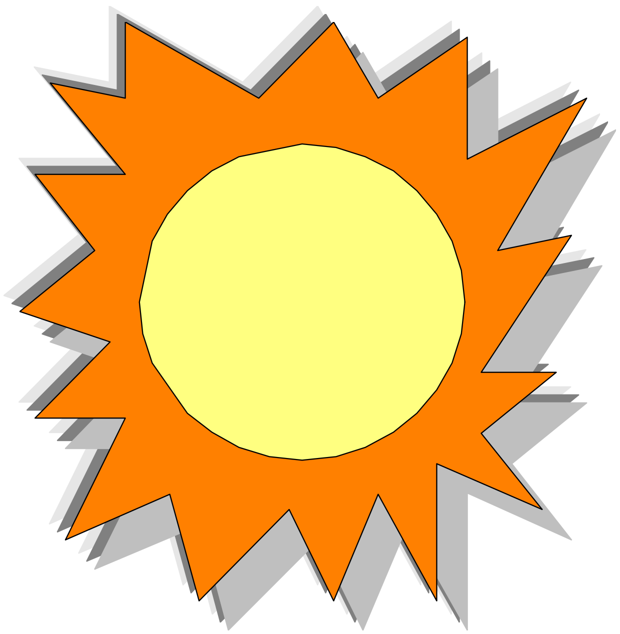
Electricity

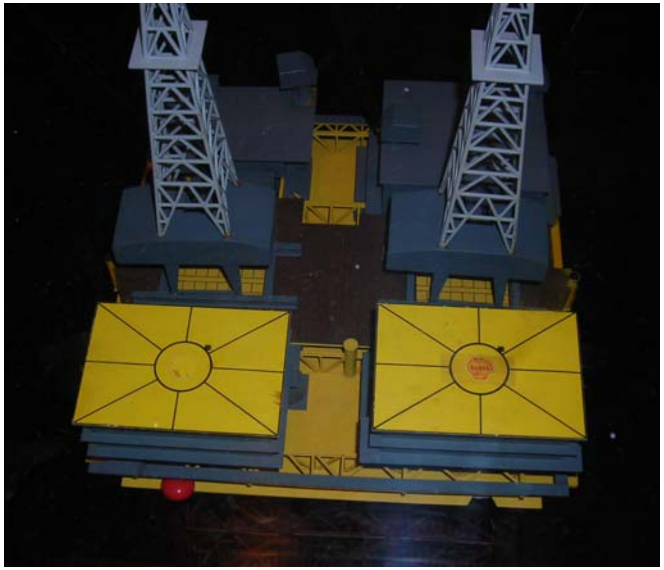
Biomass: Solid Bio-fuel

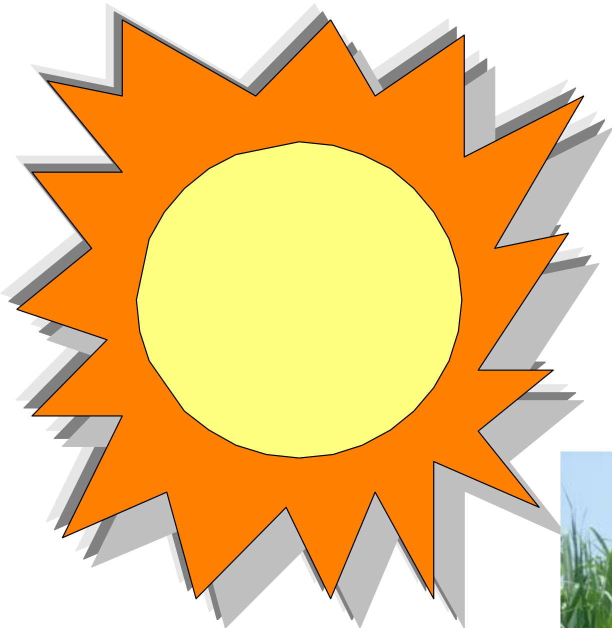
Liquid Bio-fuels
For Transportation
Fuel Ethanol

Sun → Biomass → Fossil Fuels

~ 100 Million Years







Sun → Biomass → Bio-Fuels

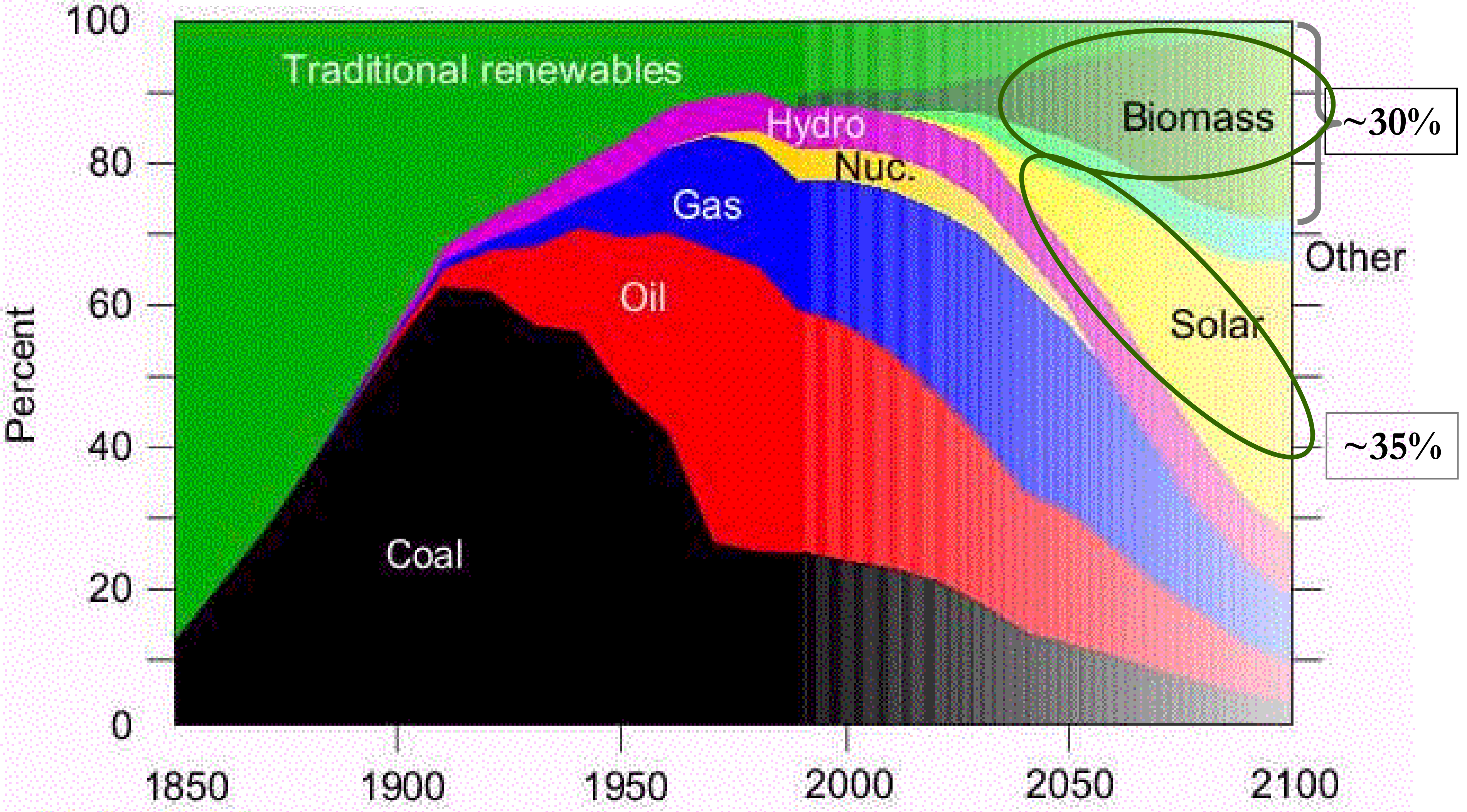
!~ or < 1 Year!

CH_3CH_2OH

BioEtanol

Energy Consumption

Mexico 2004: 8%
Sugar Cane Bagasse
Timber

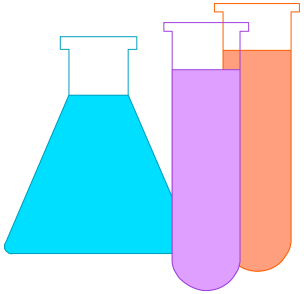


Fuel Ethanol Production

Brazil, expertise 30 years
More than 15,000 million L/year (41 million L/day)

Sucrose syrups from Sugar Cane + Yeast → Ethanol
40% car use E95. Others E22. Ford, GM, WV, Honda, etc.

Saccharomyces cerevisiae



Mature Technology

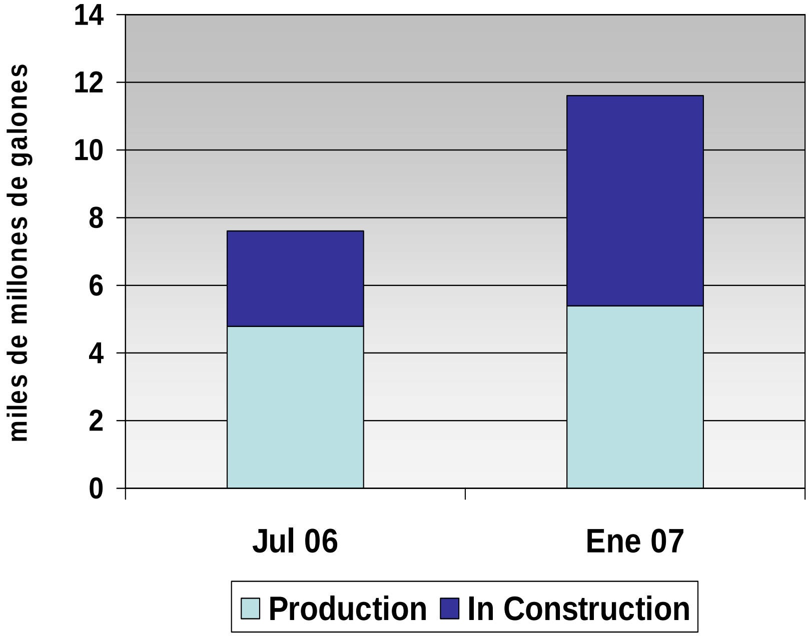
Fuel Ethanol Production

USA: expertise 20 years
2006: More than 41 million L/day (more than Brazil)

Corn Starch + Amylases → Glucose
Glucose + Yeast → Ethanol
E10 any car: 2% gasoline consumption EUA

Mature Technology

1st Generation Fuel Ethanol
USA and Brazil

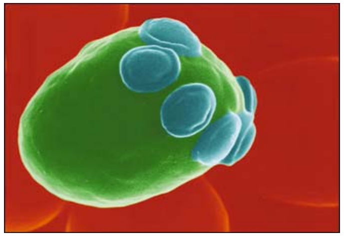


Year	Area (Mha)	Alcohol (Mm^3)
2005	5.50	15.60
2015	7.92	45.00
2025	8.64	78.00

Colombia

~2 years expertise
1 million liters/day

Sucrose syrups from Sugar Cane + Yeast → Ethanol



What about Mexico?

Mexican Scenario: Fuel Ethanol

- Mexican demonstrated oil reserves will be depleted in 9 years
- 33% of the Mexican federal income is based in oil commercialization.

Gasoline Price

8 - 10 pesos/L

Glucose → 2 Ethanol + 2 CO_2

Sucrose → 4 Ethanol + 4 CO_2

Theoretical Yield: 0.51

$g_{ETHANOL}$ / $g_{(GLC\ or\ SUC)}$

0.64 L_{EtOH}/kg

Substrate	Bulk Price in Mexico (pesos/kg)	Et-OH Prod. Cost Substrate (pesos/L)
Glucose	→	8-11
Sucrose	→	8-11

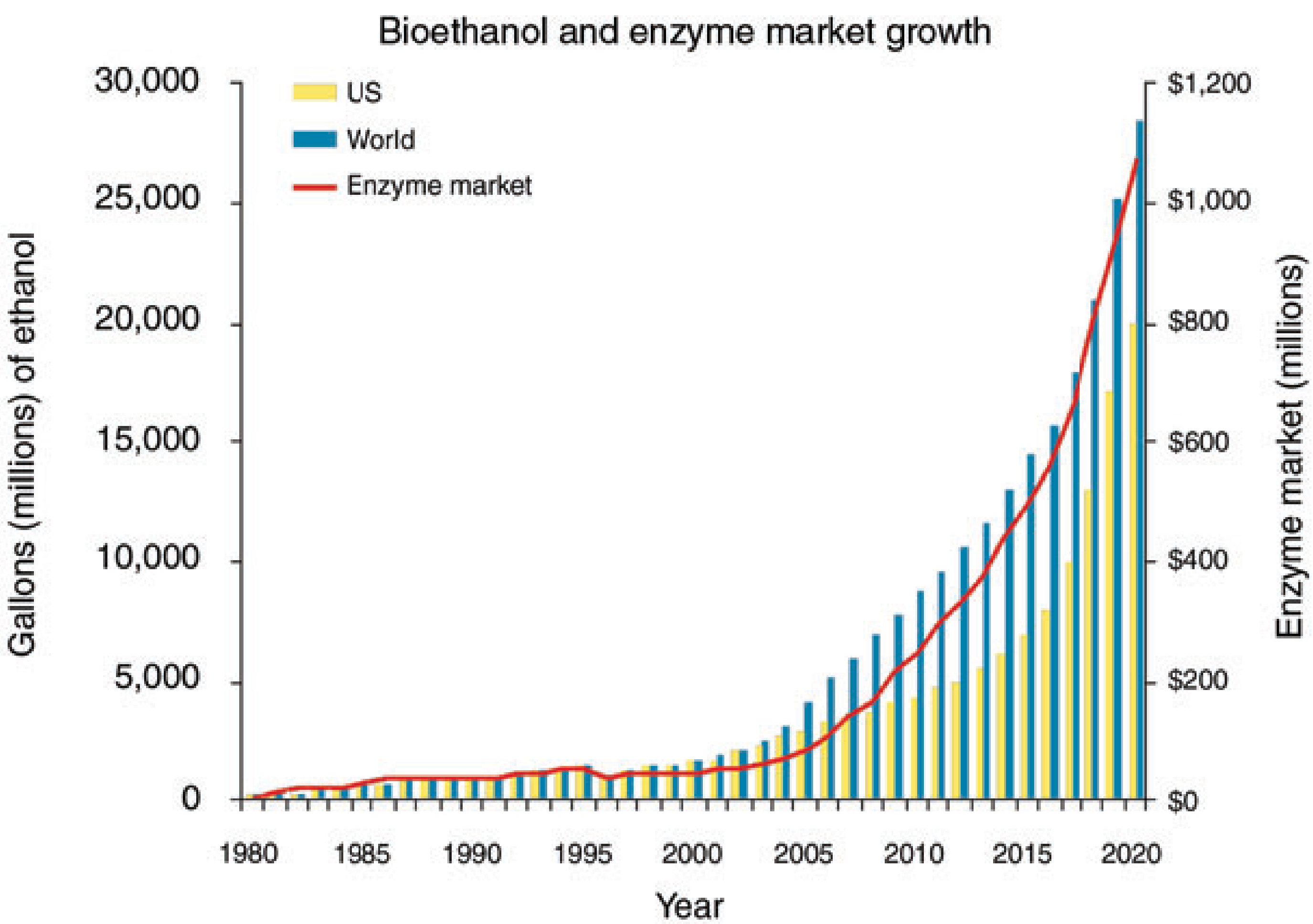
Glucose, Corn Imports: 8 x 10^6 metric tons & Corn Yield: 2-4 ton/ha *vs* 12-14 ton/ha
Sucrose, small surplus – small market & high sugar cane price (3 times the price of Brazil)

Vision 20 years

- Given that in 20 years the main form of transportation fuel will still be liquid fuel, the technological vision for 2020-30 is that bio-fuels will evolve to those that will integrate seamlessly into current transportation refining to end use fuel systems: Bio-ethanol, bio-diesel and other liquid and gaseous bio-fuels need to be produced

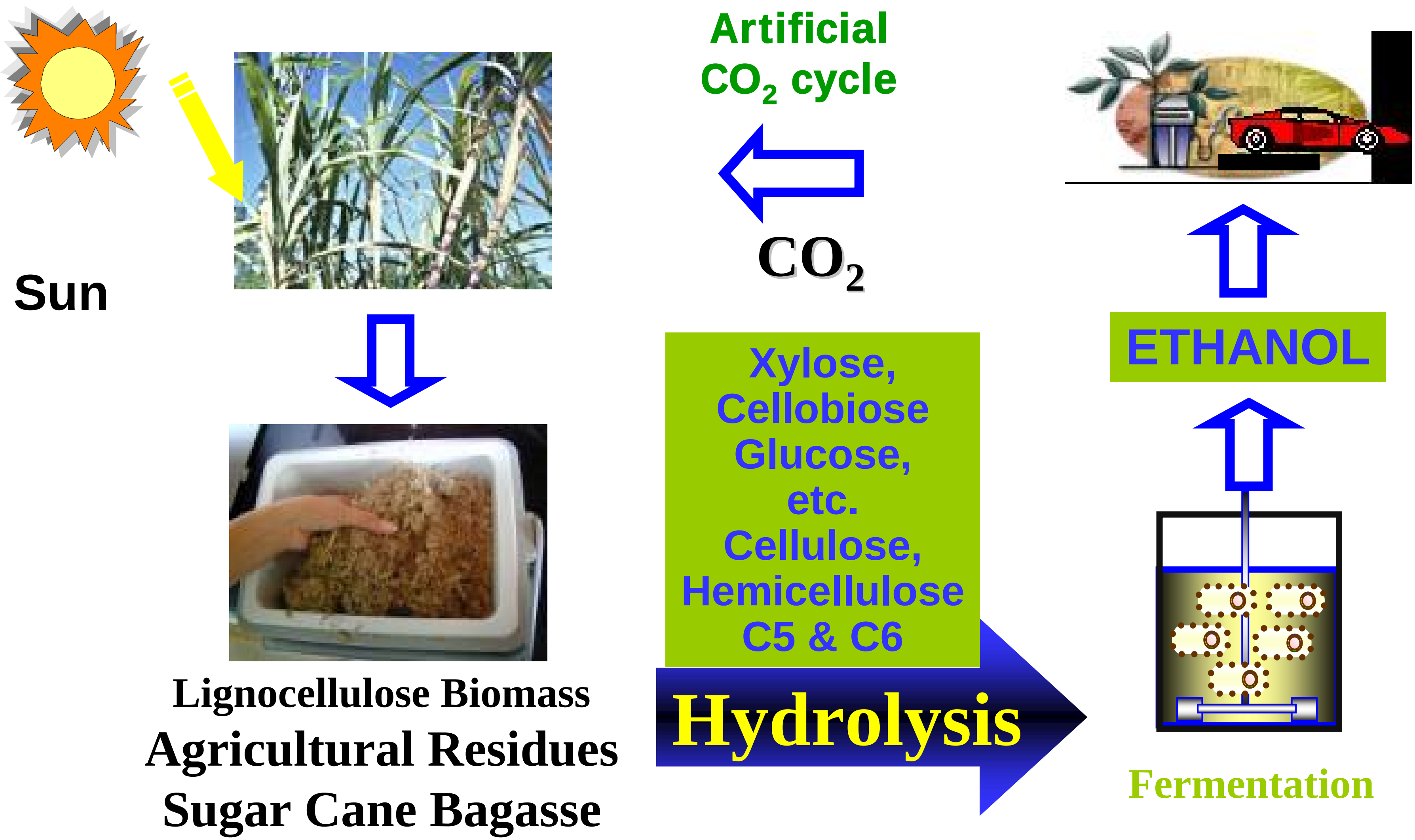
- **Look for solutions: Biotechnology**

Bio-Ethanol Market



Schubert. Nature: 2006

Second Generation Fuel Ethanol

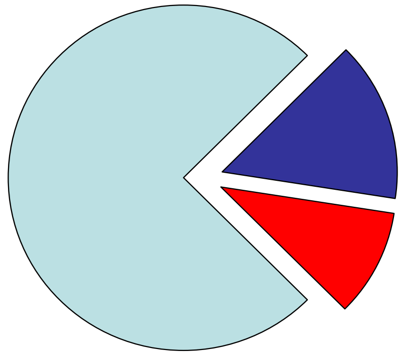


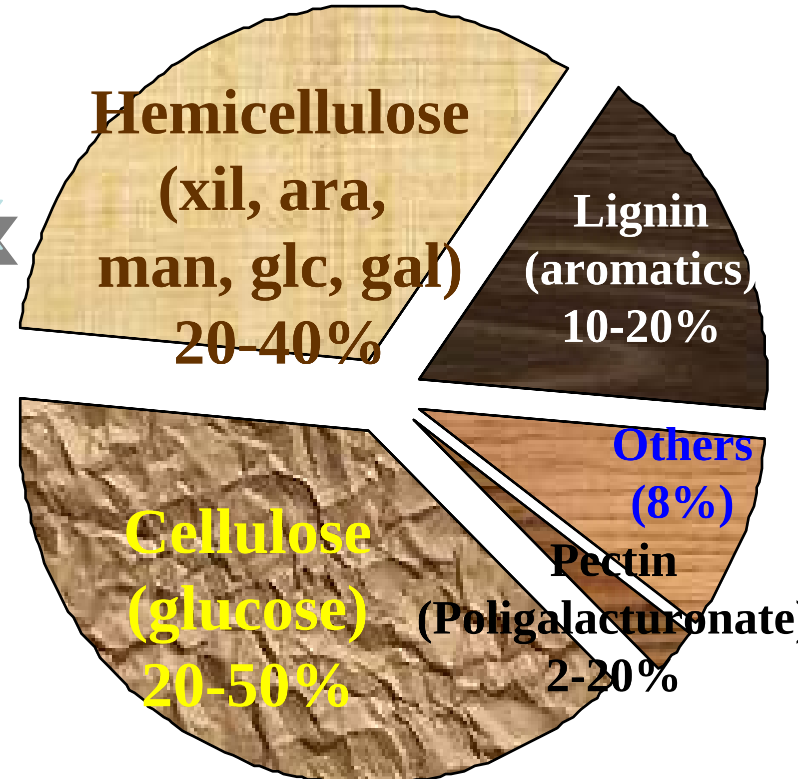
Purpose: Design microorganism and process to transform Lignocellulose (cellulose & hemicellulose: pentoses, hexoses, disaccharides) to ethanol

Martinez et al. 2006


CH3CH2OH
E10
SIN PLOMO
Con 10%
ETANOL
300ml

Lignocellulose
Sugar Cane Bagasse





Sugars in Sugar Cane Bagasse Hemicellulose Hydrolysates (g/L)

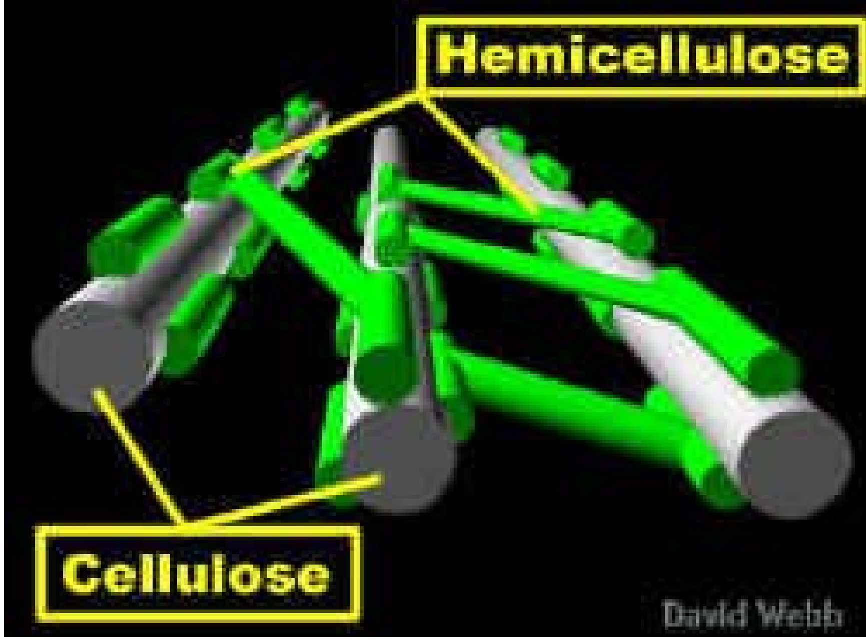
Xylose:	**80**
Arabinose:	**5**
Glucose:	**15**

Pentoses 85% + Hexoses 15%
(Xil + Ara) + (Gluc+Man+Gal)
+ Acetate + Glucuronic Acid

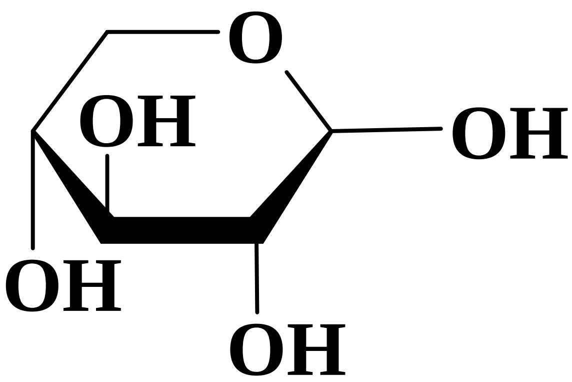
Martinez et al. 2000

Ingram et al., 1998

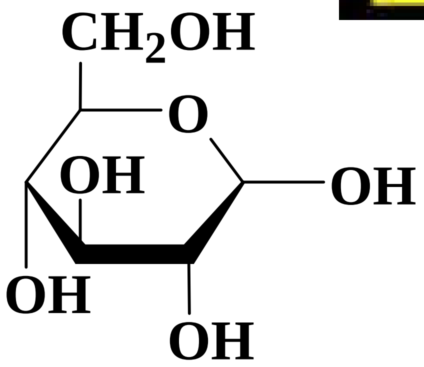
Biomass – Lignocellulose
Future Raw Material



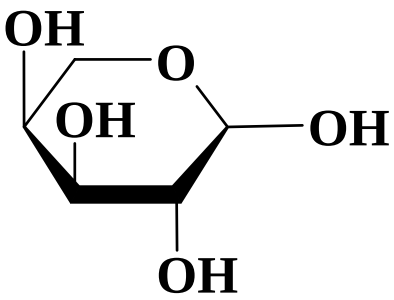
Hemicellulose Hydrolysis Products



Xilose
5-carbons



Glucose
6-carbons



Arabinose
5-carbons

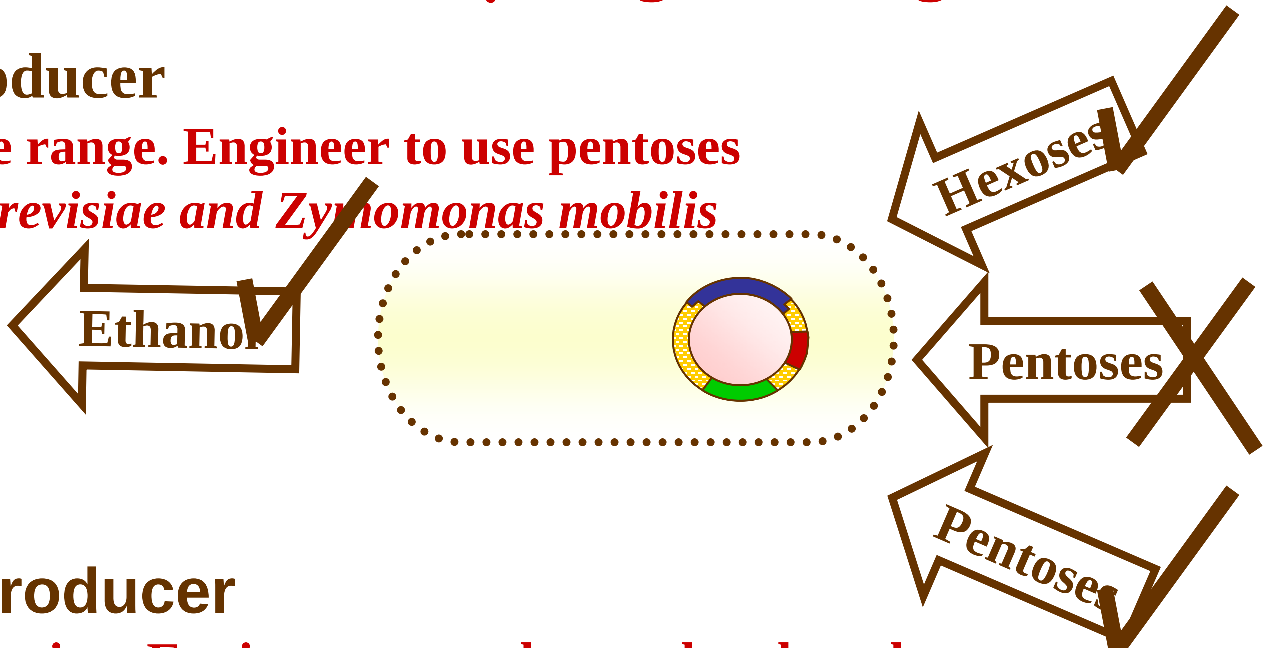
Yeast: Do not Ferment Pentose Sugars

Two main strategies: Pentoses → Ethanol
Metabolic Pathway Engineering

A: Ethanol producer

Increase substrate range. Engineer to use pentoses

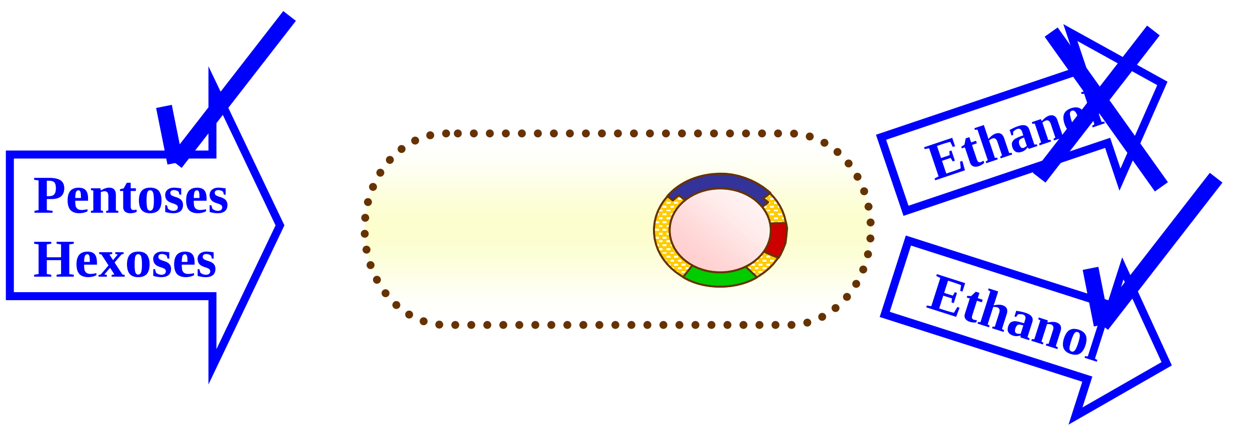
Saccharomyces cerevisiae and Zymomonas mobilis



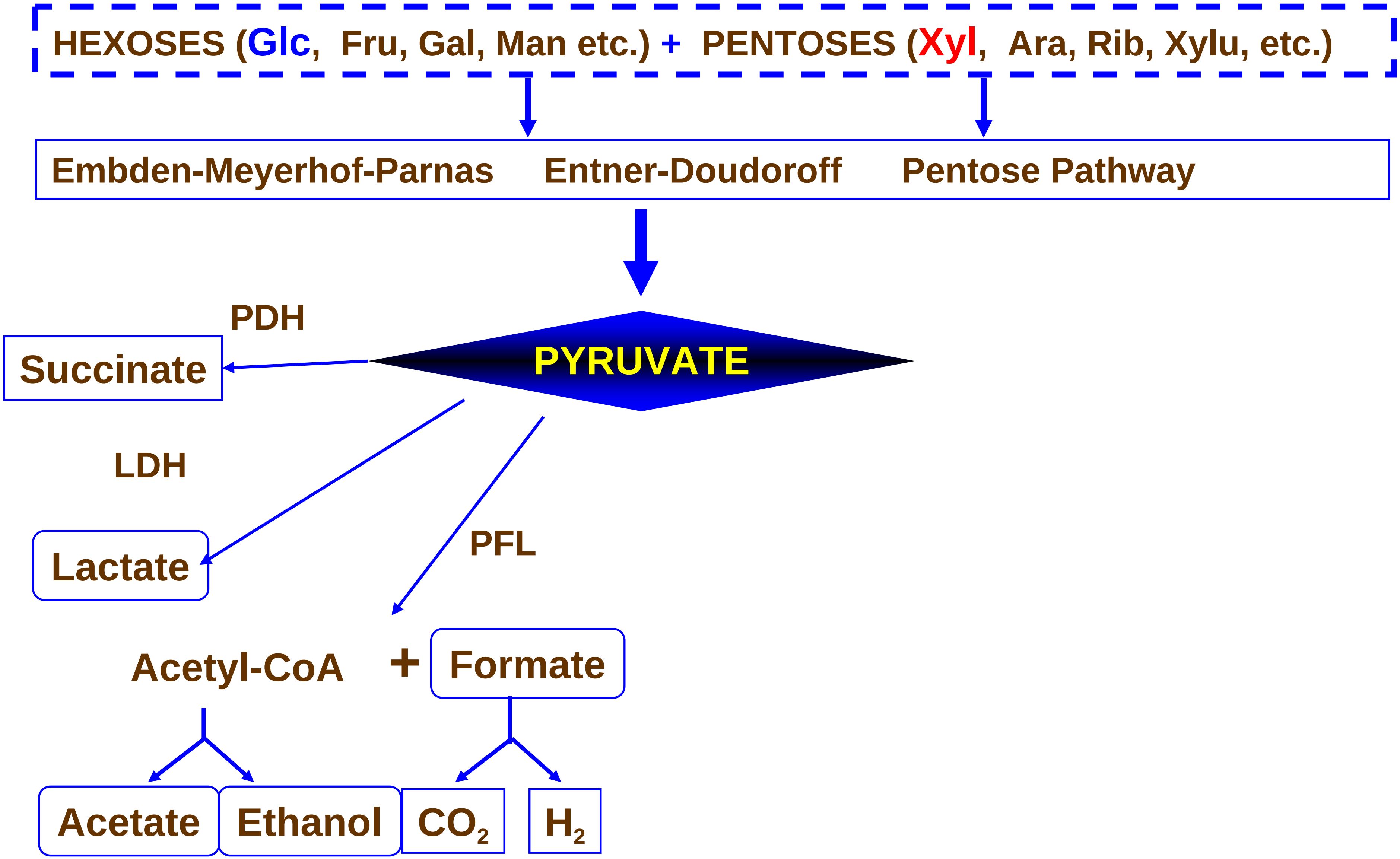
B: NO Ethanol producer

Pathway complementation. Engineer to produce only ethanol

Escherichia coli

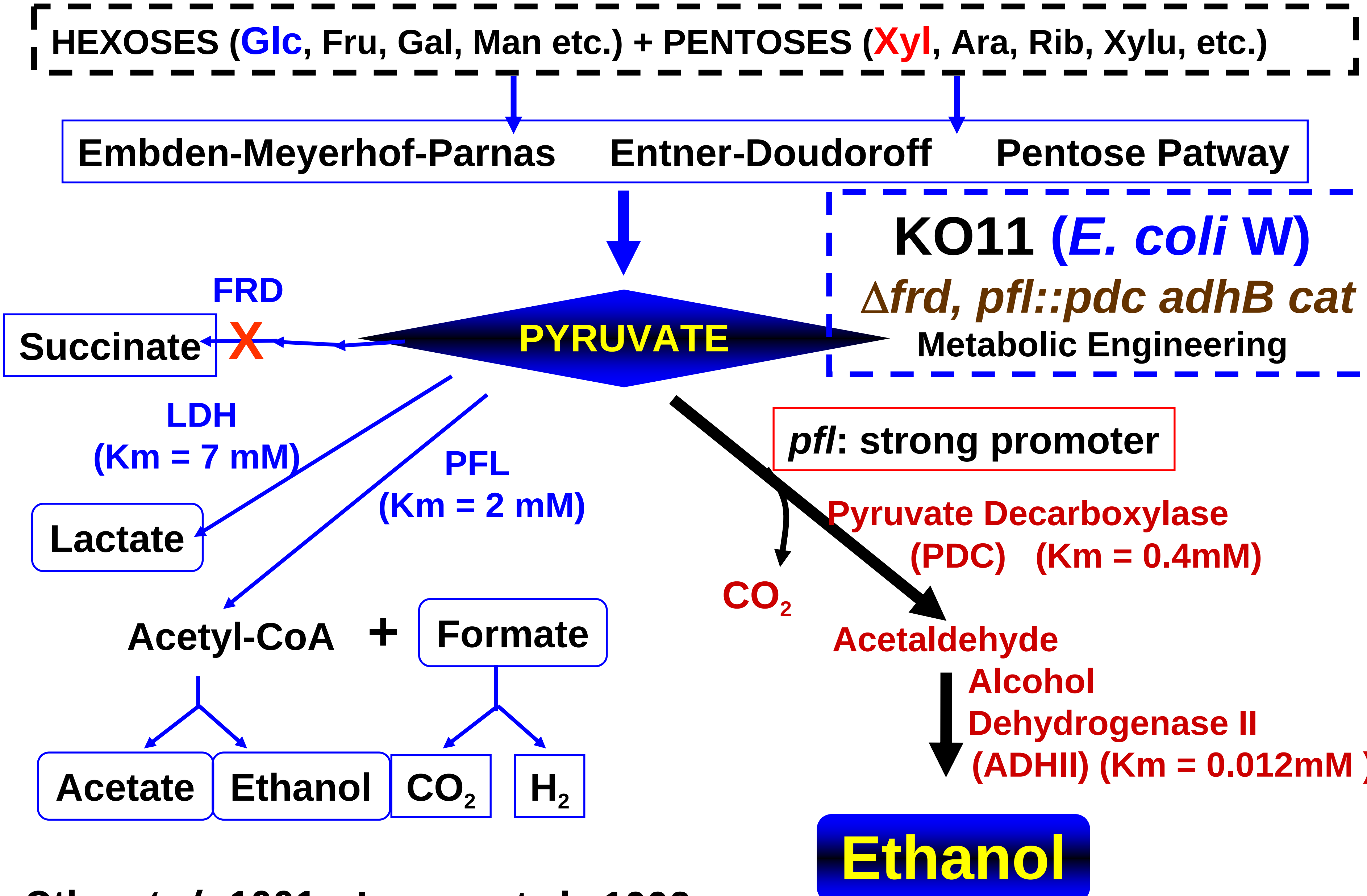


Escherichia coli: Uses a wide range of sugars



and make fermentation products, but little ethanol

Construction of ethanologenic *E. coli*: KO11

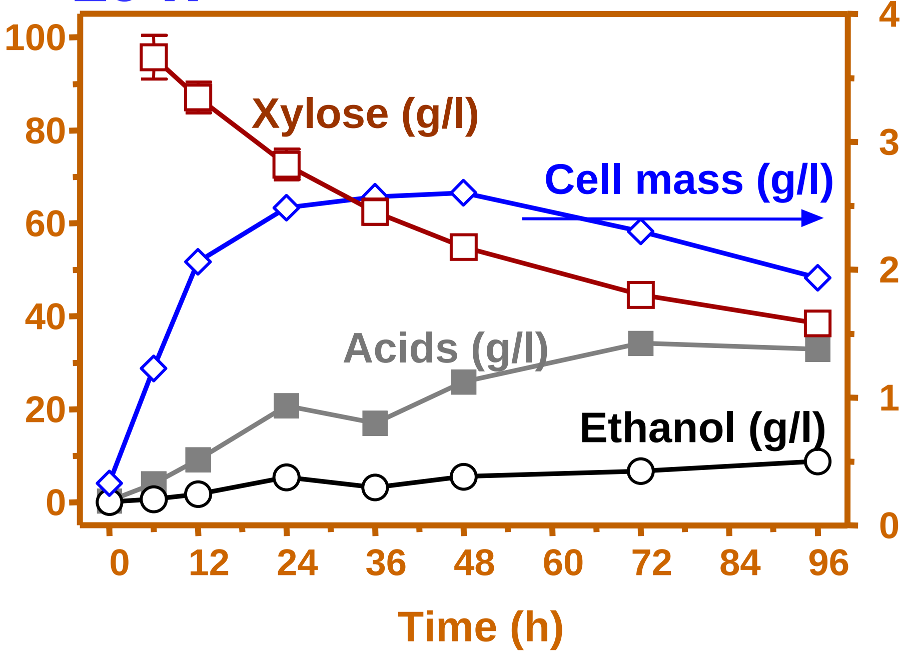


Otha *et al*., 1991 Ingram et al., 1998

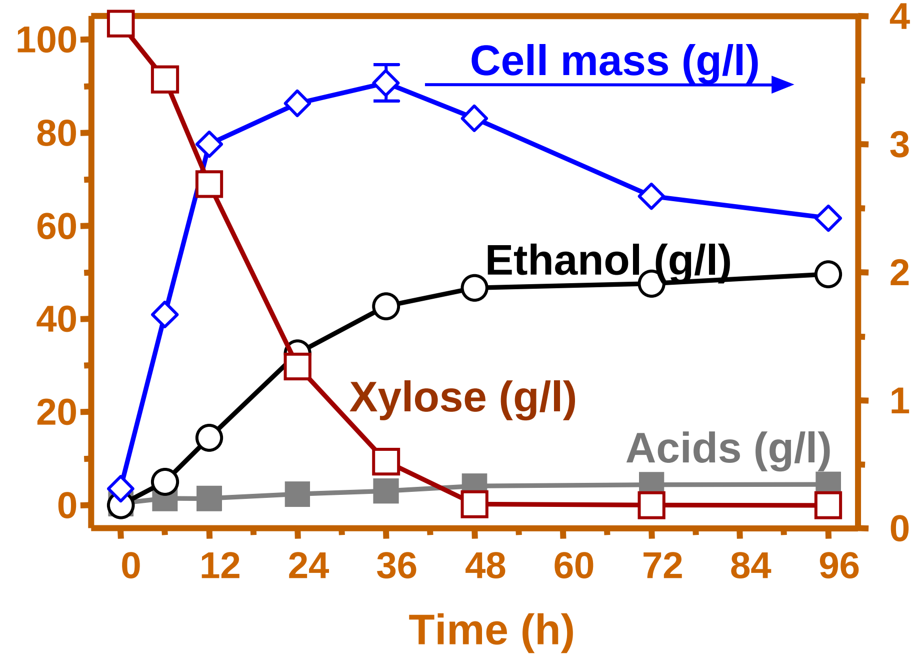
Xylose (10%) fermentation

Luria Broth, 35°C, 100 rpm, pH 7.0

Ec W



KO11



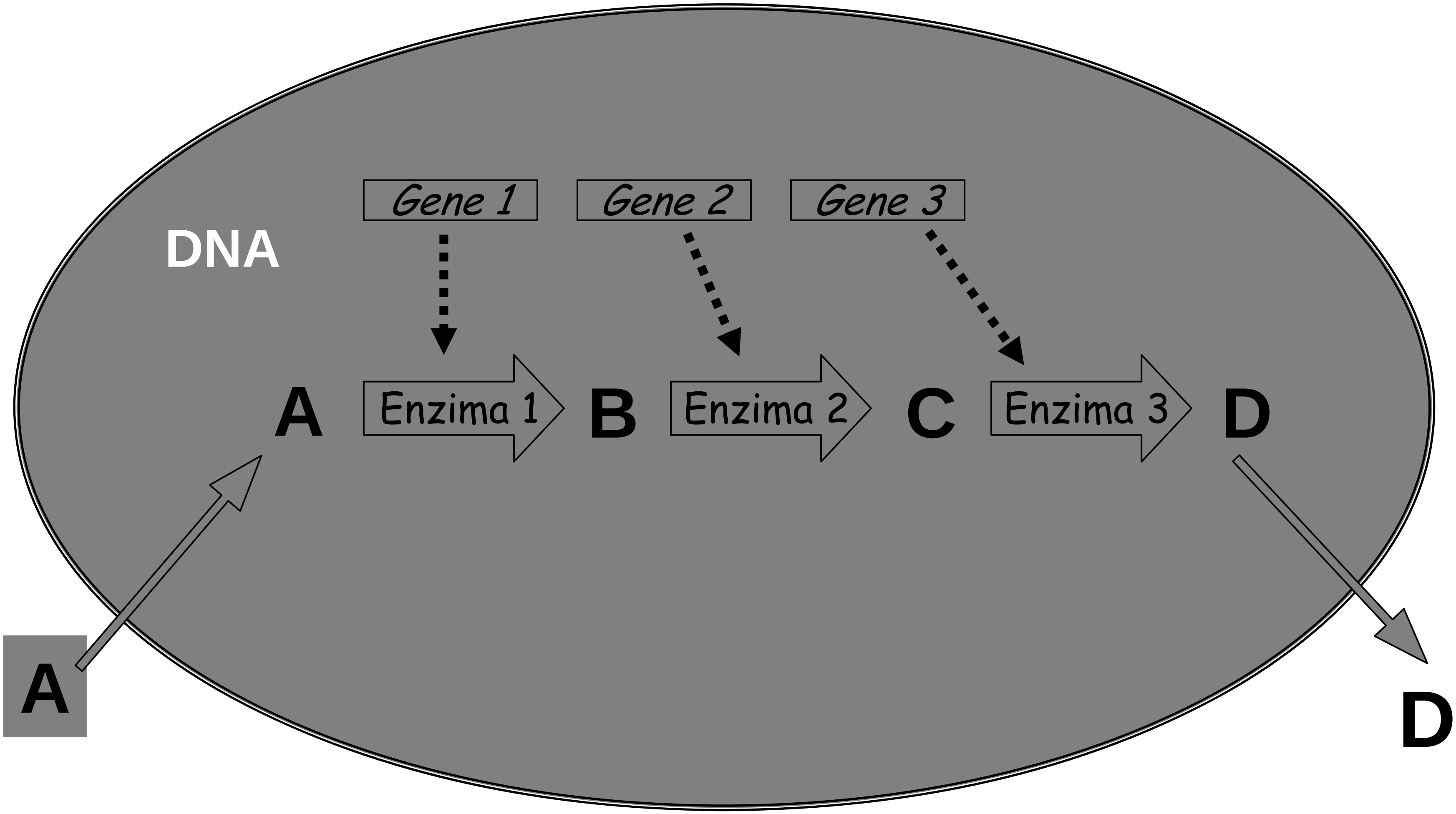
Cell mass:	**KO11 > Ec_W**	**(34%)**
μ:	**KO11 > Ec_W**	**(30%)**
q_S: (g_{Xyl}/g_{DCW}h)	**KO11 > Ec_W**	**(30%)**
Q_{EtOH} ($g_{Et\text{-}OH}$/l h)	**KO11 = 1.0**	

Theoretical Yield =
0.51 $g_{Et\text{-}OH}/g_{Glc\ or\ Xyl}$

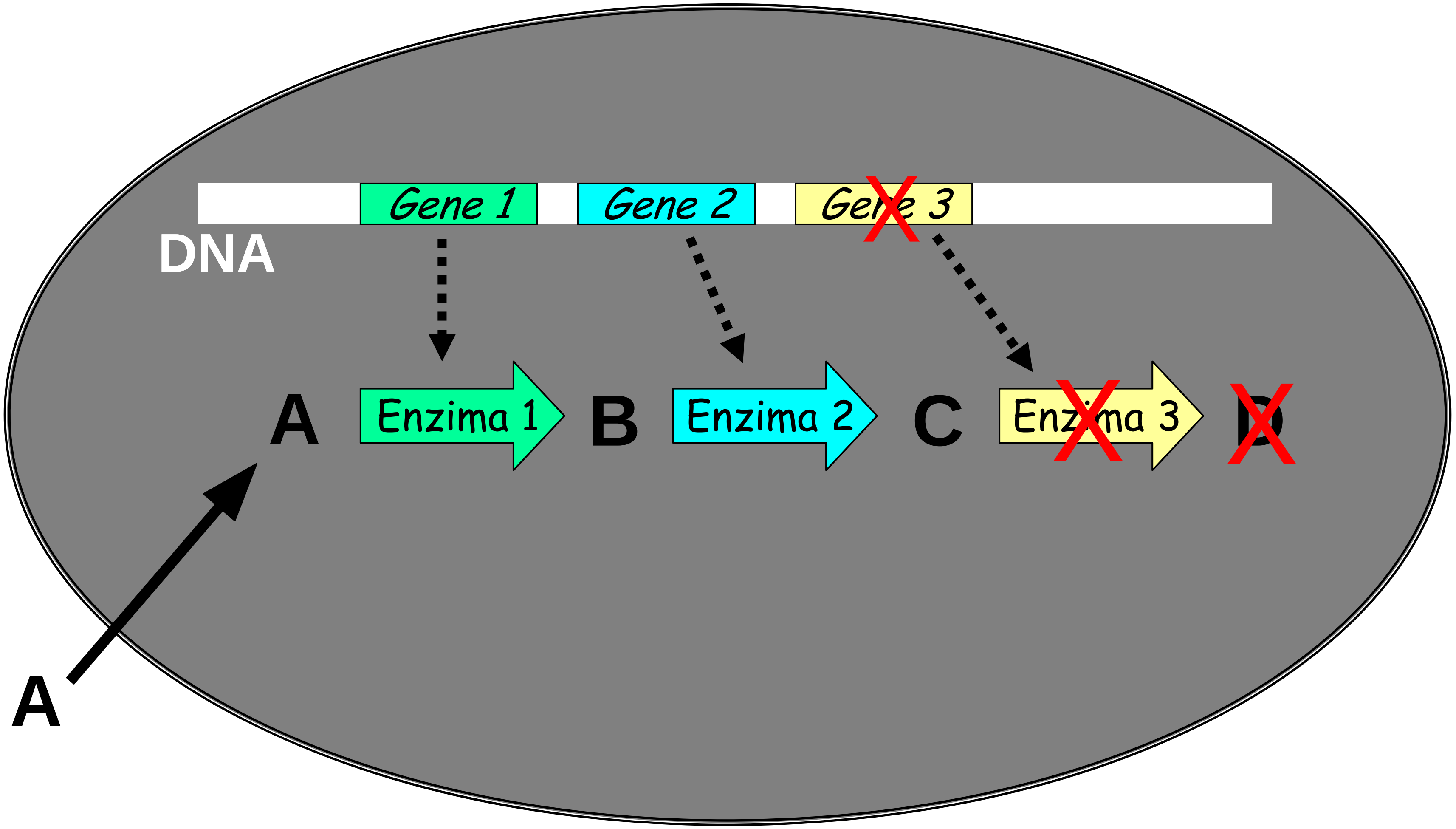
Yield 94.0%

Tao *et al.*, 2001, Martínez et al., 1999

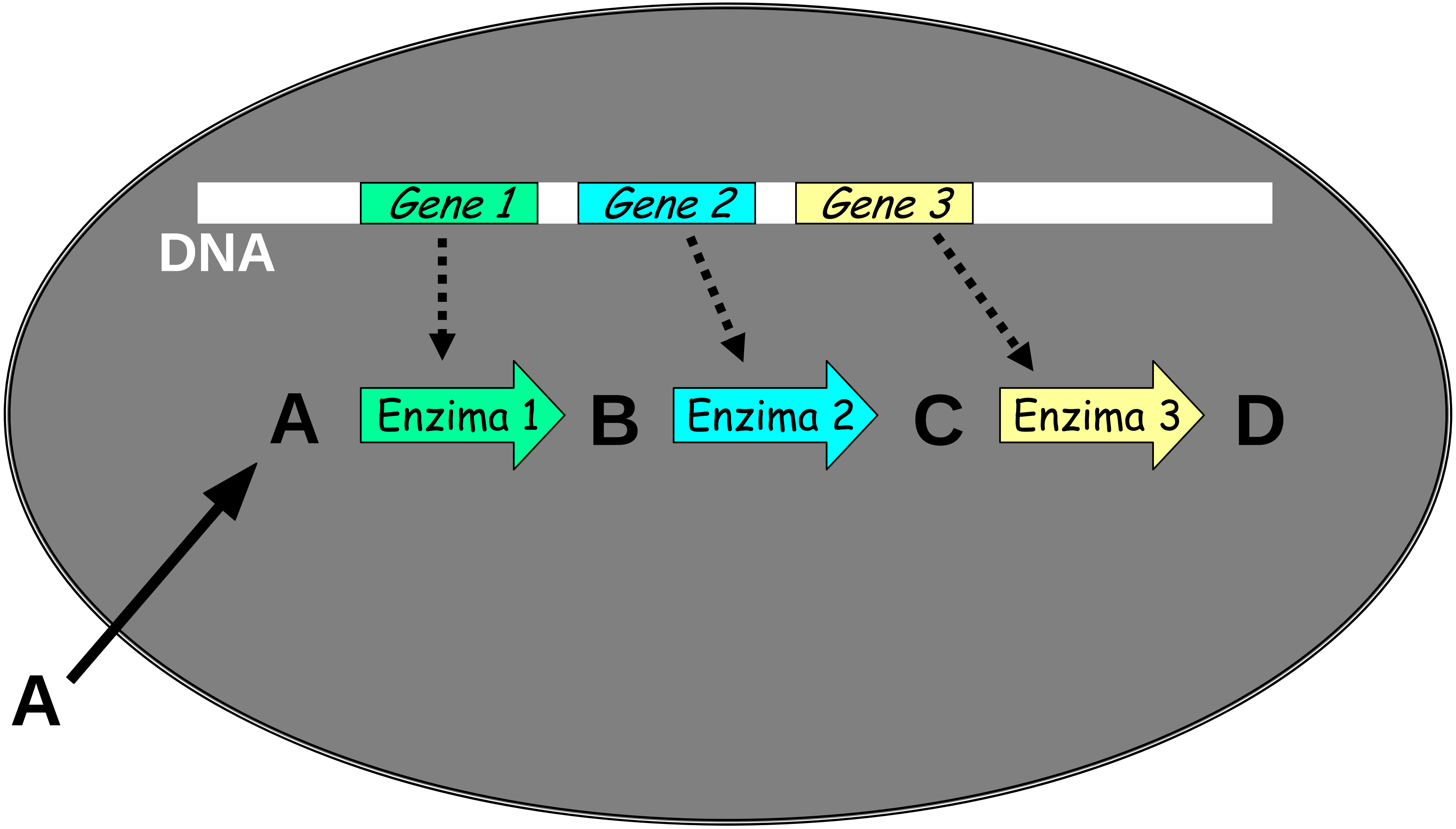
Metabolic Engineering
(Genetic Engineering Tools)



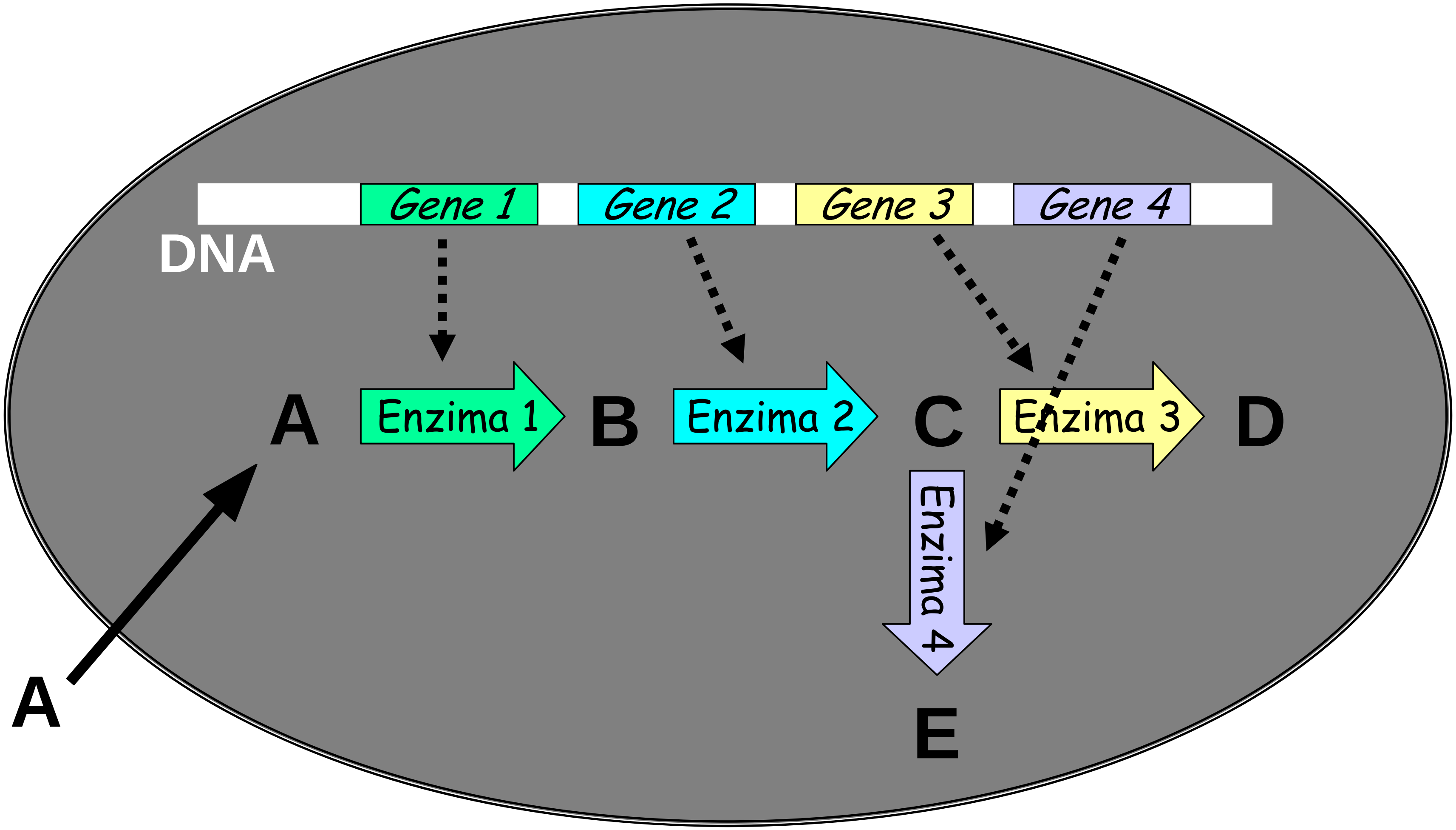
Delete - Interrupt Genes



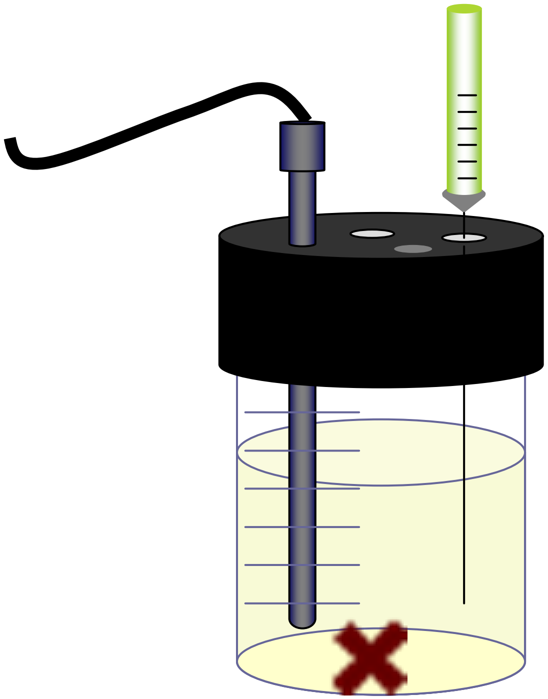
New genes



New (add foreign) genes



Strain evaluation



Fermentor setup: 6-Mini-fermentors (Fleakers)

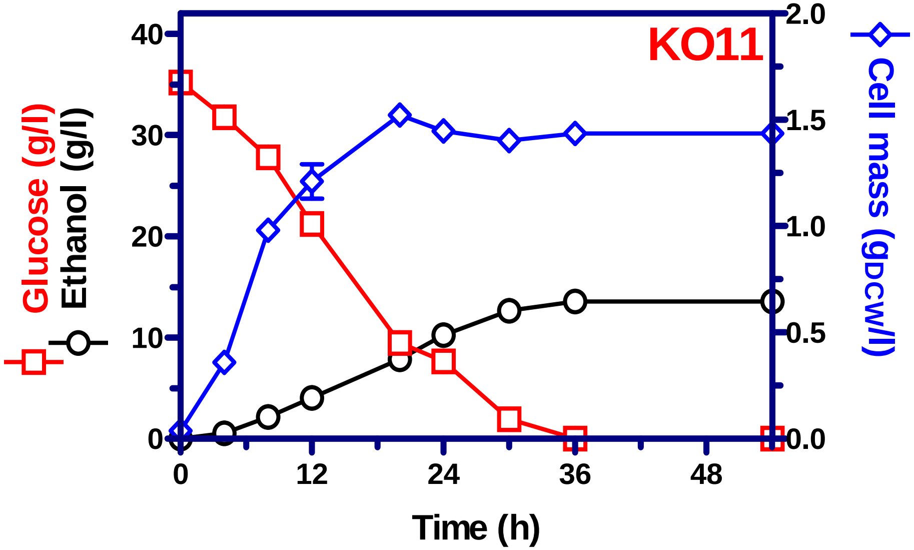
Working volume 200 ml

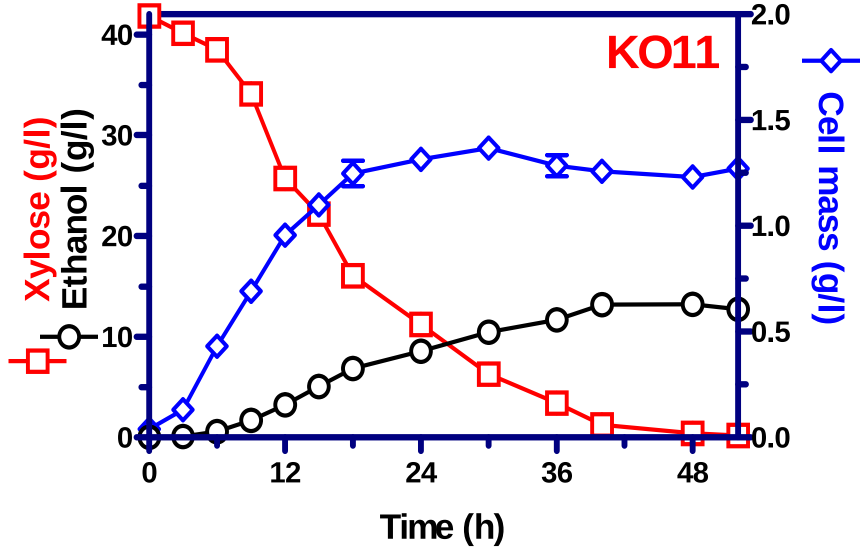
Mineral media (some with Rich Media) & Sugars (Xyl, Glc)

Temperature: 37°C; Speed: 100 rpm; pH: 7.0

Without aeration

KO11: Glc or Xyl (4%): Mineral Media
Sugar Cane Hemicellulose Hydrolyzates





In comparison with rich media, in mineral media there are reductions by:

57_{Glc} & 63_{Xyl} % in cell mass formation; 25% in the specific growth rate

70_{Glc} & 60_{Xyl} % in the specific sugar consumption rate

Q_{EtOH} is reduced to 0.42_{Glc} & 0.33_{Xyl} $g_{Et\text{-}OH}$/l h, for glucose and xylose

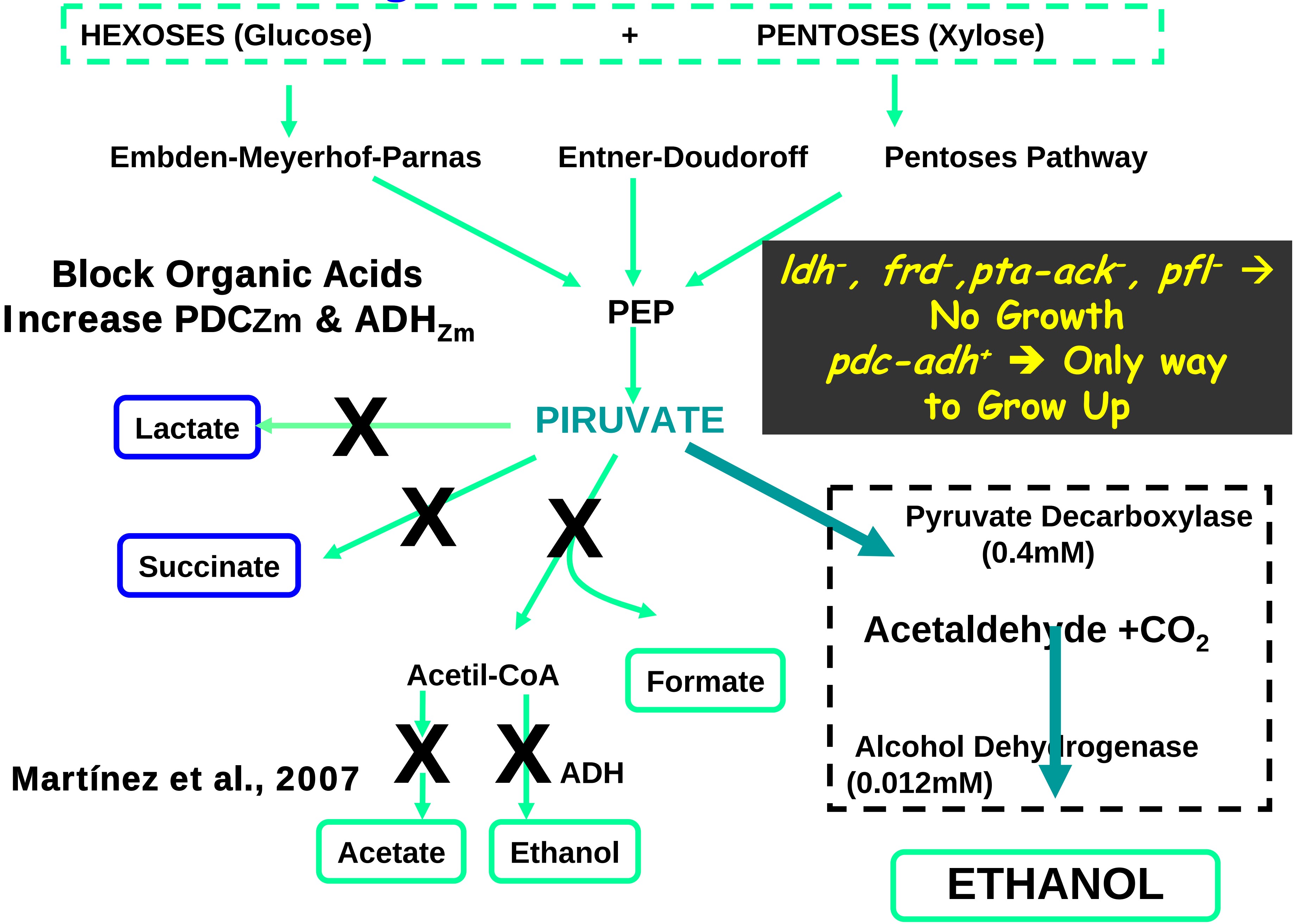
Yield	70%

Yield	60%

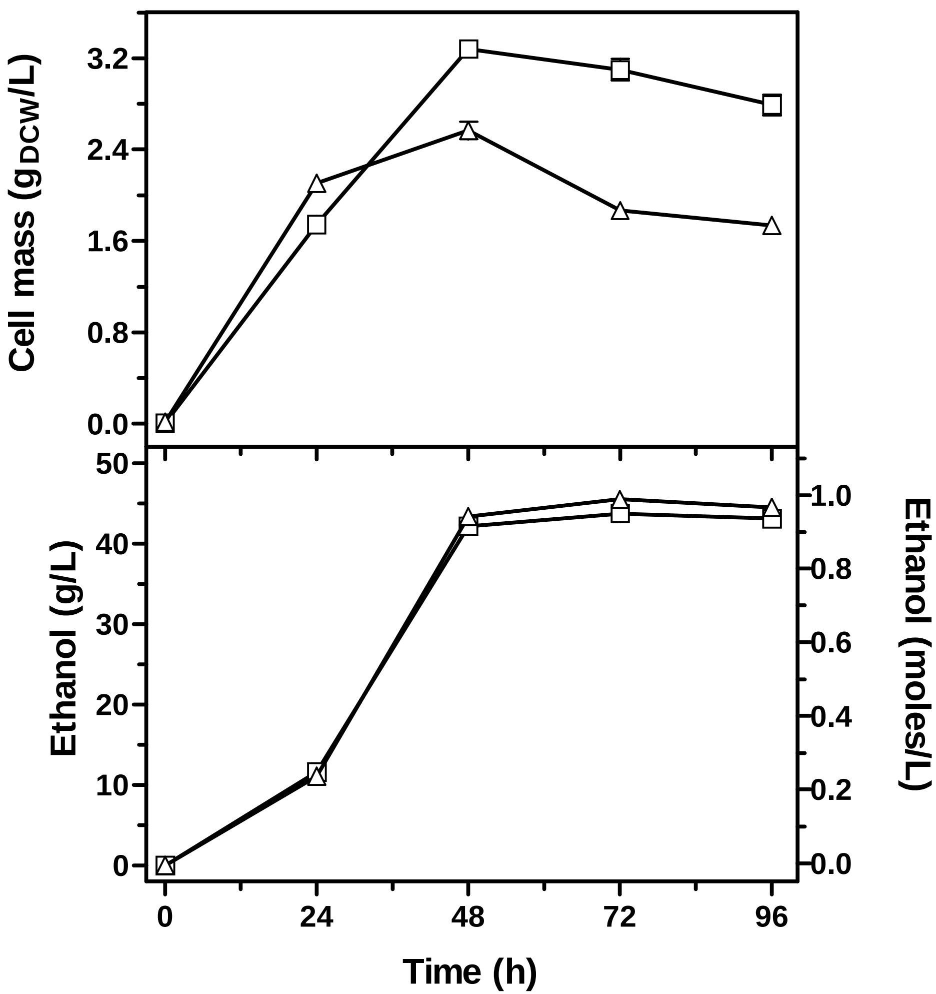
Main Conclusion

- **The study provides the basis for the implementation of appropriate genetic modifications to increase the ethanol yield when mineral media or sugar from hemicellulose syrups containing a large amount of pentoses are used, for instance, the disruption of succinate and lactate pathways that compete for ethanol production.**

Ethanologenic *E. coli* 2nd Generation



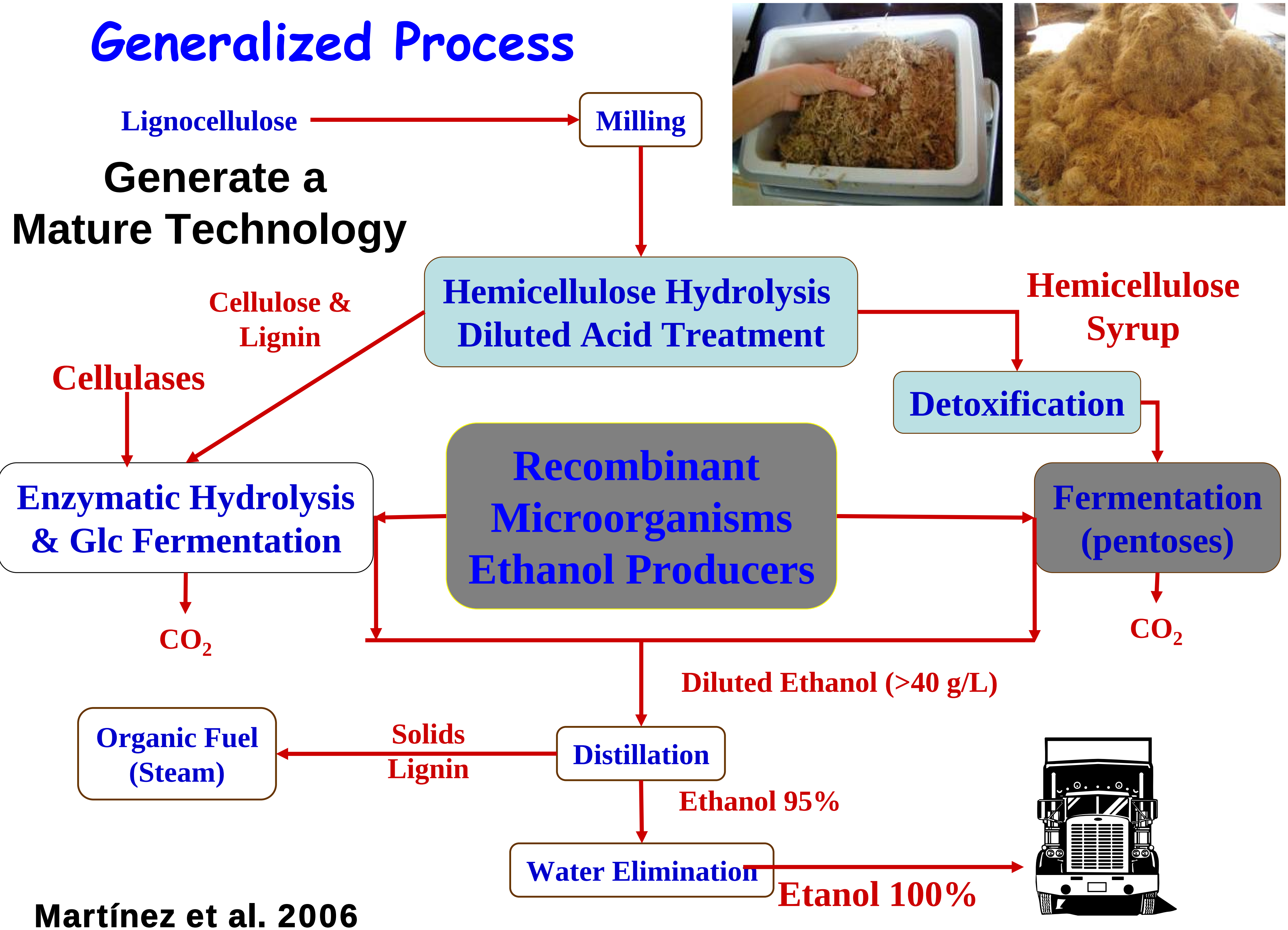
Ethanol Production in Mineral Media-Xyl-Glc Or Hemicellulose Syrups



Martinez et al., 2007

2nd Generation

- **Homo-ethanologenic strains**
- **Capable to ferment glc, xyl or mixtures (100 g/L total sugars) in 48 h**
- **Yield above 90% of the theoretical**
- **Some metabolic evolution work was performed to contend with the effects of *pfl* interruption.**


Generalized Process
Lignocellulose
Milling
Generate a
Mature Technology
Hemicellulose Hydrolysis
Diluted Acid Treatment
Hemicellulose
Syrup
Cellulose &
Lignin
Cellulases
Detoxification
Enzymatic Hydrolysis
& Glc Fermentation
Recombinant
Microorganisms
Ethanol Producers
Fermentation
(pentoses)
CO_2
CO_2
Diluted Ethanol (>40 g/L)
Organic Fuel
(Steam)
Solids
Lignin
Distillation
Ethanol 95%
Water Elimination
Etanol 100%
Martínez et al. 2006

SCB

Production Potential with Sugar Cane Base 100 Ton/Ha

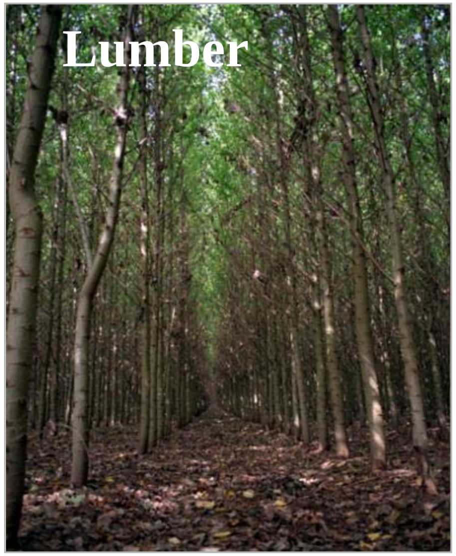
- Cane syrups: **7,500 L/Ha**
- Bagasse (300 kg/Ton, 50% humidity)
- **5,500 L/Ha**

- **Syrups + Bagasse**
- **13,000 L/Ha**

- **+ Stover**
- 300 kg/Ton, ~20% humidity
- + 7,000 L/ha

- **~ 20,000 L/Ha**

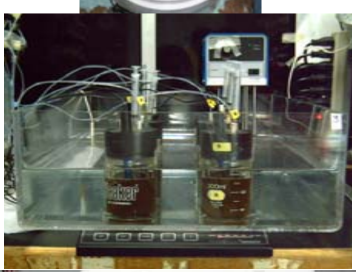
Sources



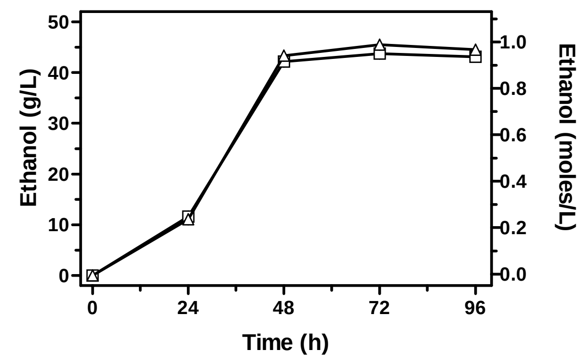
Any lignocellulosic material, biomass from semi-arid areas

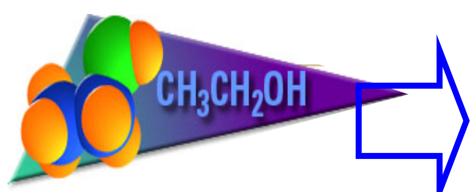
Bio-Ethanol Activities at the IBt - UNAM






- The Biotechnology Institute has been involved in the research project since 1999.
- It has a fermentation pilot plant based in 100 and 350 liters fermentors.
- We had demonstrated the generation of fermentable sugars using thermohemical and enzymatic hydrolysis at laboratory and pilot plant scale.
- We have develop (and patented) bacterial strains with the capacity to ferment all sugars present in hydrolyzates with conversions yields above 90% of the theoretical and with volumetric productivities reaching 1 g/L/h.
- Actual research focus in:
  - Other raw materials
  - Improve and integrate a process
  - Cost reduction





Technological goals

1. Many opportunities remains to optimize ethanologenic biocatalyst
2. Raw materials (lignocellulose) is different for each country or region. So process development is case specific.
3. Thermochemical (pre-treatments and distillation) need to be energy efficient
4. Enzymatic hydrolysis of cellulose needs to be optimized

Wyman. Trends in Biotechnol. 2007.

Alfredo Martínez 2006

My vision:

Politics are very important. Biomass production is fundamental. We need to avoid competence with food and feed, and take care of land usage. Sustainable methodologies are essential for all the chain process.

BUT DEVELOPING COUNTRIES ALSO CAN DEVELOP THEIR OWN TECHNOLOGIES, WE DO NOT NEED TO WAIT FOR THE BIG COMPANIES TO DEVELOP THEM AND LATER DEPEND ON THEM. BIOTECHNOLOGY IS FUNDAMENTAL FOR THIS TECHNOLOGIES.

CONACYT
Consejo Nacional de Ciencia y Tecnología

!!Thanks!!

Acknowledgments

- CONACyT Grants
- SAGARPA 2004-C01-224
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- PAPIIT – DGAPA - UNAM IN220908-3

Collaborations

Instituto de Biotecnología
University of Florida

Students

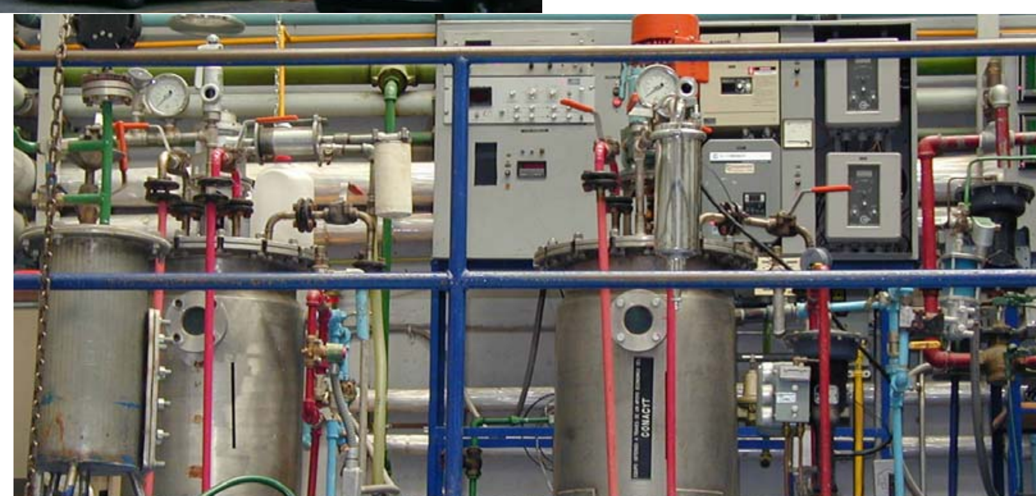
Red Mexicana de Bioenergía

COMPETE

Questions


Instituto de Biotecnología
UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO


LIGHTNIN



Production Cost and Energy Balance

Table 2. Ethanol costs and energy balances.

Feedstock	Cost (US$ per gallon)	Energy balance (renewable output to fossil input)
Sugarcane, Brazil		10.2 (*18*)
2006, without import tax	0.81 (*17*)	
2006, with U.S. import tax	1.35 (*9, 17*)	
Sugar beet, Europe, 2003	2.89 (*17*)	2.1 (*19*)
Corn, U.S., 2006	1.03 (*17*)	1.4 (*9, 11*)
Cellulose ethanol, U.S.		10.0 (*11*)
Achieved in 2006	2.25 (*11*)	
Target for 2012	1.07 (*11*)	

Science 2007

Estimated Production Costs

Fuel Ethanol	(dol/gal)	Pesos/Liter
NREL-DOE Corn Stover:	1.44	4.20
NREL-California Lumber:	1.07	3.10

Sugar Cane Bagasse Mexico

Production Cost $3.7 / Liter
From Molasses (2001)
Production Cost $2.3 /Liter
Cabrera, Gómez & Quintero 2001

Brazil Prices March 2005

	($/L)	(R/L)
Alcohol:	5.9	1.3
Gasoline:	10.4	2.3

CH_3CH_2OH BioEthanol

Bio-ethanol: Mexico

Sugar Cane

- Planta Sn. Juan Bautista Tuxtepec (Tuxtepec, Oaxaca)
- Planta Tecnol del Sureste S.A de C.V (Huehuetan, Chiapas)
- Ingenio La Gloria (Veracruz)

Corn

- Planta Mex-Starch (Sinaloa)
- Planta Destilmex (Navolato, Sinaloa)

Sorgum

- Bioenergéticos Mexicanos S.A.P.I. de C.V (Valle Hermoso, Tamaulipas)

Cianobacterias

BioFields (Sonora

Lignocellulose

Planta Piloto IBt – UNAM