Emerging Platforms for Biofuels and Biochemicals: The Role of Metabolic Engineering and Systems Biology

Ramon Gonzalez

Department of Chemical and Biomolecular Engineering

Rice University

Biomass to Chemicals and Fuels: Science, Technology and Public Policy
September 25-26, 2006
Baker Institute International Conference Facility
Rice University, Houston, TX

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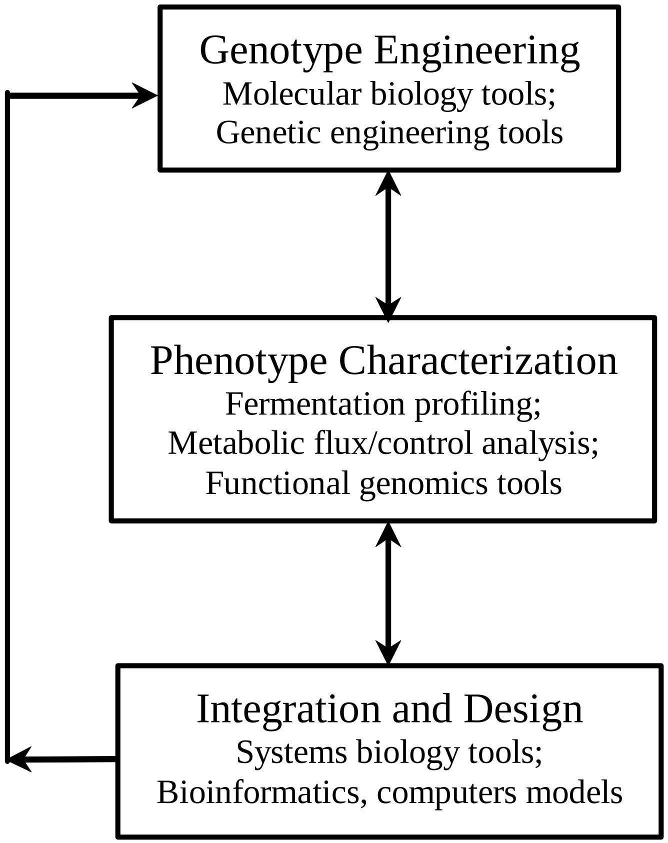
Metabolic Engineering (ME)

Metabolic engineering is the improvement of cellular activities by manipulation of enzymatic, transport, and regulatory functions of the cell with the use of recombinant DNA technology. The oppor-

Rice Alumnus!!



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Systems Biology

A relatively new field that aims at systems level understanding of biological processes

The two Determinants of Systems Biology

1. New technologies for comprehensive, high-throughput, quantitative measurements at system/cellular levels: i.e., functional genomics tools.
2. Advances in theory, modeling, softwares, and computational power for data analysis and integration.

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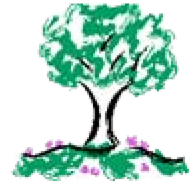
Renewables in Our Future?

Linking *PetroChemical* and *BioBased* Industries



Petrochemical Industry

Crude oil and natural gas

↓

Hydrocarbons

↓

Biobased Industry

Plant biomass and wastes

↓

Sugars, oils, etc.

↓

Feedstocks and intermediates

↓

Fuels

Bulk and Specialty Chemicals

- **National energy security**
- **Economic growth**
- **Climate protection**

Fuels:

Gasoline, diesel

Chemicals

Materials

Biofuels:

Ethanol, biodiesel

Chemicals

Bio-based Materials

Three Main Platforms for Fuels and Chemicals from Biomass

• **The sugar platform** is based on extracting sugars from plant biomass and using them as substrates (feedstocks) for the production of fuels and chemicals, predominantly via fermentation processes. This is the platform that includes cellulosic ethanol.

• **The syngas platform** proposes to process the plant biomass thermally (pyrolysis/combustion) to obtain heat, power and a gas mix (syngas or synthesis gas) containing CO, CO_2, H_2, and other compounds. Syngas will then be processed via various chemical or fermentation routes to produce ethanol and other chemicals.

• **The oil platform** is related to biodiesel or bio-distillate production. Vegetable oils or animal fats are used biodiesel (a mixture of fatty acid methyl esters) or process it into bio-distillates via conventional refinery technology.

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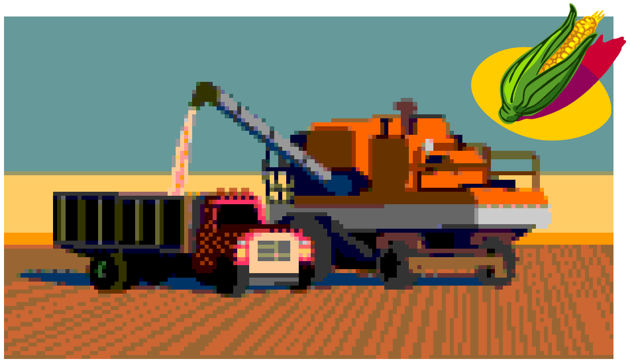
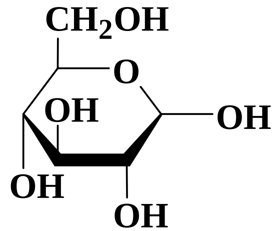
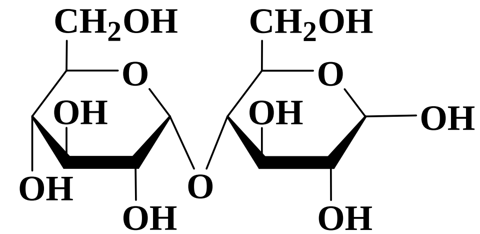
US Feedstocks

<u>Today</u>: Corn





Glucose

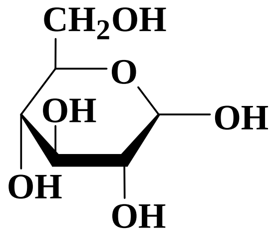


Maltose

<u>Tomorrow</u>: Lignocellulosic biomass

Lignin: 20-30%, Cellulose: 30-50%, Hemicellulose: 20-40%

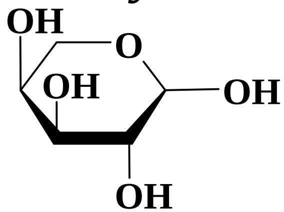
Cellulose: Glucose



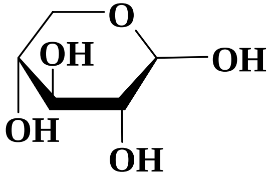
Glucose

Hemicellulose: Xylose, arabinose, mannose, glucose, etc.

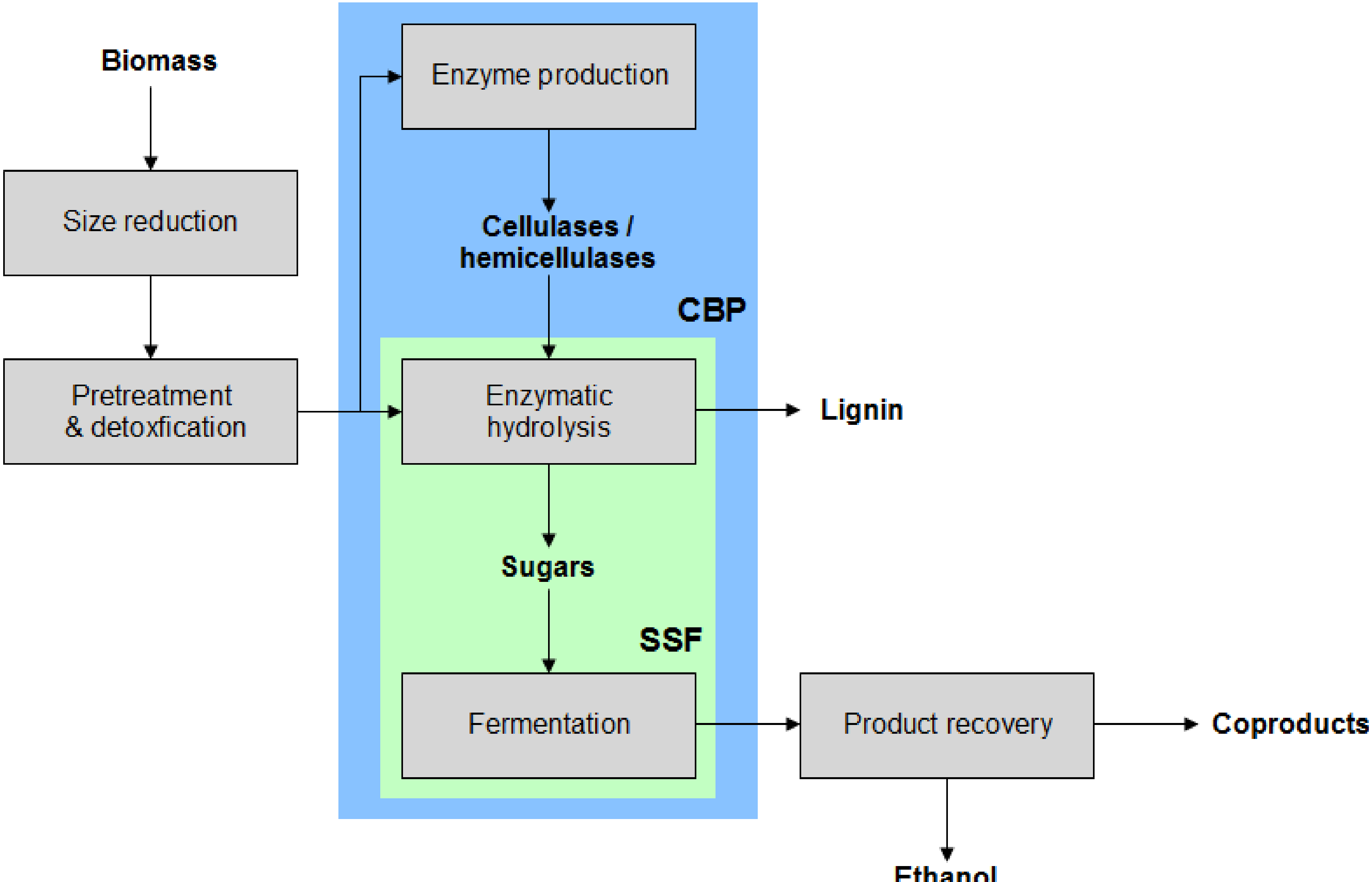
Xylose



Arabinose



EtOH/BuOH…Fuels or Chemicals from Cellulosic Biomass



Dharmadi and Gonzalez (2006)

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Fuels & Chemicals from Biorenewables

Feedstock Engineering

Plant Biotechnology

→

Feedstock Deconstruction

Pretreatment and Hydrolysis

→

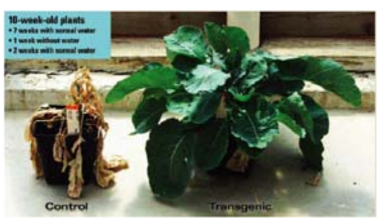
Microbial Fermentation

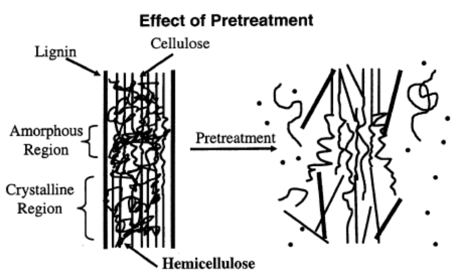
Biocatalysis

→

Recovery

Distillation
Extraction





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Conversion of Plant Biomass Sugars into Fuels & Chemicals via Fermentation

Plant biomass

↓

5-C and 6-C-sugars

Microbial ↓ fermentation

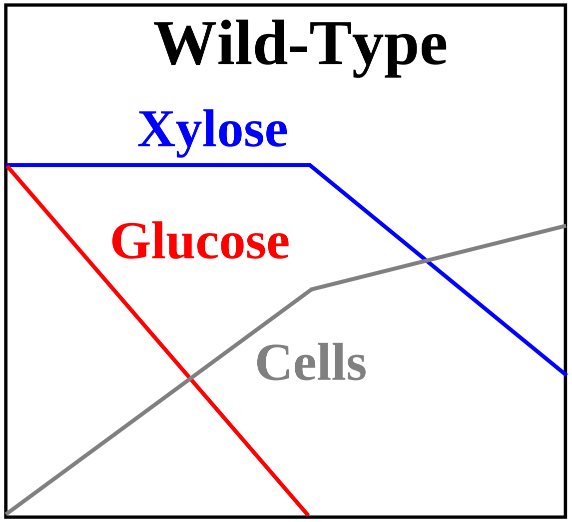
Chemicals and fuels

***Escherichia coli, Zymomonas, Lactobacillus, Clostridium, Saccharomyces,* Xylose-Assimilating Yeasts**

Search for the ideal biocatalyst

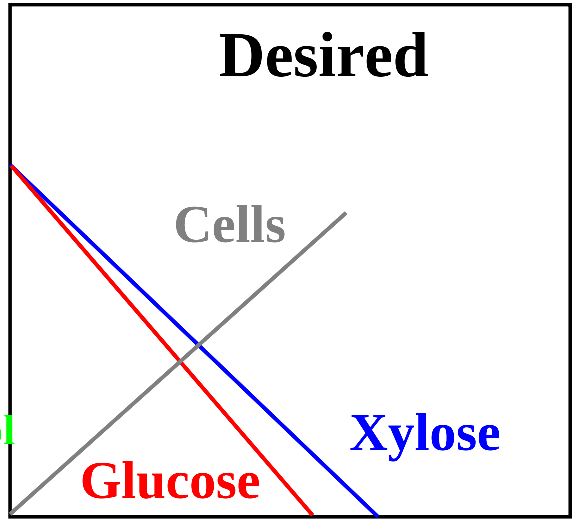
- Broad substrate utilization range (C5/C6)
- Co-fermentation of C5/C6
- High titer, yield, and productivities
- High tolerance to final product
- Minimal nutrients requirements
- Resistance to inhibitors in hydrolysates
- No oxygen requirement
- Available genetic tools and physiological knowledge
- Low fermentation pH
- Use in large-scale fermentations and production on an industrial level

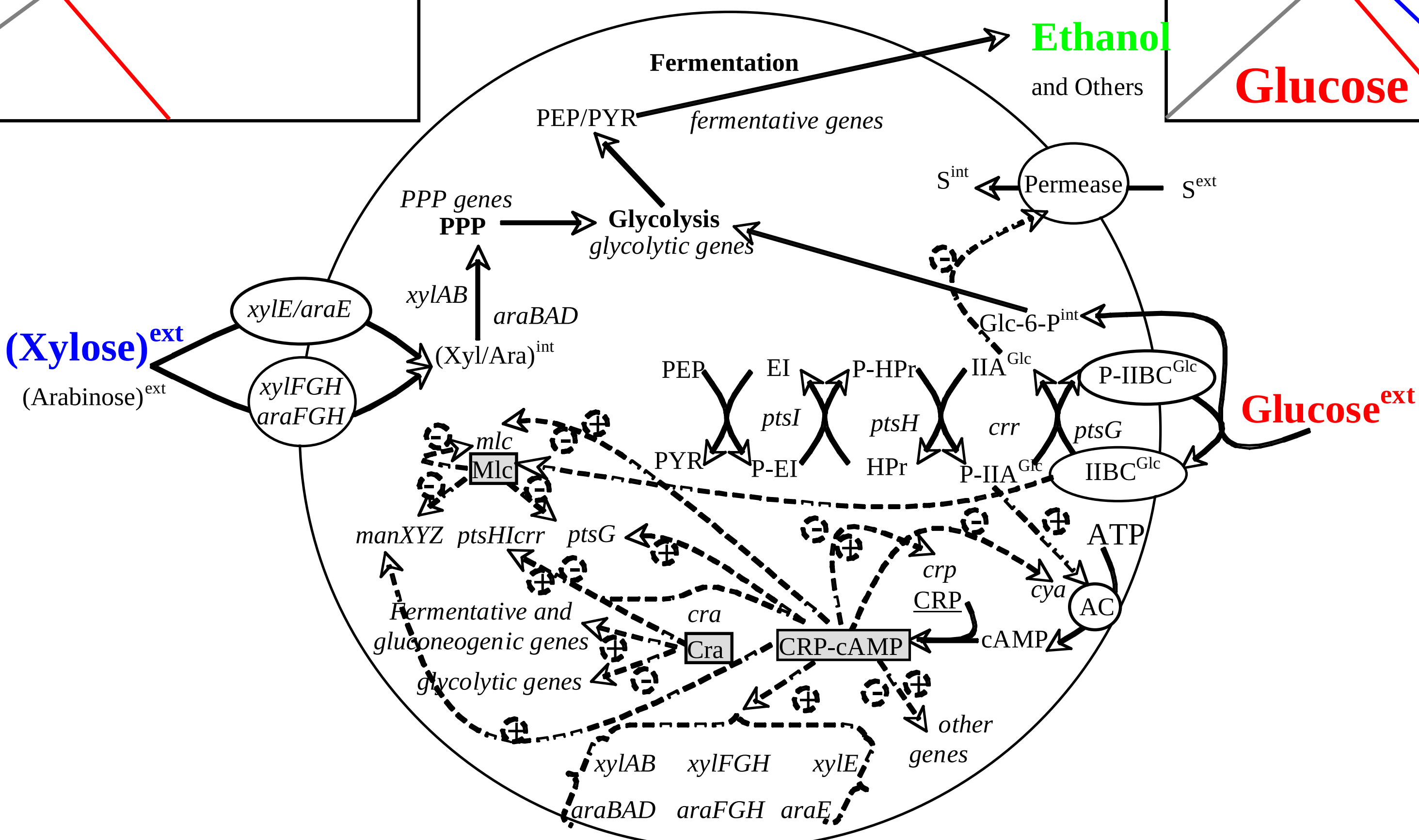
Sugar-Utilization Regulatory Systems (SURS)



Understanding

Harnessing





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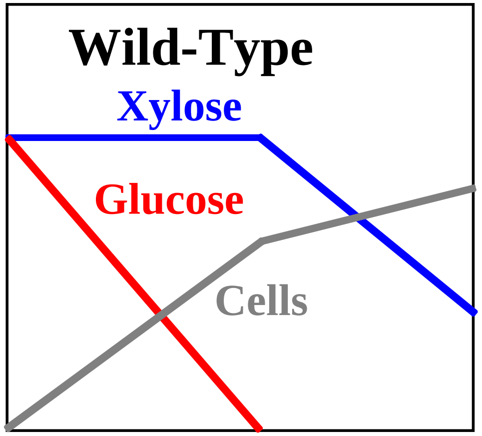
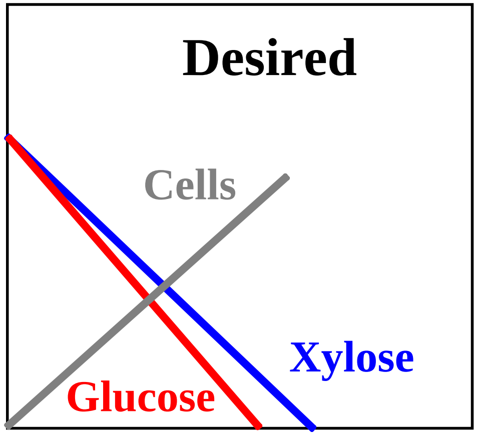
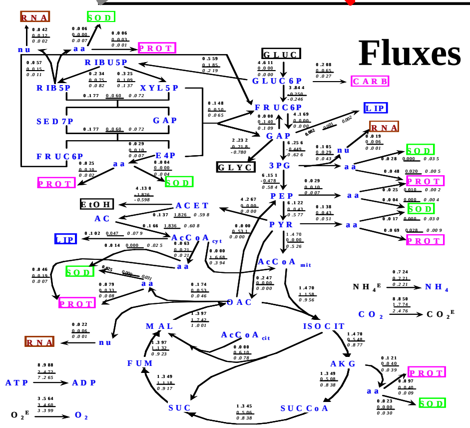
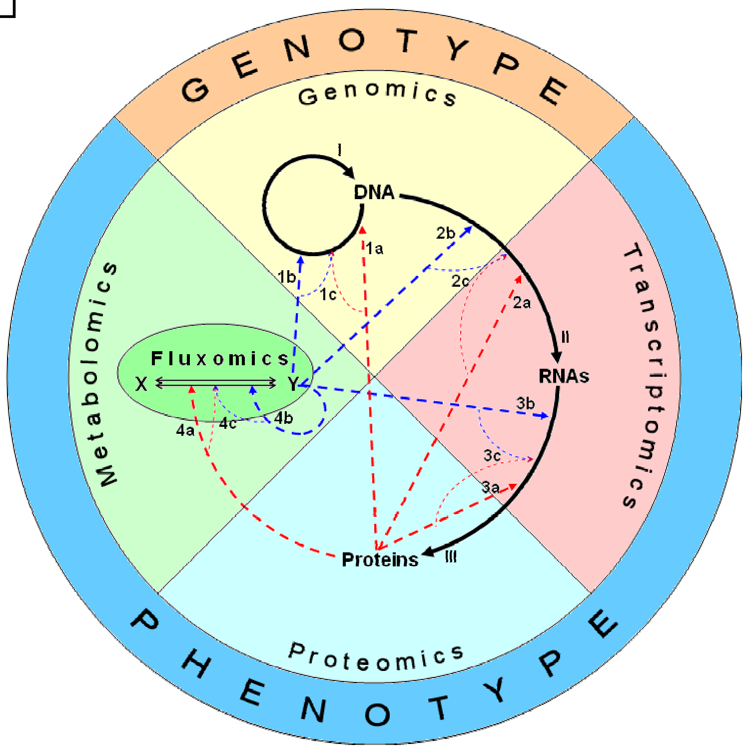
Systems Biology-Based Approach

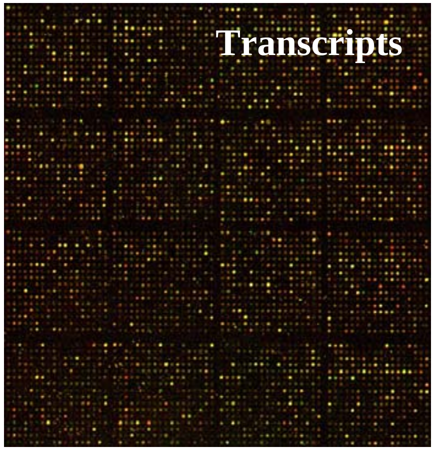


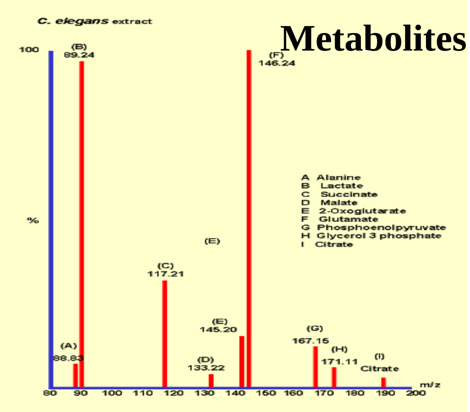
Systems Biology Approach to Understand SURS

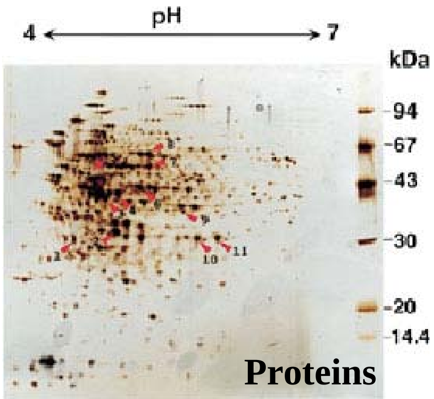












Systems Biology-Based Approach

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Systems Biology Approach to Understand SURS

A Priori Knowledge

Updated Knowledge

Wild-Type

Xylose

Glucose

Cells

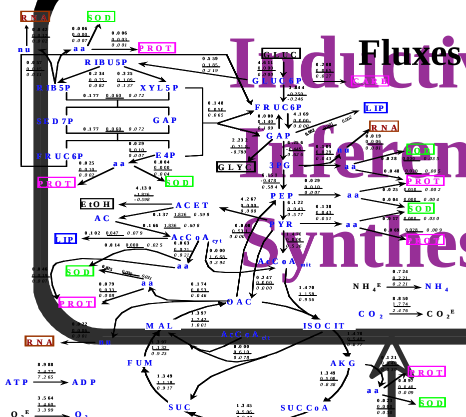
Desired

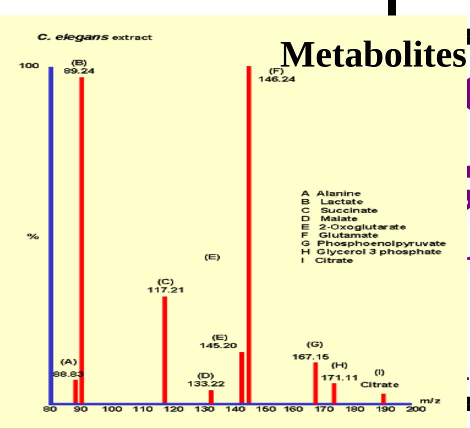
Cells

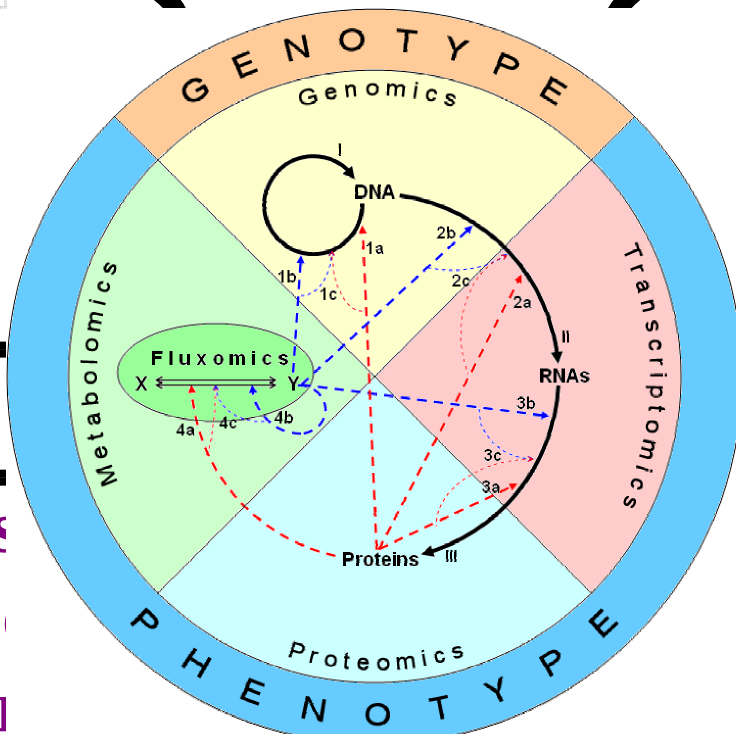
Xylose

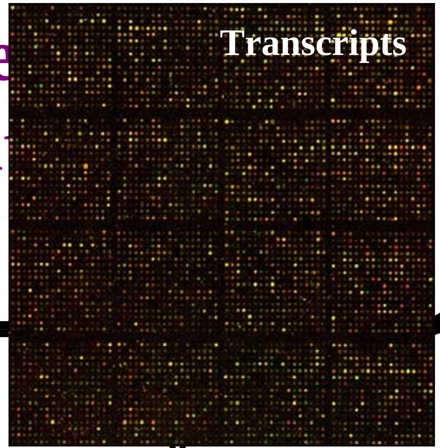
Glucose

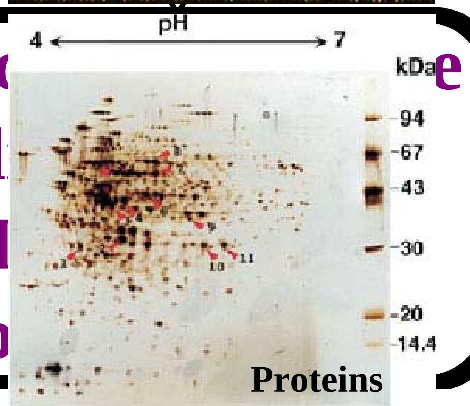
Inductive Inference Synthesis











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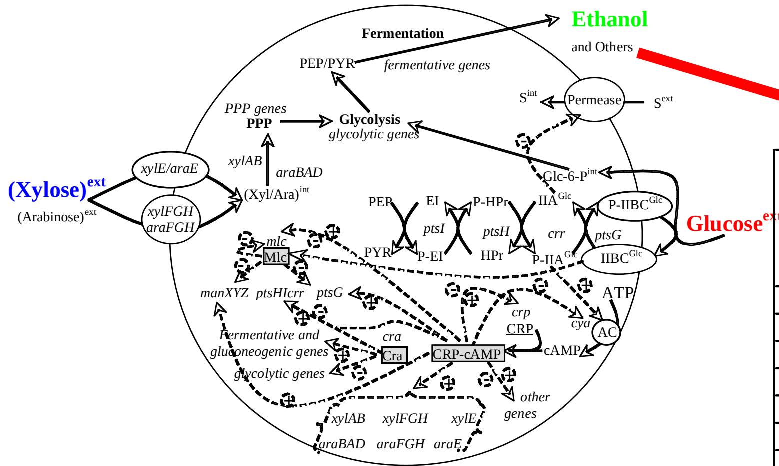
SURS and Ethanol Tolerance

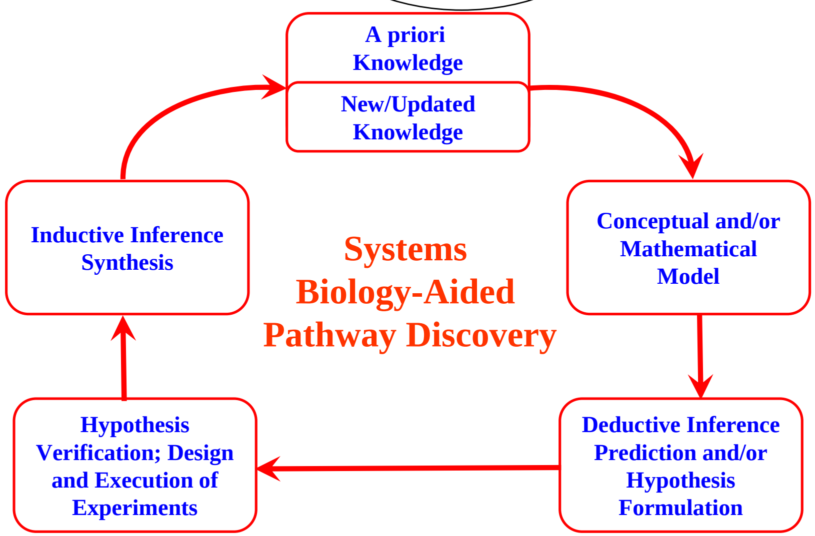


Elementary Network Decomposition (END)

	crp	CRP	CRP-cAMP	*cyaA*	AC	cAMP	*crr*	IIA^{glc}	IIA^{glc}~P	$glucose^{ext}$
crp		1	1	-0.2	-0.7	-1.1				
CRP			1.0	-0.1	-0.3	-0.5				
CRP-cAMP	-1	-1		-0.1	-0.2	-0.4			-0.1	
cyaA					1	1				
AC						1				
cAMP			1.0							
crr					1			1	1	
IIA^{glc}										
IIA^{glc}~P					1	1				
$glucose^{ext}$	-1	-1	-1	0.1	0.2	0.2				

$$\left\langle \frac{\text{CRP - cAMP}}{\text{AC}} \right\rangle = \left\langle \frac{\text{CRP - cAMP}}{cyaA} \right\rangle \left\langle \frac{cyaA}{\text{AC}} \right\rangle + \left\langle \frac{\text{CRP - cAMP}}{\text{IIA}^{glc} \sim \text{P}} \right\rangle \left\langle \frac{\text{IIA}^{glc} \sim \text{P}}{\text{AC}} \right\rangle$$

= (-0.1)(1) + (-0.1)(1) = -0.1 Š 0.1 = -0.2



Elementary Network Decomposition (END)

To elucidate the topology of a regulatory network

- The network is broken down to the smallest elements: genes, proteins, metabolites, phoshorylated/unphosphorylated species, allosteric effectors, transcriptional repressors/activators, etc.
- By arrangement of these n elements into a $n \times n$ table, the network is then rebuilt from the ground up through complete enumeration of all possible $n(n\text{-}1)$ interactions.
- This is easily done using a spreadsheet, which also prevents circular reference in building inferences, and accommodates network expansion/reduction by simple row and column insertion/deletion.

Dharmadi and Gonzalez. (2006).

Elementary Network Decomposition (END)

	crp	CRP	CRP-cAMP	*cyaA*	AC	cAMP	*crr*	IIA^{glc}	IIA^{glc}~P	$glucose^{ext}$
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CRP			1.0	-0.1	-0.3	-0.5				
CRP-cAMP	-1	-1		-0.1	-0.2	-0.4			-0.1	
cyaA					1	1				
AC						1				
cAMP			1.0							
crr					1			1	1	
IIA^{glc}										
IIA^{glc}~P					1	1				
$glucose^{ext}$	-1	-1	-1	0.1	0.2	0.2				

$$\left\langle \frac{\text{CRP-cAMP}}{\text{AC}} \right\rangle = \left\langle \frac{\text{CRP-cAMP}}{cyaA} \right\rangle \left\langle \frac{cyaA}{\text{AC}} \right\rangle + \left\langle \frac{\text{CRP-cAMP}}{\text{IIA}^{glc} \sim \text{P}} \right\rangle \left\langle \frac{\text{IIA}^{glc} \sim \text{P}}{\text{AC}} \right\rangle$$

$$= (-0.1)(1) + (-0.1)(1) = -0.1 - 0.1 = -0.2$$

Dharmadi and Gonzalez. (2006).

"END" Features

(1) All knowledge about regulatory interactions in the network can be catalogued/summarized/integrated within a compact representation.

(2) Construction of END requires no aesthetic effort in spatial arrangement of the network elements.

(3) New elements and knowledge can be easily appended, and conversely, smaller subsets of the network can be easily extracted.

(4) Correlations between network elements are self-consistent and explicitly presented.

(5) Once mechanistic interactions are set as highest-level entries, other interactions can be inferred in a self-consistent manner through simple rules of arithmetic multiplication and addition.

(6) END can show how exertion of regulatory control can propagate through cooperativity with lower-level interactions.

(7) END is potentially useful as discovery tool for uncovering hidden regulatory structures: emerging properties.

Dharmadi and Gonzalez. (2006).

END of SURS: Role of Mlc

	crp	CRP	CRP-cAMP	*cyaA*	AC	cAMP	*crr*	IIA^{glc}	IIA^{glc}~P	$glucose^{ext}$	*ptsI*	EI	EI~P	PEP	Pyruvate	*ptsH*	HPr	HPr~P	*ptsG*	IIB^{glc}	IIB^{glc}~P	G6P	*xyl*	*lacY*	LacY	*mlc*	Mlc
crp		1	1	-0.2	-0.7	-1.1																					
CRP			1	-0.1	-0.3	-0.5																					
CRP-cAMP	-1	-1		-0.1	-0.2	-0.4			-0.1														1	1			
cyaA					1	1																					
AC						1																	1				
cAMP			1																				1				
crr					1	1		1	1																		
IIA^{glc}									-1											-1	1				-1		
IIA^{glc}~P					1	1		-1									1	-1					1				
$glucose^{ext}$	-1	-1	-1	0.1	-0.8	-1.8		1	-1											-1	1	-1	-1	-1	-2		
ptsI			1		1	1			1			1	1														
EI													-1				-1	1									
EI~P									1			-1		-1	1			-1									
PEP																											
Pyruvate												-1	1	-1													
ptsH			1		1	1			1								1	1									
HPr								-1	1									-1									
HPr~P									-1			1	-1				-1										
ptsG			-1		-1	-1			-1											1	1		-2				
IIB^{glc}										-1											-1	1					
IIB^{glc}~P								1	-1											-1							
G6P										-1										1	-1						
xyl																											
lacY																									1		
LacY																											
mlc																							2				1
Mlc																			-1				2				

Dharmadi and Gonzalez. (2006).

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END of SURS: Role of Mlc

	CRP-cAMP	AC	cAMP	IIAglc~P	*ptsG*	*xyl*	*mlc*	Mlc
CRP-cAMP		-0.2	-0.4	-0.1		1		
AC			1			1		
cAMP	1					1		
IIAglc~P		1	1			1		
ptsG	-1	-1	-1	-1		-2		
xyl								
mlc						2		1
Mlc					-1	2		

$$\left\langle \frac{\Delta mlc}{xyl} \right\rangle = \left\langle \frac{\Delta mlc}{mlc} \right\rangle \left\langle \frac{mlc}{\mathrm{Mlc}} \right\rangle \left\langle \frac{\mathrm{Mlc}}{ptsG} \right\rangle \left\langle \frac{ptsG}{xyl} \right\rangle = (-1)(1)(-1)(-2) = -2$$

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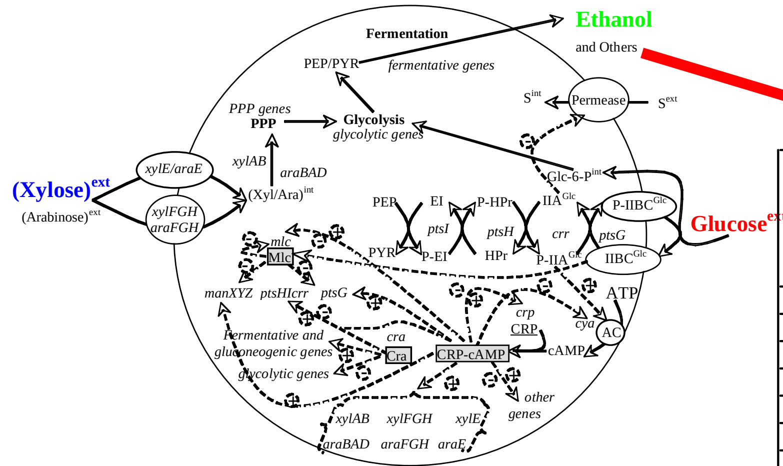
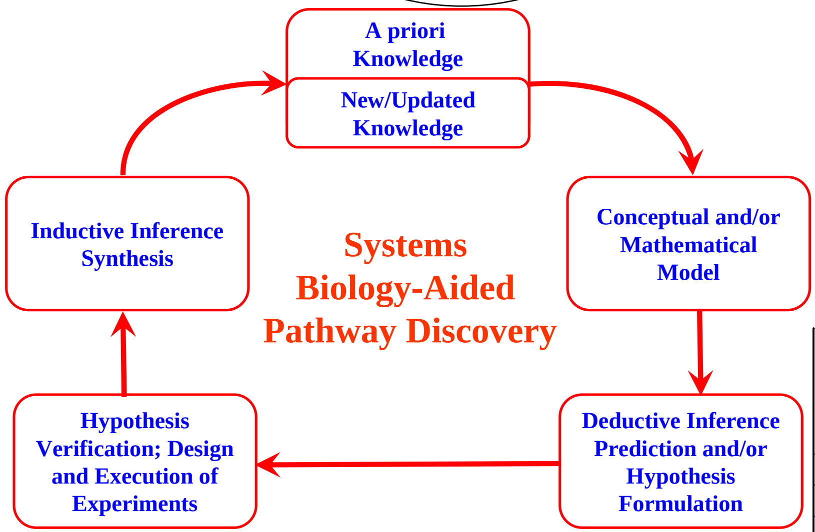
SURS and Ethanol Tolerance





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cAMP			1.0							
crr					1			1	1	
IIA^{glc}										
$IIA^{glc}\sim P$					1	1				
$glucose^{ext}$	-1	-1	-1	0.1	0.2	0.2				

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END of SURS: Role of Mlc

	CRP-cAMP	AC	cAMP	IIAglc~P	*ptsG*	*xyl*	*mlc*	Mlc
CRP-cAMP		-0.2	-0.4	-0.1		1		
AC			1			1		
cAMP	1					1		
IIAglc~P		1	1			1		
ptsG	-1	-1	-1	-1		-2		
xyl								
mlc						2		1
Mlc					-1	2		

$$\left\langle \frac{\Delta mlc}{xyl} \right\rangle = \left\langle \frac{\Delta mlc}{mlc} \right\rangle \left\langle \frac{mlc}{\text{Mlc}} \right\rangle \left\langle \frac{\text{Mlc}}{ptsG} \right\rangle \left\langle \frac{ptsG}{xyl} \right\rangle = (-1)(1)(-1)(-2) = -2$$

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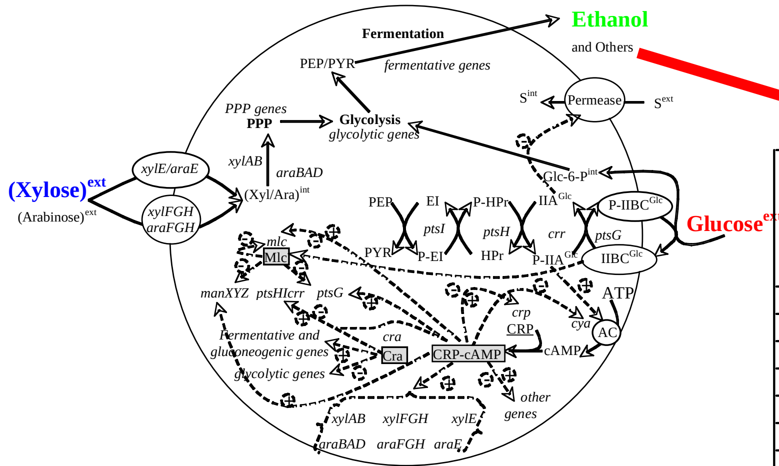
SURS and Ethanol Tolerance



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cyaA					1	1				
AC						1				
cAMP			1.0							
crr					1			1	1	
IIA^{glc}										
IIA^{glc}~P					1	1				
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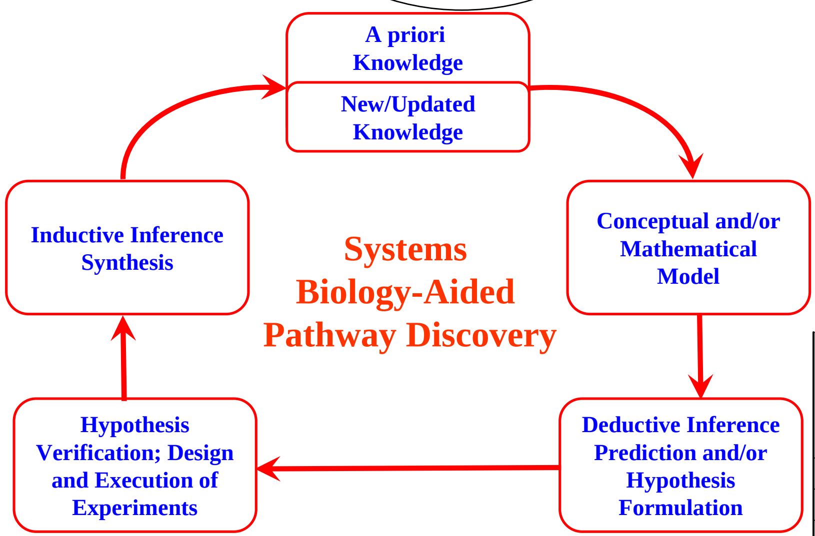

END of SURS: Role of Mlc

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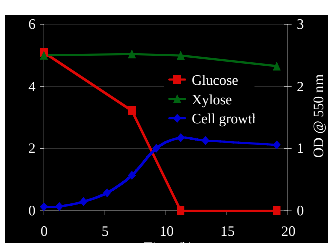
AC			1			1		
cAMP	1					1		
IIAglc~P		1	1			1		
ptsG	-1	-1	-1	-1		-2		
xyl								
mlc						2		1
Mlc					-1	2		



Growth of W3110	
Gluc.	yes
Xyl.	yes

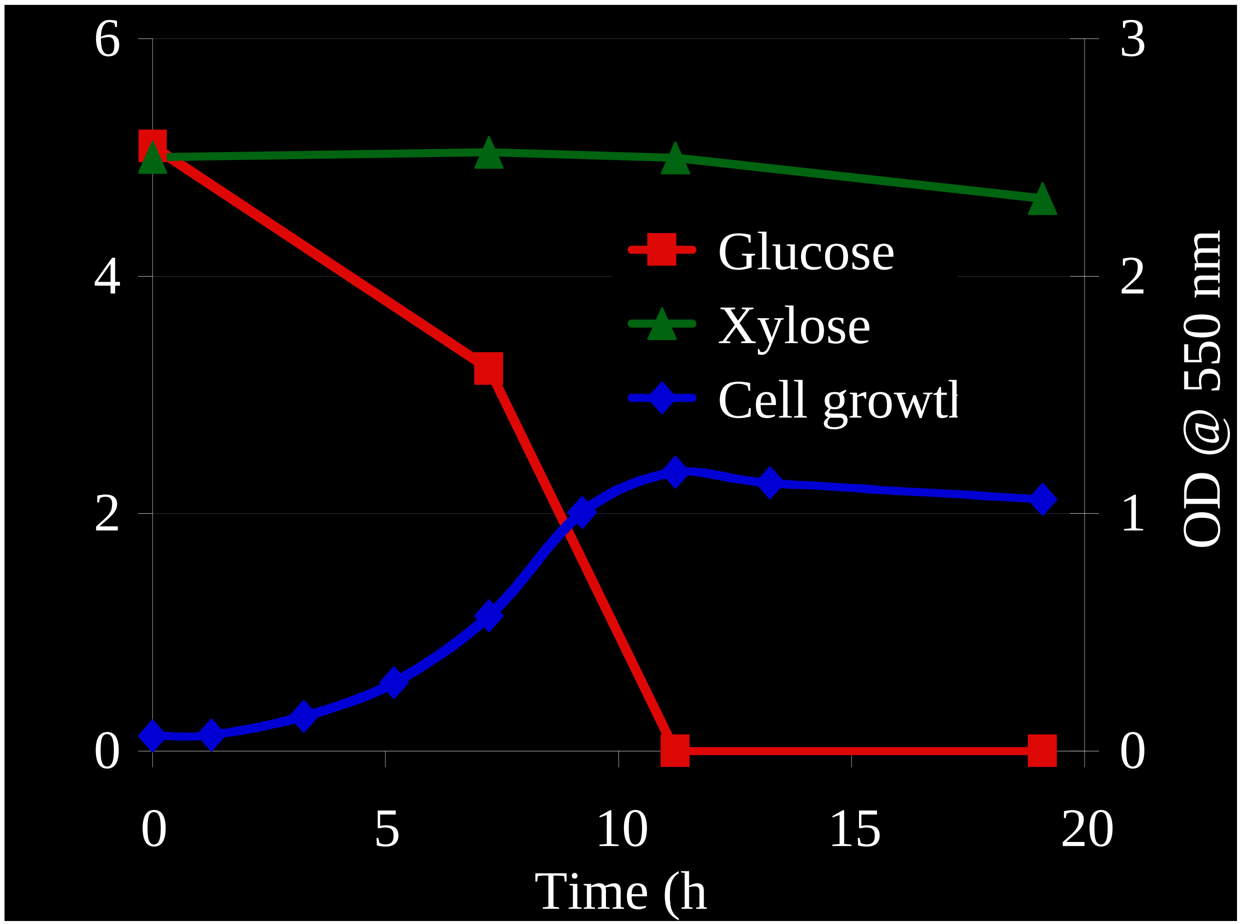
Growth of Δ*mlc*	
Gluc.	yes
Xyl.	no

$$\left\langle \frac{\Delta mlc}{xyl} \right\rangle = \left\langle \frac{\Delta mlc}{mlc} \right\rangle \left\langle \frac{mlc}{\text{Mlc}} \right\rangle \left\langle \frac{\text{Mlc}}{ptsG} \right\rangle \left\langle \frac{ptsG}{xyl} \right\rangle = (-1)(1)(-1)(-2) = -2$$

Role of Mlc on Xylose Utilization

Growth of W3110	
Glucose	yes
Xylose	yes

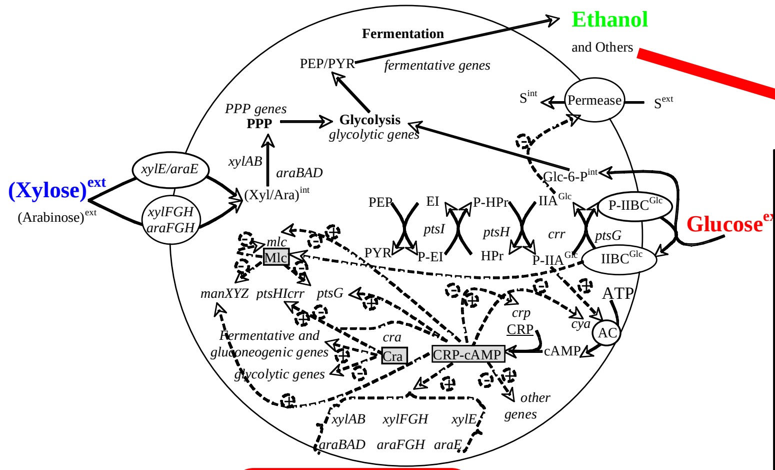
Growth of Δ*mlc*	
Glucose	yes
Xylose	no



Dharmadi and Gonzalez. (2006).

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

SURS and Ethanol Tolerance



A priori Knowledge

New/Updated Knowledge

Conceptual and/or Mathematical Model

Deductive Inference Prediction and/or Hypothesis Formulation

Hypothesis Verification; Design and Execution of Experiments

Inductive Inference Synthesis

Systems Biology-Aided Pathway Discovery

Elementary Network Decomposition (END)

	crp	CRP	CRP-cAMP	*cyaA*	AC	cAMP	*crr*	IIA^{glc}	IIA^{glc}~P	glucose^{ext}
crp		1	1	-0.2	-0.7	-1.1				
CRP			1.0	-0.1	-0.3	-0.5				
CRP-cAMP	-1	-1		-0.1	-0.2	-0.4			-0.1	
cyaA					1	1				
AC						1				
cAMP			1.0							
crr					1			1	1	
IIA^{glc}										
IIA^{glc}~P					1	1				
glucose^{ext}	-1	-1	-1	0.1	0.2	0.2				

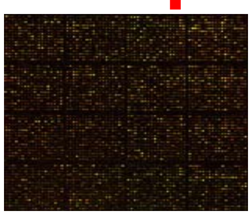
$$\left\langle \frac{\text{CRP-cAMP}}{\text{AC}} \right\rangle = \left\langle \frac{\text{CRP-cAMP}}{cyaA} \right\rangle \left\langle \frac{cyaA}{\text{AC}} \right\rangle + \left\langle \frac{\text{CRP-cAMP}}{\text{IIA}^{glc} \sim \text{P}} \right\rangle \left\langle \frac{\text{IIA}^{glc} \sim \text{P}}{\text{AC}} \right\rangle$$

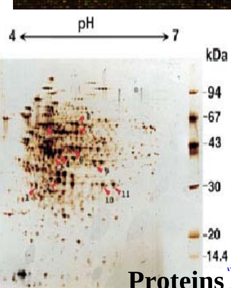
= (-0.1)(1) + (-0.1)(1) = -0.1 Š 0.1 = -0.2

END of SURS: Role of Mlc

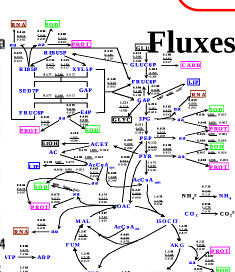
	CRP-cAMP	AC	cAMP	IIAglc~P	*ptsG*	*xyl*	*mlc*	Mlc
CRP-cAMP		-0.2	-0.4	-0.1		1		
AC			1			1		
cAMP	1					1		
IIAglc~P		1	1			1		
ptsG	-1	-1	-1	-1		-2		
xyl								
mlc						2		1
Mlc					-1	2		

$$\left\langle \frac{\Delta mlc}{xyl} \right\rangle = \left\langle \frac{\Delta mlc}{mlc} \right\rangle \left\langle \frac{mlc}{\text{Mlc}} \right\rangle \left\langle \frac{\text{Mlc}}{ptsG} \right\rangle \left\langle \frac{ptsG}{xyl} \right\rangle = (-1)(1)(-1)(-2) = -2$$





Proteins



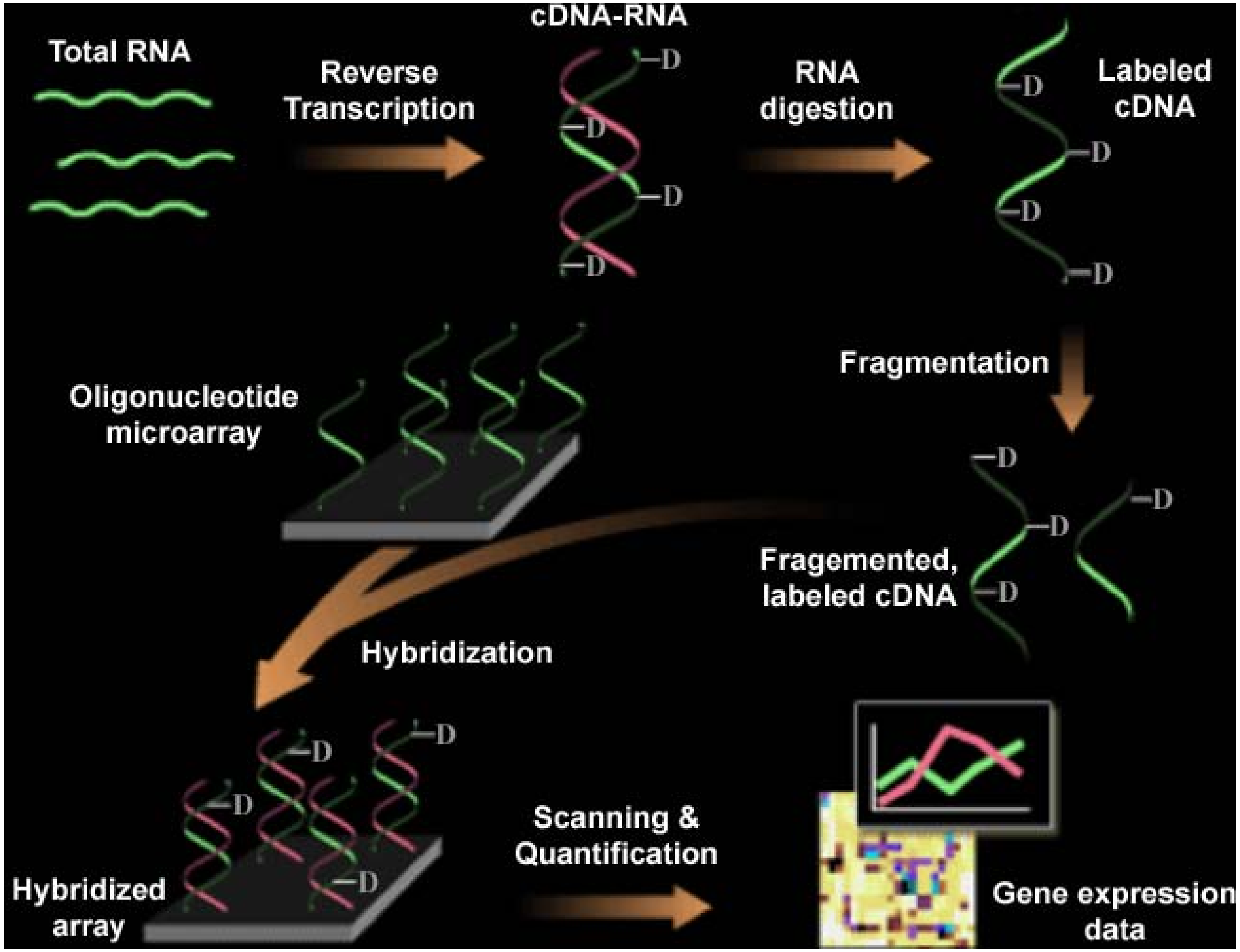
Fluxes

Growth of W3110	
Gluc.	yes
Xyl.	yes

Growth of Δ*mlc*	
Gluc.	yes
Xyl.	no

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

DNA Microarrays: Experimental procedure



Adapted from Affymetrix (www.affymetrix.com)

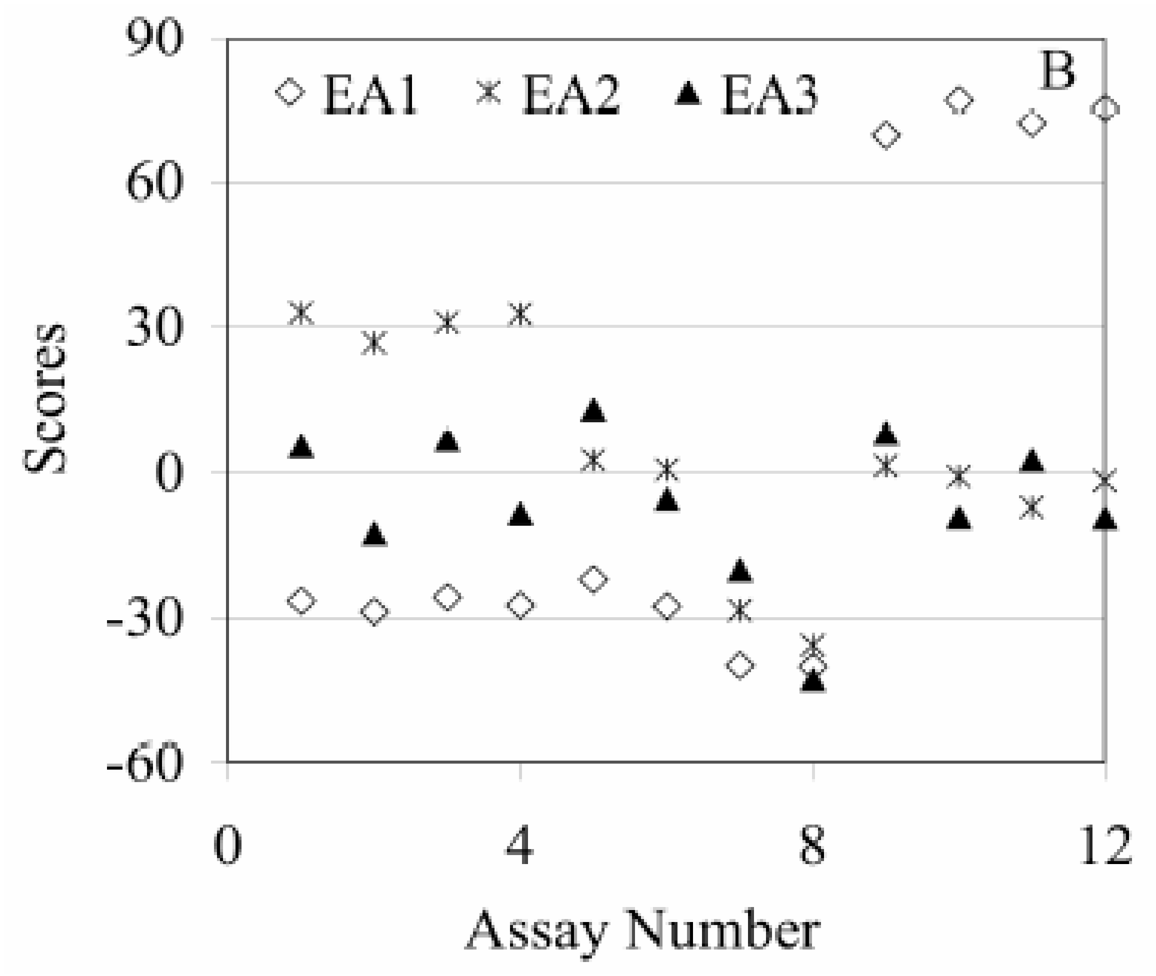
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

A Novel Data Mining Method to Identify Assay-Specific Signatures in Functional Genomic Studies

		Assay characteristics		
Assay	Strain	Sugar	1% EtOH in the initial medium	2% EtOH challenge
1	KO11	Glucose	NO	NO
2	LY01	Glucose	NO	NO
3	KO11	Xylose	NO	NO
4	LY01	Xylose	NO	NO
5	KO11	Glucose	YES	NO
6	LY01	Glucose	YES	NO
7	KO11	Xylose	YES	NO
8	LY01	Xylose	YES	NO
9	KO11	Glucose	NO	YES
10	LY01	Glucose	NO	YES
11	KO11	Xylose	NO	YES
12	LY01	Xylose	NO	YES

Rollins, Zhai, Joe, Guidarelli, Murarka, and Gonzalez. **(2006). BMC Bioinformatics: 7, 377.**

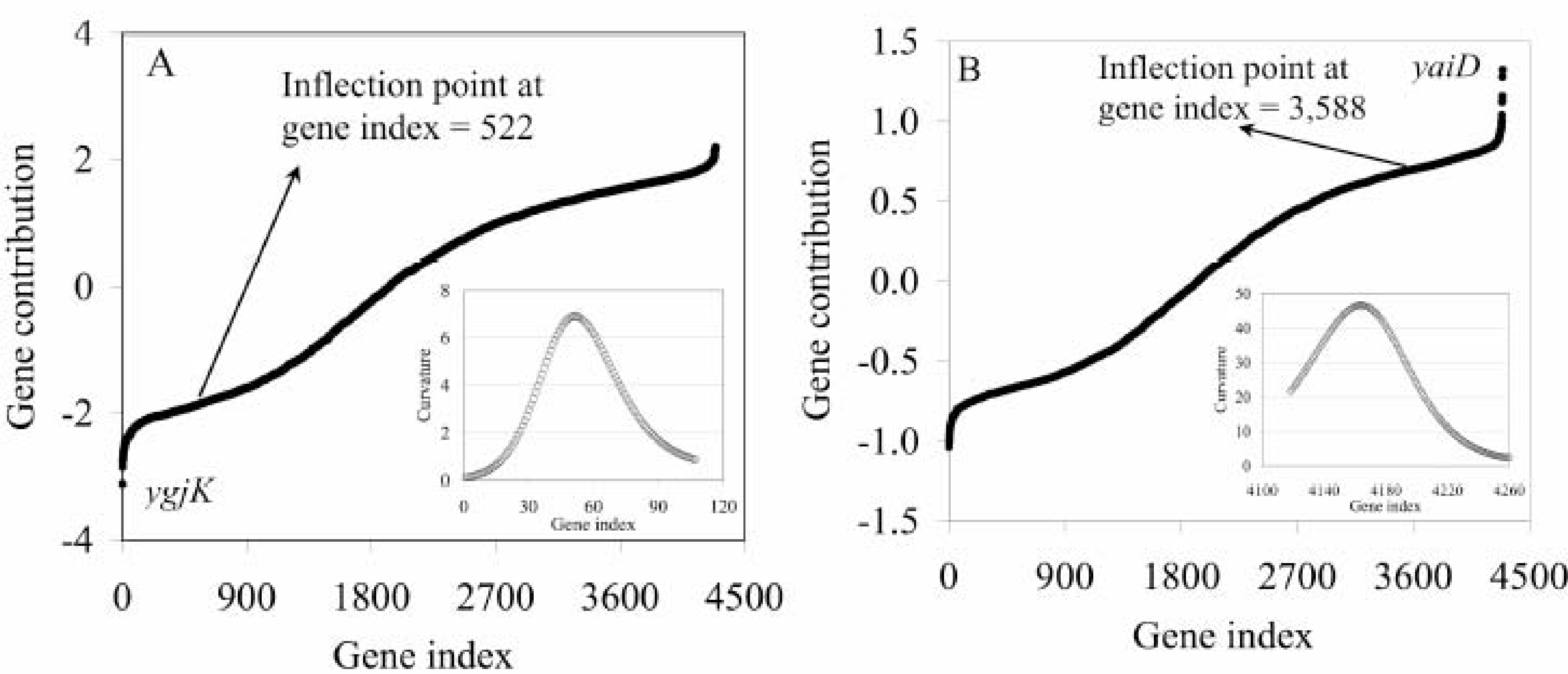
Assay Contribution: Identifying Associating Between Assays and Principal Components



The EAs efficiently identify signatures corresponding to ethanol- and non-ethanol-challenged cultures, presence and absence of ethanol in the initial culture medium, and a strain-specific signature

Rollins, Zhai, Joe, Guidarelli, Murarka, and Gonzalez**. (2006). BMC Bioinformatics: 7, 377.**

Identifying Response-to-Ethanol Signature



Complete gene signatures and subset of genes contributing the most to each signature are identified by the existence of points of inflection and maximum curvature, respectively.

Rollins, Zhai, Joe, Guidarelli, Murarka, and Gonzalez**. (2006). BMC Bioinformatics: 7, 377.**

Identifying Response-to-Ethanol Signature

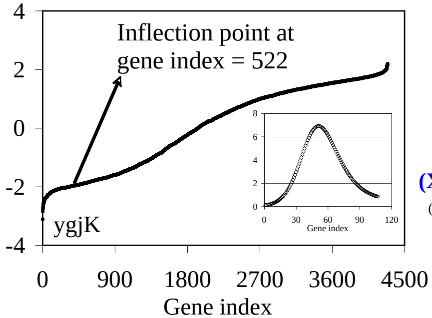
Gene Name	Rank	Expression Ratio	Gene Name	Rank	Expression Ratio
yaiD	1	298.3	*ygjK*	1	-20.9
argH	2	170.0	*tktA*	2	-5.2
mngA	3	85.6	*dsbC*	3	-8.5
plsC	4	76.9	*cvrA*	4	-2.3
caiA	5	300.3	*nrfE*	5	-11.9
yebU	6	51.6	*yehI*	6	-19.2
ylbF	7	29.9	*ybbA*	7	-4.1
nrfG	8	46.8	*evgS*	8	-16.2
yaiY	9	27.6	*ynfE*	9	-14.4
pnp	10	4.9	*pqiB*	10	-3.7

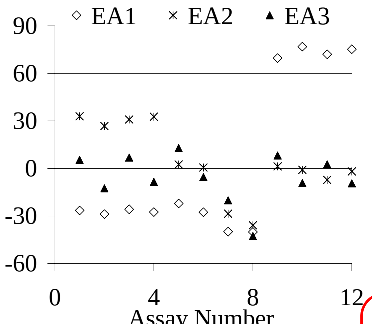
Many of the top-ranked genes encode functions that one would expect to be involved in the cellular response to an ethanol challenge such as the metabolism and transport of osmolytes (*mngA*, *cvrA*, and *caiA*), the biosynthesis of phospholipids (*plsC*) which are major constituents of the cell membrane, and the repairing of misfolded proteins (*dsbC*). In fact, increased tolerance to ethanol in certain E.coli strains is related to the increased availability of osmolytes like betaine and trehalose.

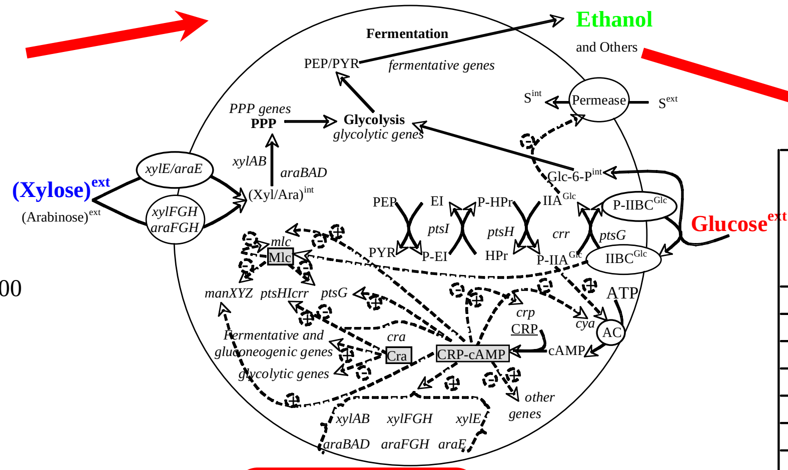
Rollins, Zhai, Joe, Guidarelli, Murarka, and Gonzalez**. (2006). BMC Bioinformatics: 7, 377.**

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

SURS and Ethanol Tolerance





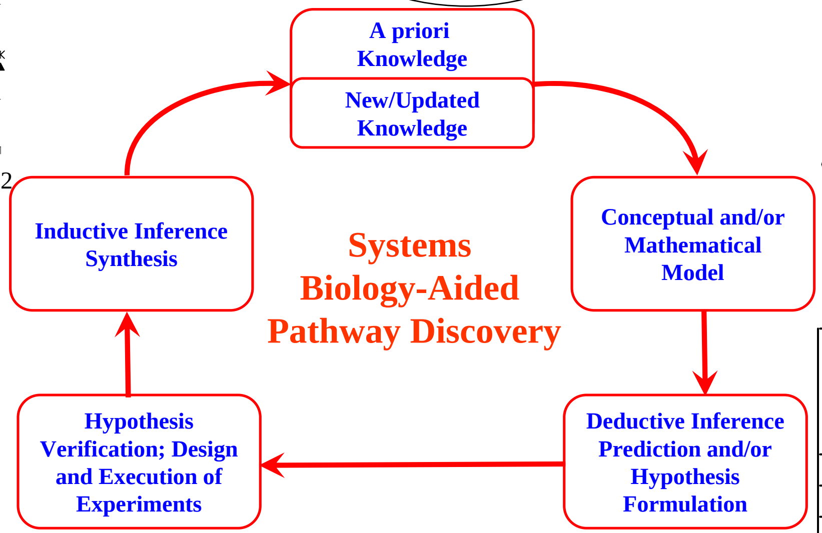


Elementary Network Decomposition (END)

	crp	CRP	CRP-cAMP	cyaA	AC	cAMP	crr	IIA^{glc}	IIA^{glc}~P	$glucose^{ext}$
crp		1	1	-0.2	-0.7	-1.1				
CRP			1.0	-0.1	-0.3	-0.5				
CRP-cAMP	-1	-1		-0.1	-0.2	-0.4			-0.1	
cyaA					1	1				
AC						1				
cAMP			1.0							
crr					1			1	1	
IIA^{glc}										
IIA^{glc}~P					1	1				
$glucose^{ext}$	-1	-1	-1	0.1	0.2	0.2				

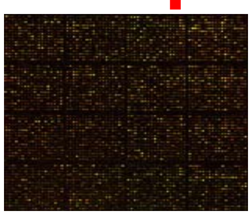
$$\left\langle \frac{\text{CRP-cAMP}}{\text{AC}} \right\rangle = \left\langle \frac{\text{CRP-cAMP}}{cyaA} \right\rangle \left\langle \frac{cyaA}{\text{AC}} \right\rangle + \left\langle \frac{\text{CRP-cAMP}}{\text{IIA}^{glc} \sim \text{P}} \right\rangle \left\langle \frac{\text{IIA}^{glc} \sim \text{P}}{\text{AC}} \right\rangle$$

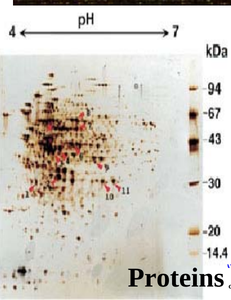
= (-0.1)(1) + (-0.1)(1) = -0.1 Š 0.1 = -0.2

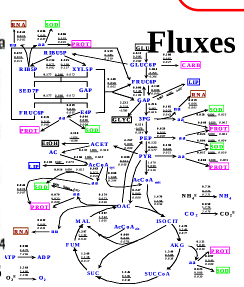


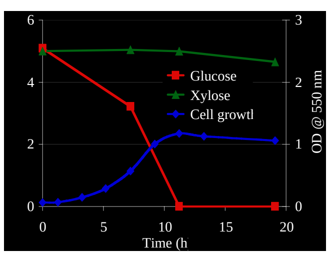
END of SURS: Role of Mlc

	CRP-cAMP	AC	cAMP	IIAglc~P	ptsG	xyl	mlc	Mlc
CRP-cAMP		-0.2	-0.4	-0.1		1		
AC			1			1		
cAMP	1					1		
IIAglc~P		1	1			1		
ptsG	-1	-1	-1	-1		-2		
xyl								
mlc						2		1
Mlc					-1	2		









Growth of W3110	
Gluc.	yes
Xyl.	yes

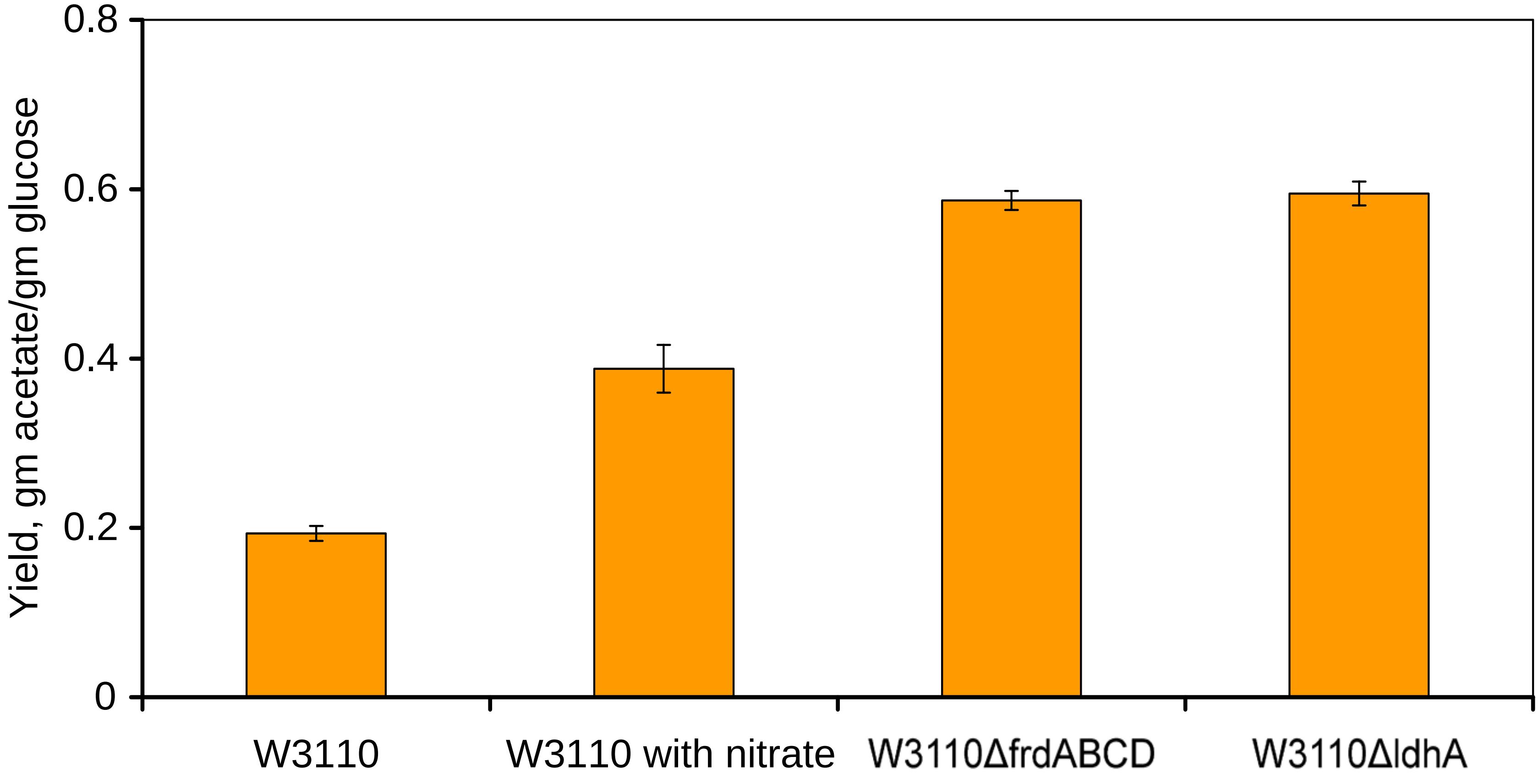
Growth of Δmlc	
Gluc.	yes
Xyl.	no

$$\left\langle \frac{\Delta mlc}{xyl} \right\rangle = \left\langle \frac{\Delta mlc}{mlc} \right\rangle \left\langle \frac{mlc}{\text{Mlc}} \right\rangle \left\langle \frac{\text{Mlc}}{ptsG} \right\rangle \left\langle \frac{ptsG}{xyl} \right\rangle = (-1)(1)(-1)(-2) = -2$$

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

Biorefinery

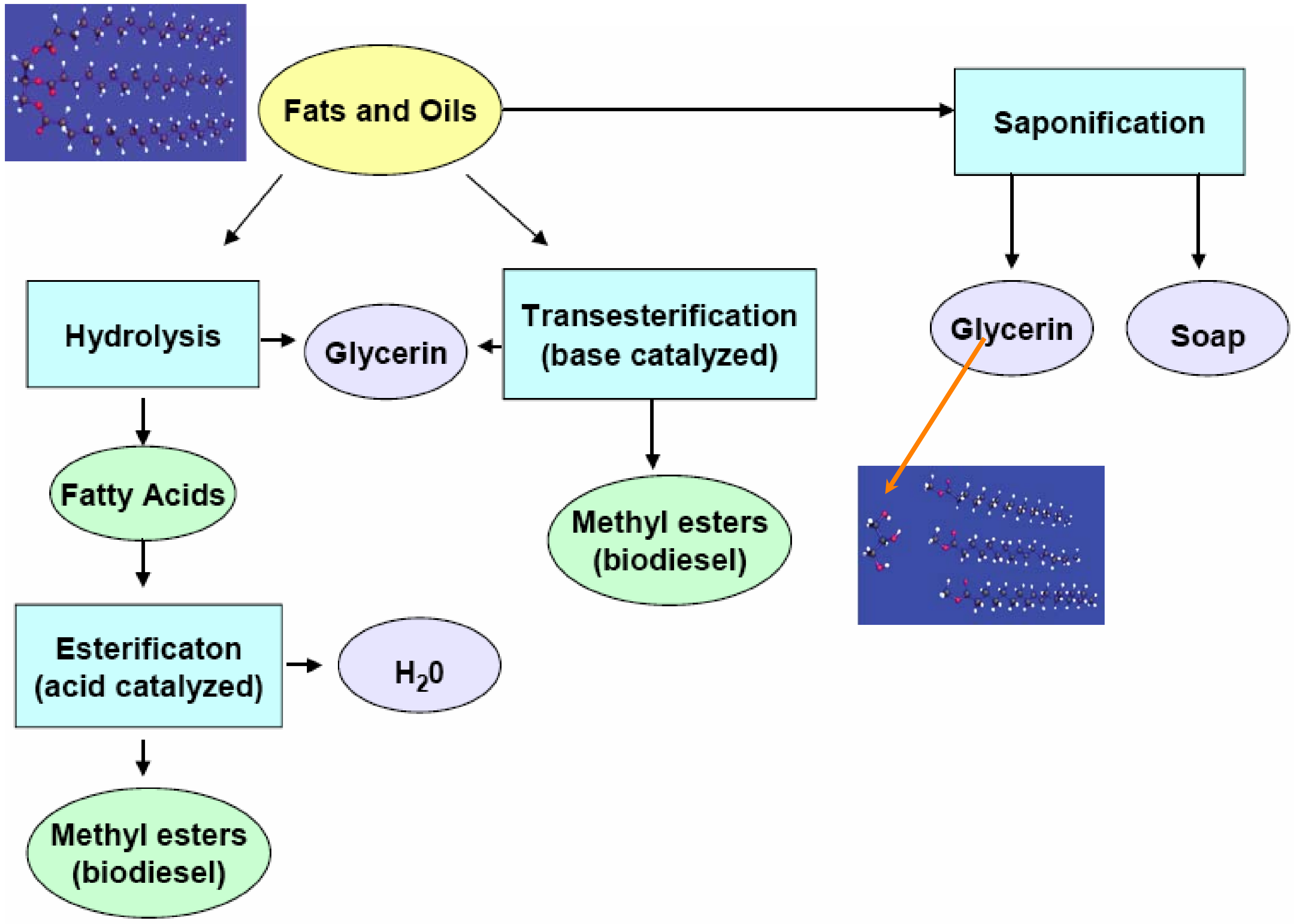
Production of Acetate: Yields



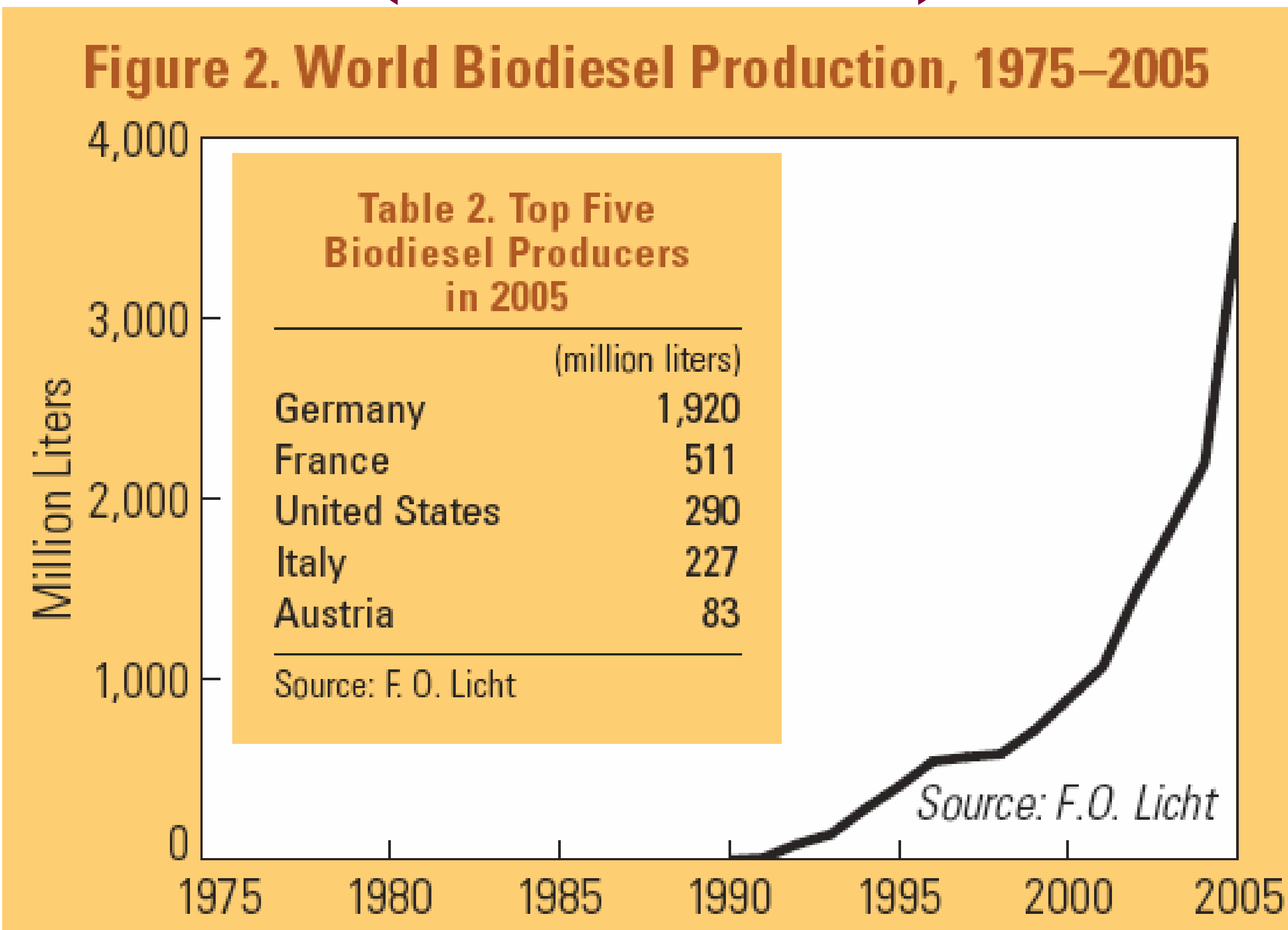
Obtained acetate yields ~90% of the maximum theoretical value

Smith et al., (2006). (*in prep.)*

The "Oil" Platform



World Production of Biodiesel (Million liters)



Biofuels For Transportation: Global Potential and Implications for Sustainable Agriculture and Energy in the 21st Century. Worldwatch Institute and German Agency for Technical Cooperation: www.worldwatch.org/pubs/biofuels.

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are needed to see this picture.

It is Not a Garage Industry!!

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Holmes, E. T. (Holmes Associates LLC). (2005). Biodiesel Southeast Perspective. Biofuels Workshop & Trade Show in Atlanta, Georgia, October 11, 2005

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Biodiesel Process Flow (Lurgi PSI)

95-100 lb

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

10 lb

Holmes, E. T. (Holmes Associates LLC). (2005). Biodiesel Southeast Perspective. Biofuels Workshop & Trade Show in Atlanta, Georgia, October 11, 2005

QuickTime™ and a
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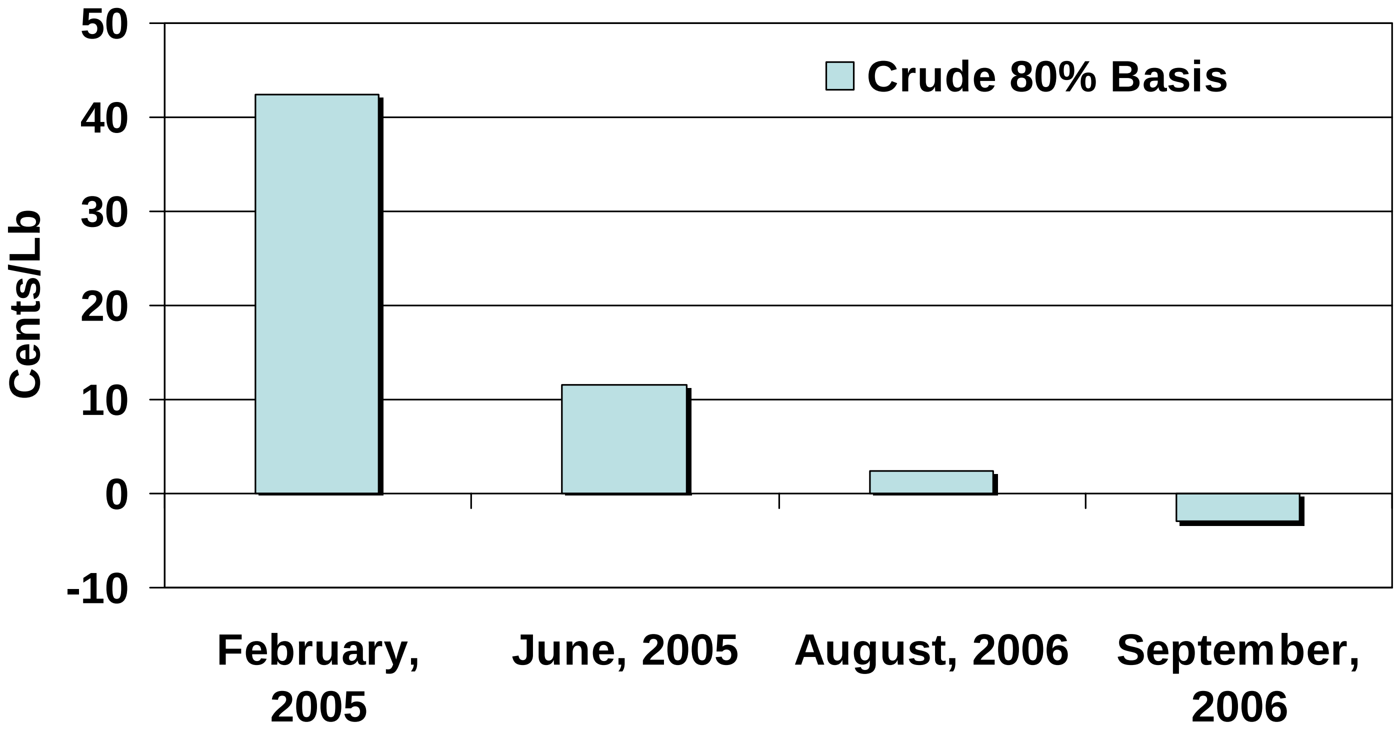
are needed to see this picture.

Crude Glycerin Prices



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Since 1865, The Jacobsen has been recognized as the **most insightful and accurate resource for** commentary and daily spot **market prices for the agricultural by-products industry**.

June 2004 • NREL/TP-510-34796

Technical Report

Biomass Oil Analysis: Research Needs and Recommendations

K. Shaine Tyson, Joseph Bozell, Robert Wallace,
Eugene Petersen, and Luc Moens

National Renewable Energy Laboratory
1617 Cole Boulevard, Golden, Colorado 80401-3393
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New Glycerol Platforms

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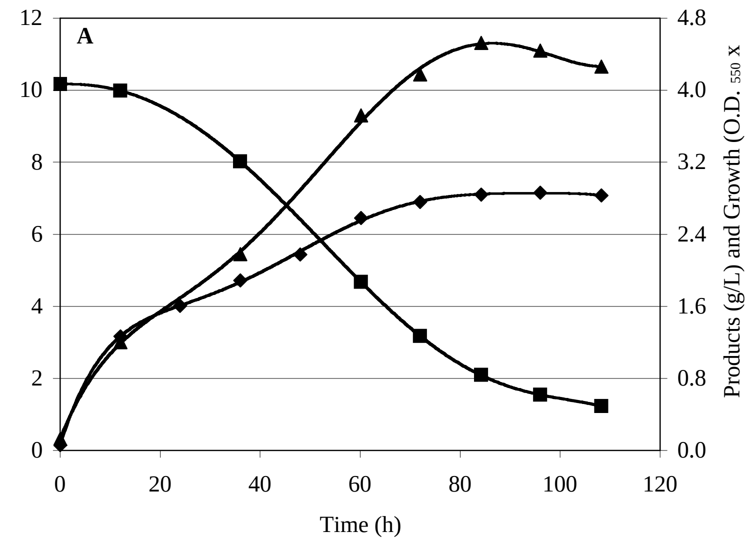
TIFF (LZW) decompressor
are needed to see this picture.

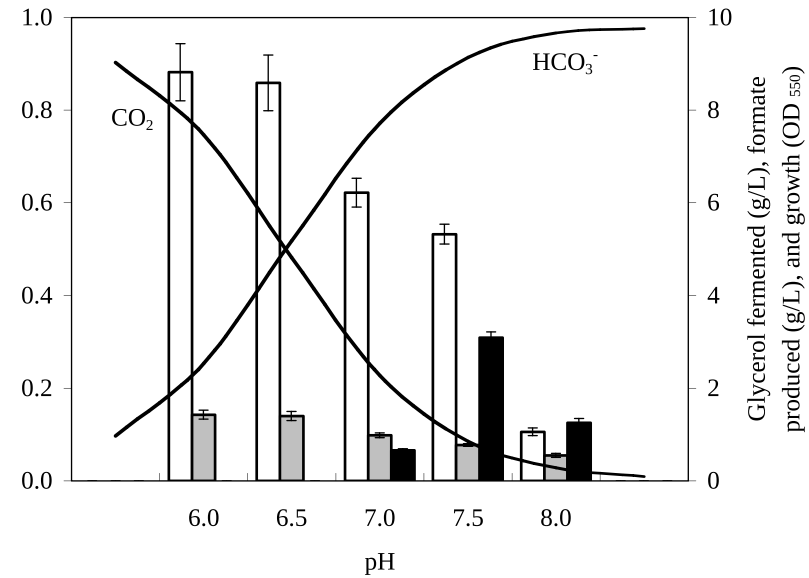
QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

Anaerobic Fermentation of Glycerol in *E. coli*

For nearly 80 years, researchers have believed that the metabolism of glycerol in *E. coli* is restricted to respiratory conditions: i.e., an external electron acceptor is needed.



- **Quastel JH, Stephenson M (1925). Biochem J. 19:660.**
- **Quastel JH, Stephenson M, Whetham MD (1925). Biochem J. 14:304.**
- **Lin EC (1976). Annu Rev Microbiol. 30:535-78.**
- **Booth I (2005). In Neidhardt FC, Curtiss III R, Ingraham JL, Lin ECC, Low KB, Magasanik B, Reznikoff WS, Riley M, Schaechter M, Umbarger HE (ed.), *E. coli* and Salmonella: cellular and molecular biology, Web Edition. ASM Press, Washington, DC. Available at www.ecosal.org.**



Dharmadi, Murarka, and Gonzalez. *Biotechnol. Bioeng.*, 2006: 94(5): 821-829.

Acknowledgements

Graduate Students:

Y. Dharmadi
A. Gupta
B. A. Murarka
V. Rigou
R. Sun

Undergraduate Students:

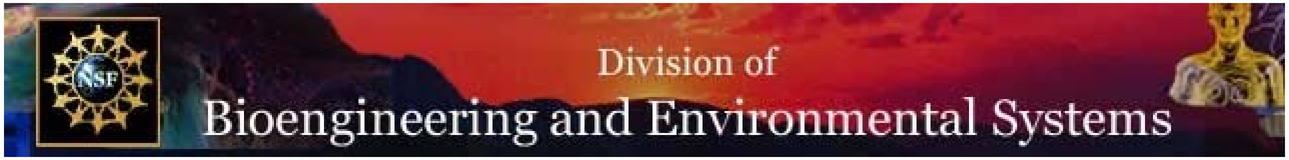
K. Smith
M. Yu

Collaborators:

J.V. Shanks, K-Y. San, M. Lamn, D. Rollins

USDA - National Research Initiative of the USDA Cooperative State Research, Education and Extension Service (2005-35504-15222/16698)

NSF - Division of Bioengineering and Environmental Systems (BES-0331388/0601549)



US Department of Energy (DEFG3603GO13159)