Glycolytic Flux in *Escherichia coli*: A Gene Array Perspective Fermenting Glucose & Xylose

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and Lonnie Ingram

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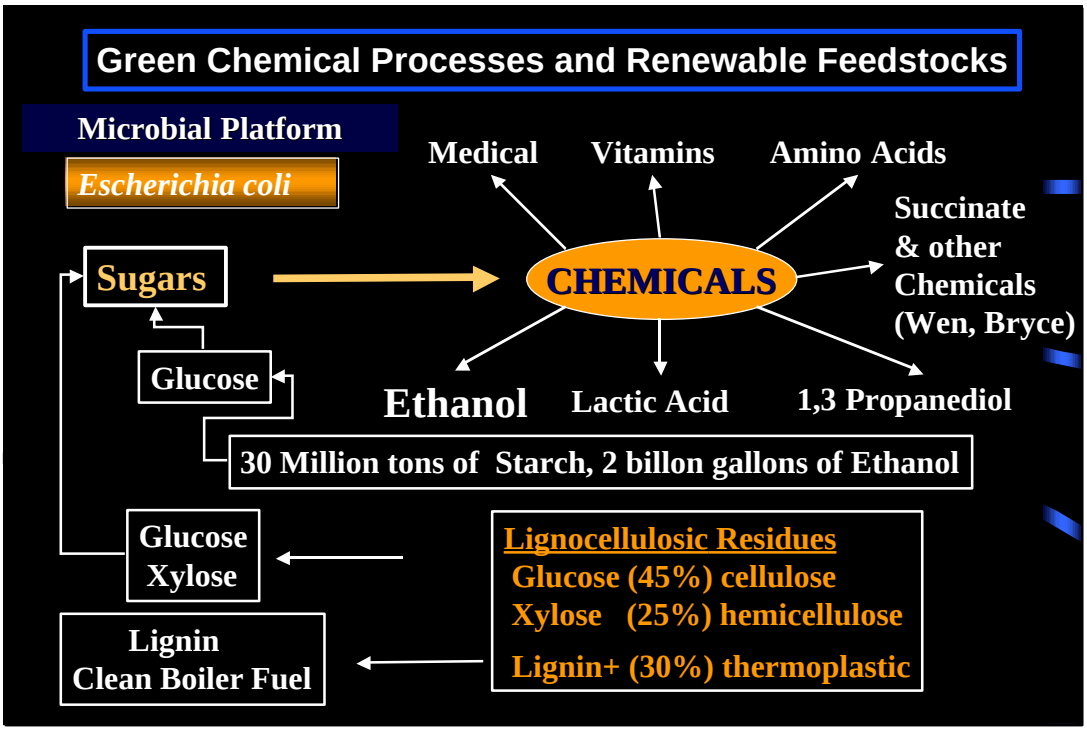
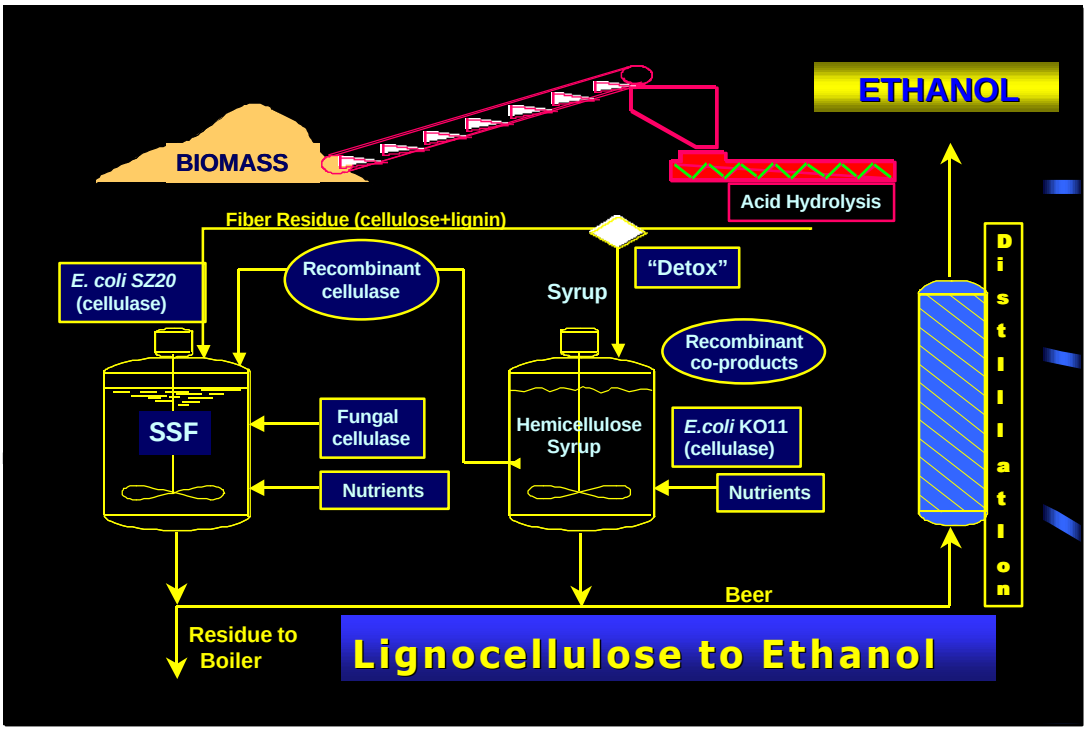
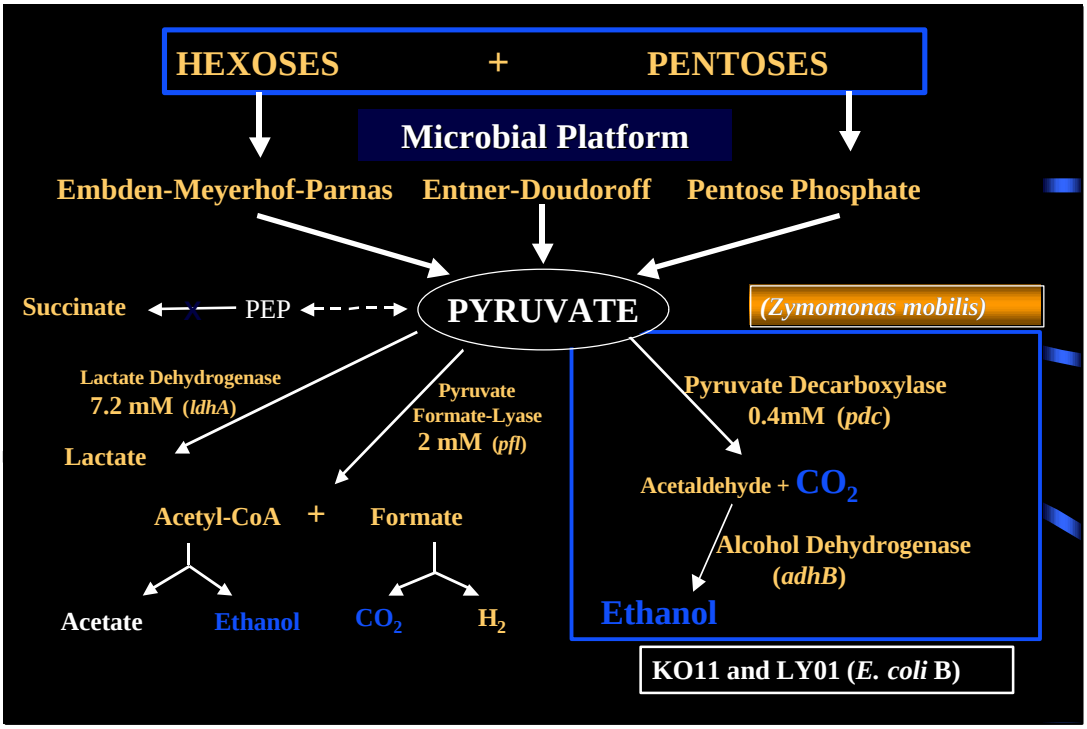
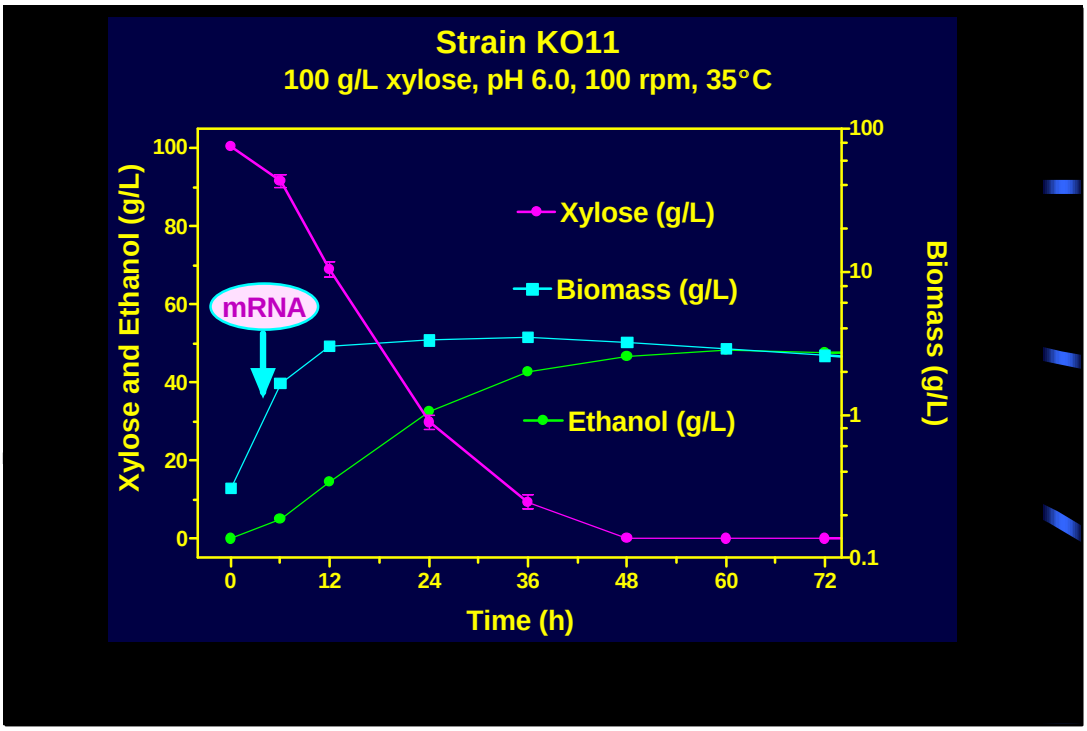


Sugar Cane Residues – Bagasse

near Lake Okeechobee, Florida



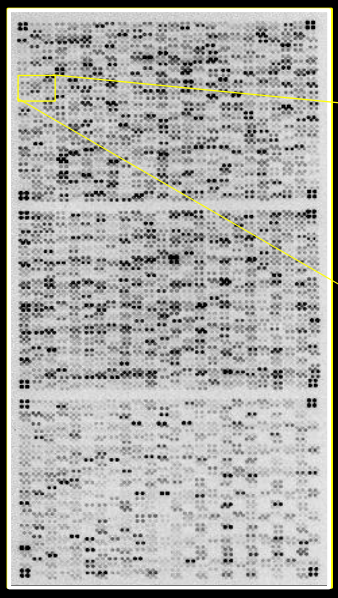

HEXOSES + PENTOSES
Microbial Platform
Embden-Meyerhof-Parnas
Entner-Doudoroff
Pentose Phosphate
Succinate
PEP
PYRUVATE
(Zymomonas mobilis)
Lactate Dehydrogenase
7.2 mM (ldhA)
Lactate
Pyruvate
Formate-Lyase
2 mM (pfl)
Acetyl-CoA + Formate
Acetate
Ethanol
CO2
H2
Pyruvate Decarboxylase
0.4mM (pdc)
Acetaldehyde + CO2
Alcohol Dehydrogenase
(adhB)
Ethanol
KO11 and LY01 (E. coli B)


Strain KO11
100 g/L xylose, pH 6.0, 100 rpm, 35°C
Xylose and Ethanol (g/L)
Biomass (g/L)
Time (h)
Xylose (g/L)
Biomass (g/L)
Ethanol (g/L)
mRNA
0
20
40
60
80
100
0.1
1
10
100
0
12
24
36
48
60
72

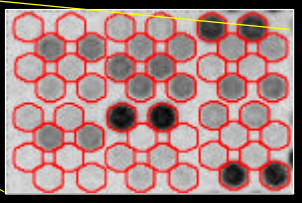
TRANSCRIPTOME ANALYSIS

Cells
↓
Hot Phenol
↓
DNase I
↓
Qiagen Column
↓
total RNA
↓
SuperScript II
(primers to 3' end,
33-P dCTP-label)
↓
cDNA

4,290 ORFs



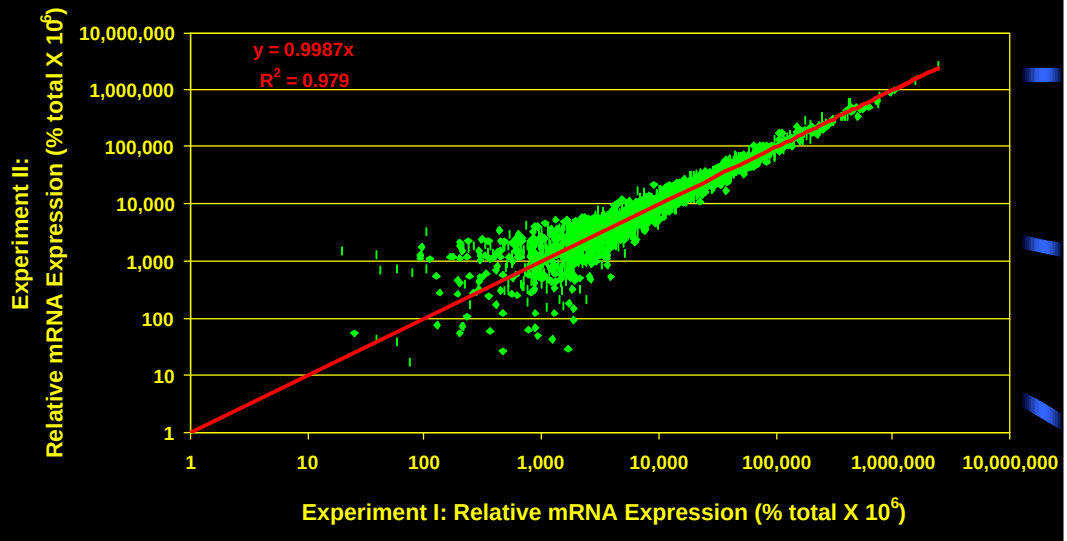
Hybridization for
16 h at 65C



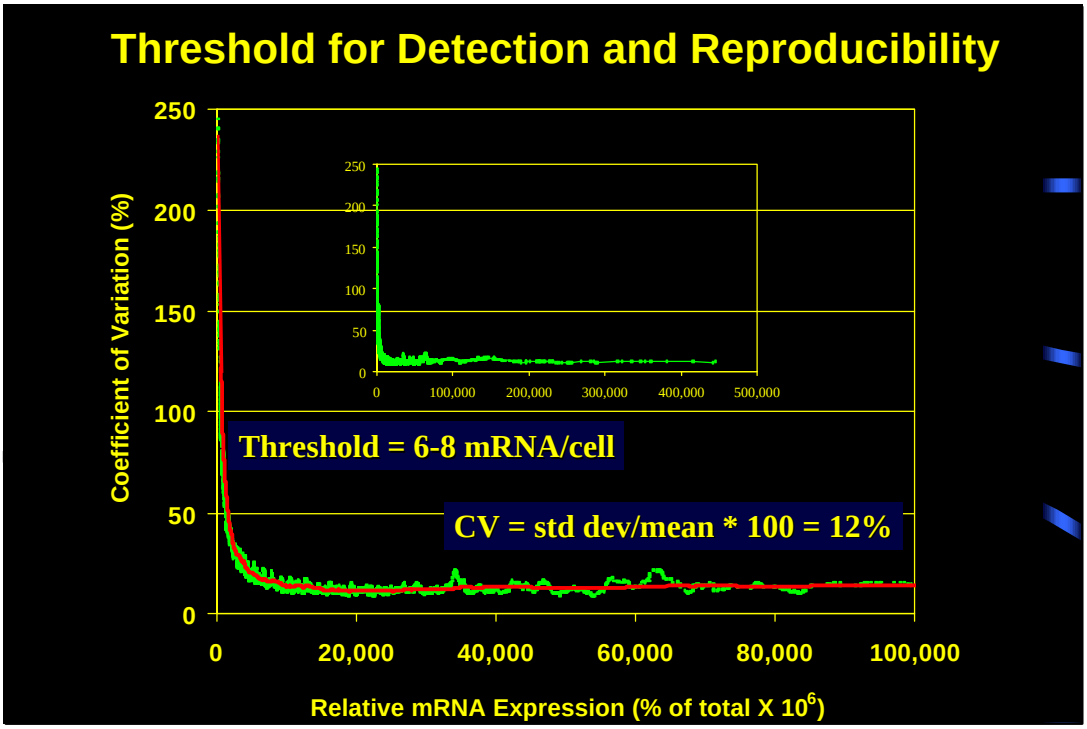
Sum of volumes
assigned value of
100 million

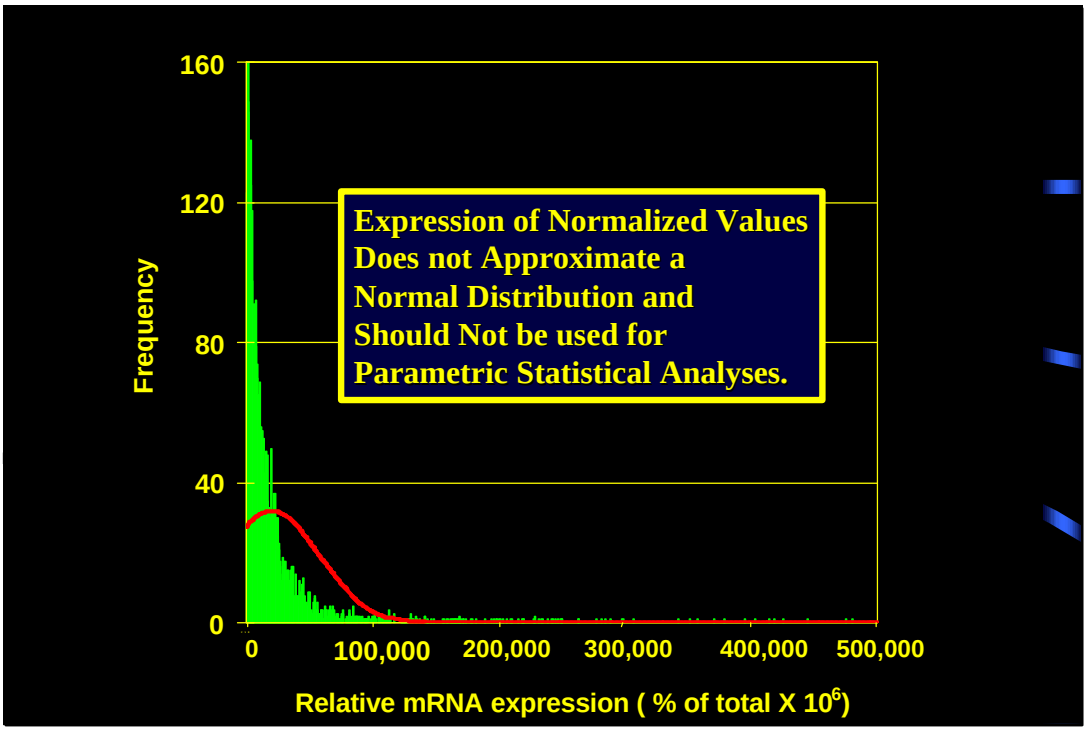
Values for each gene
expressed relative to
this total volume

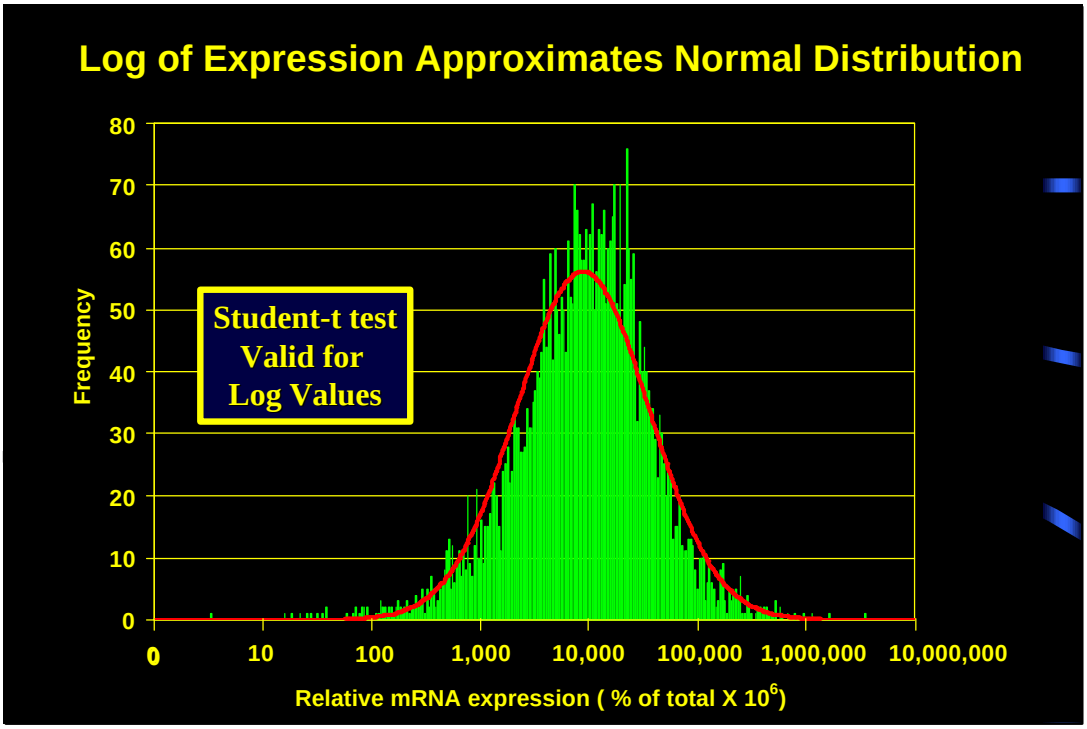
Reproducibility of Gene Array Measurements

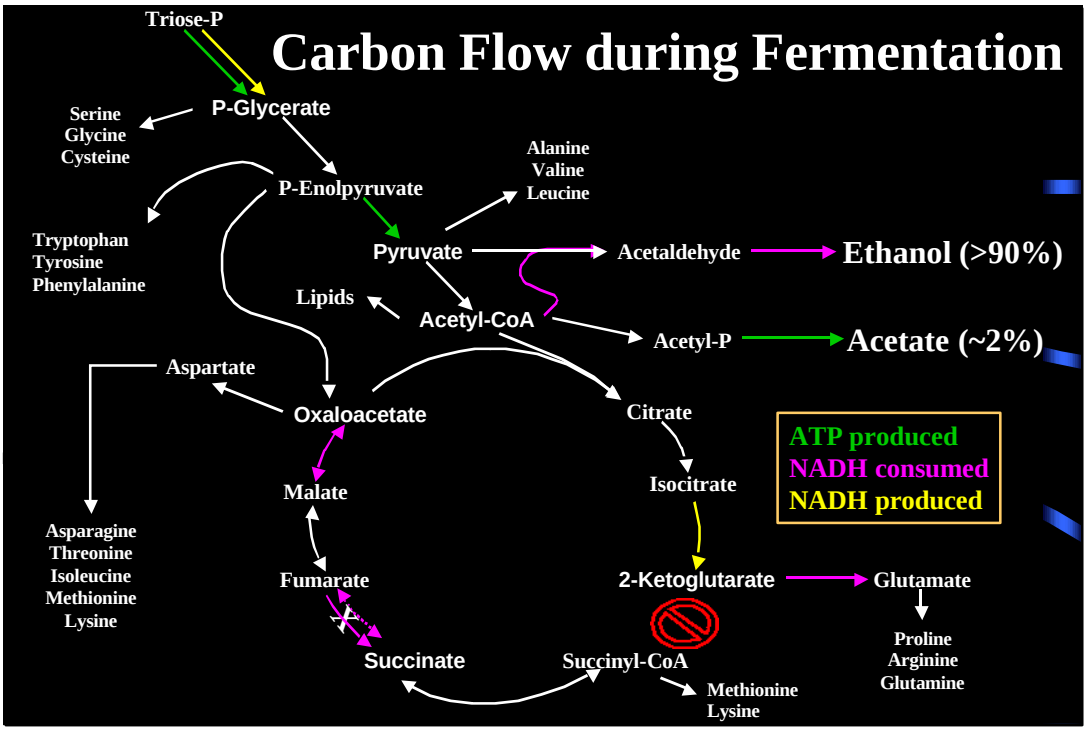


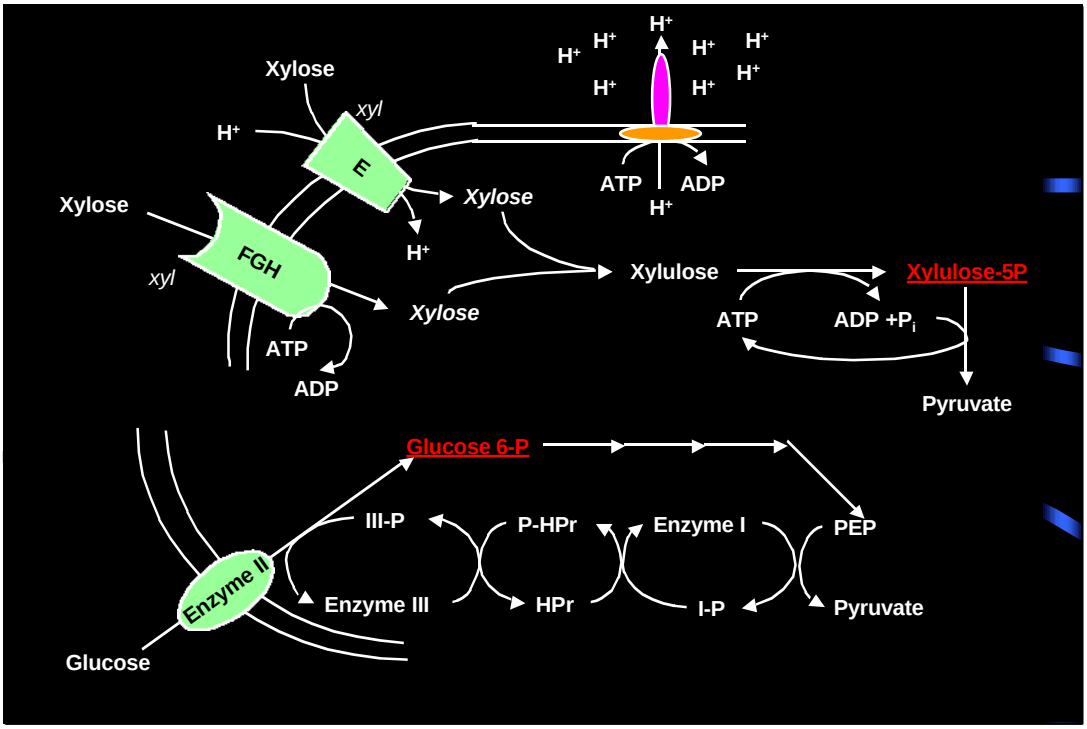
Threshold for Detection and Reproducibility

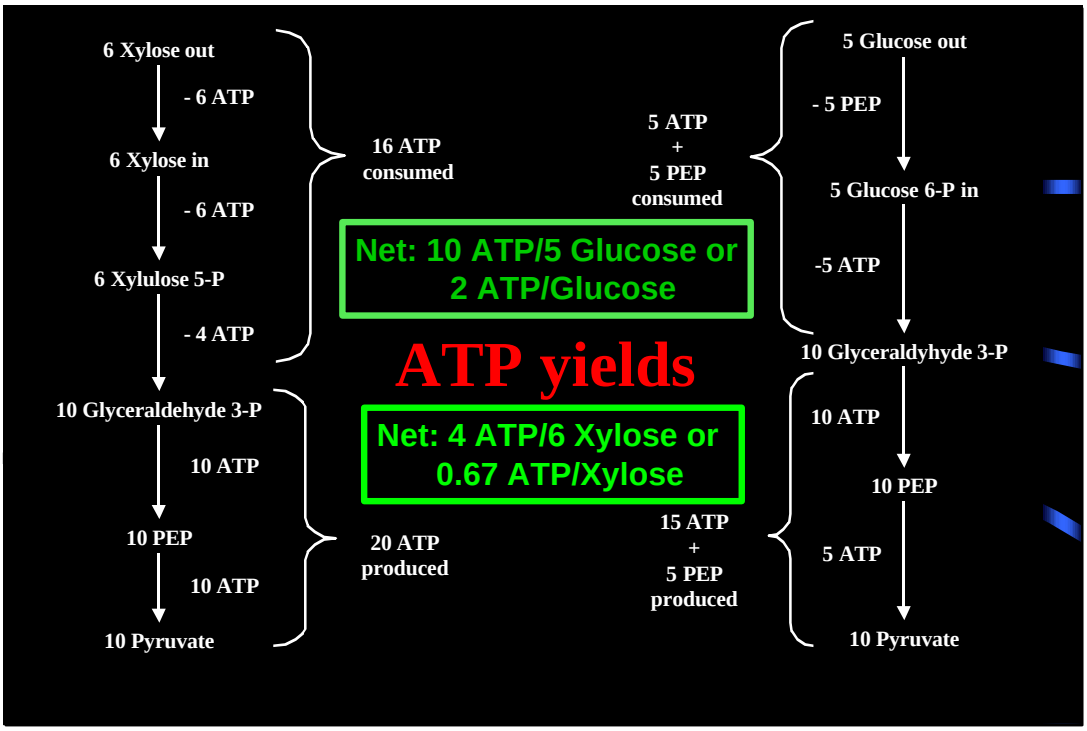


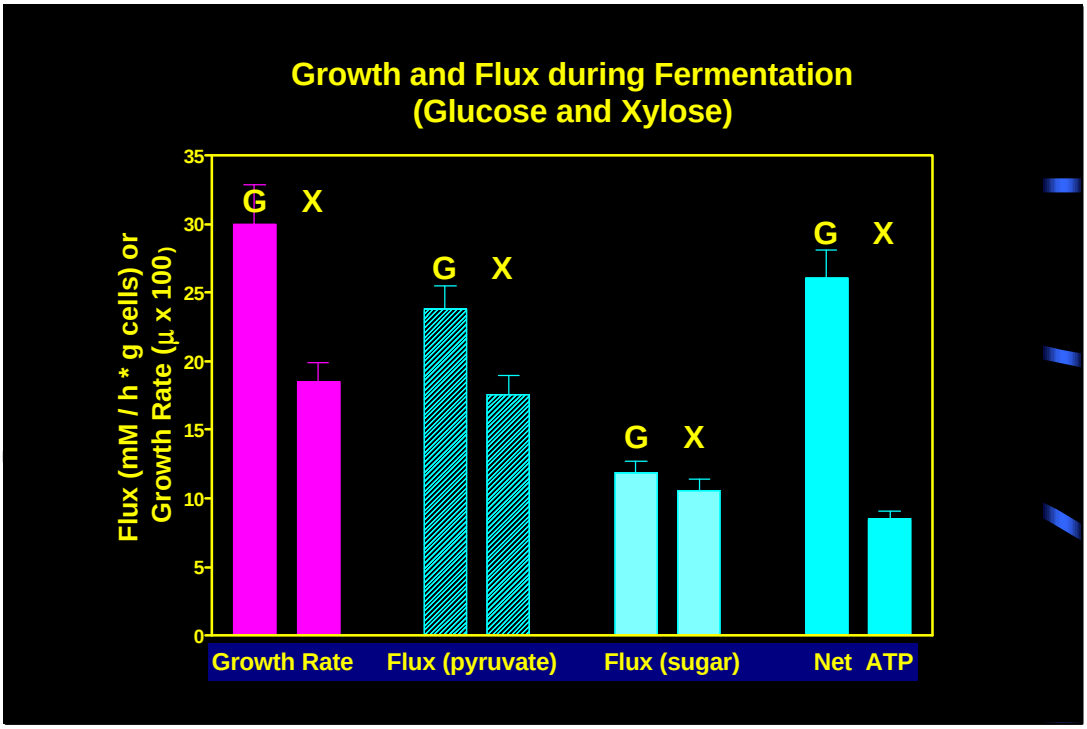


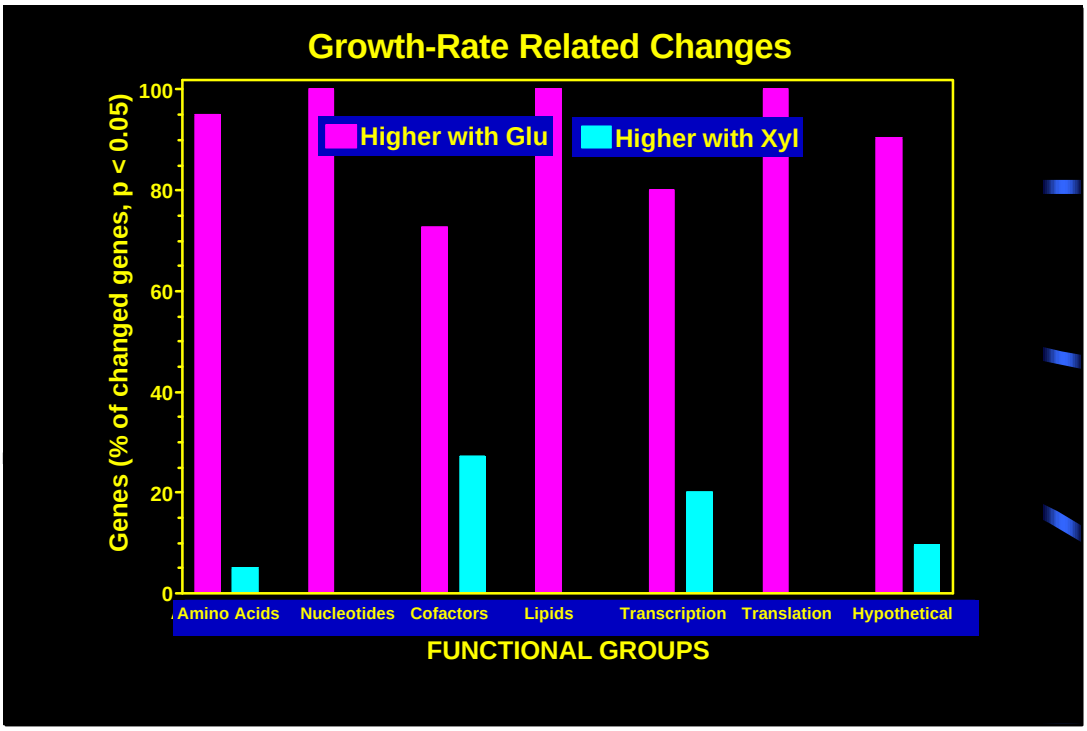

Log of Expression Approximates Normal Distribution
Frequency
80
70
60
50
40
30
20
10
0
Student-t test
Valid for
Log Values
0
10
100
1,000
10,000
100,000
1,000,000
10,000,000
Relative mRNA expression (% of total X 10^6)

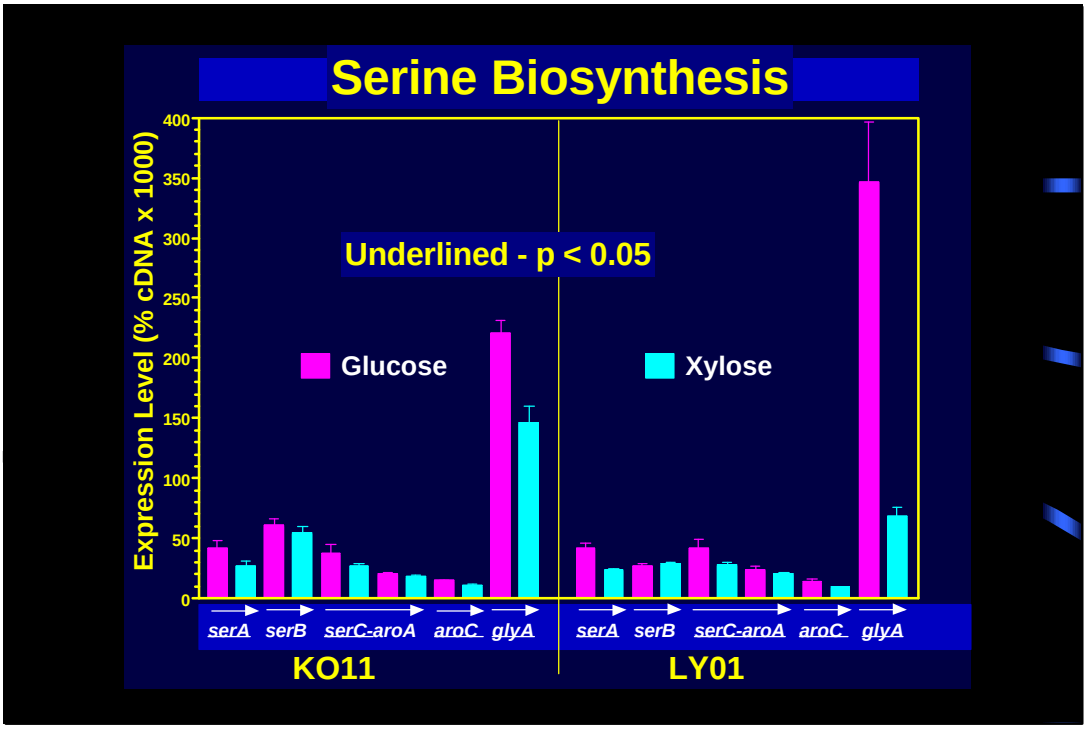

Carbon Flow during Fermentation
Triose-P
P-Glycerate
Serine
Glycine
Cysteine
P-Enolpyruvate
Alanine
Valine
Leucine
Tryptophan
Tyrosine
Phenylalanine
Pyruvate
Acetaldehyde
Ethanol (>90%)
Lipids
Acetyl-CoA
Acetyl-P
Acetate (~2%)
Aspartate
Oxaloacetate
Citrate
ATP produced
NADH consumed
NADH produced
Malate
Isocitrate
Asparagine
Threonine
Isoleucine
Methionine
Lysine
Fumarate
2-Ketoglutarate
Glutamate
Proline
Arginine
Glutamine
Succinate
Succinyl-CoA
Methionine
Lysine

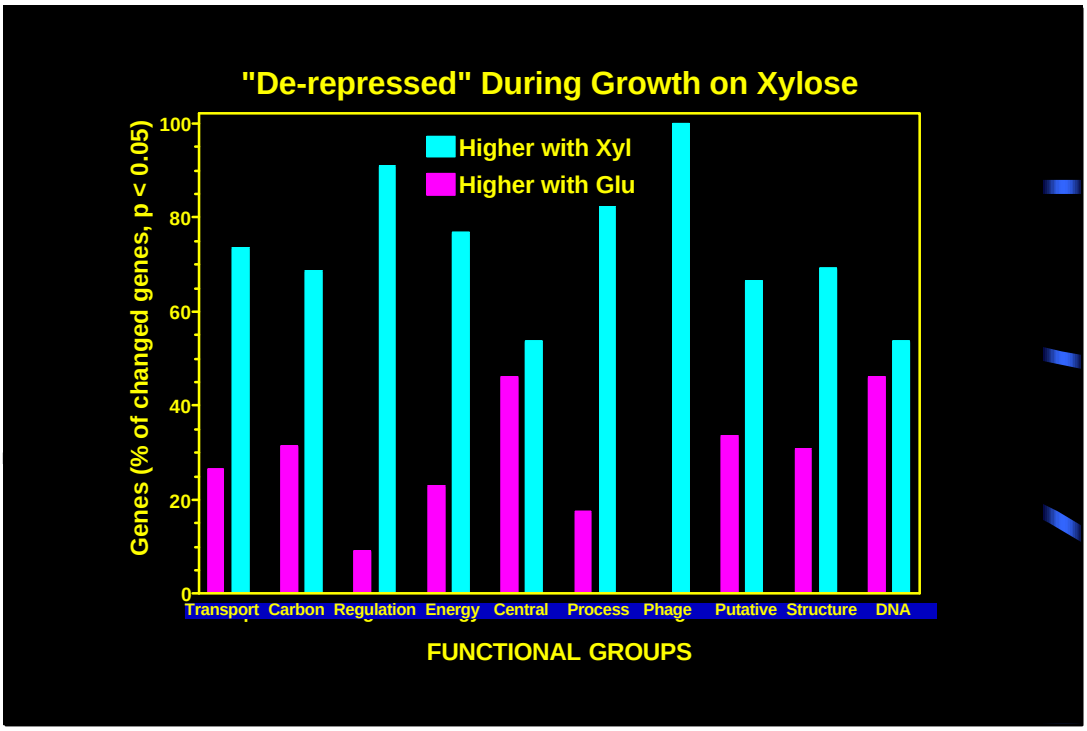

Xylose
xyl
H+
E
Xylose
H+
ATP
ADP
H+
Xylose
FGH
xyl
Xylose
Xylulose
Xylulose-5P
ATP
ADP +Pi
Pyruvate
ATP
ADP
Glucose 6-P
III-P
P-HPr
Enzyme I
PEP
Enzyme II
Enzyme III
HPr
I-P
Pyruvate
Glucose

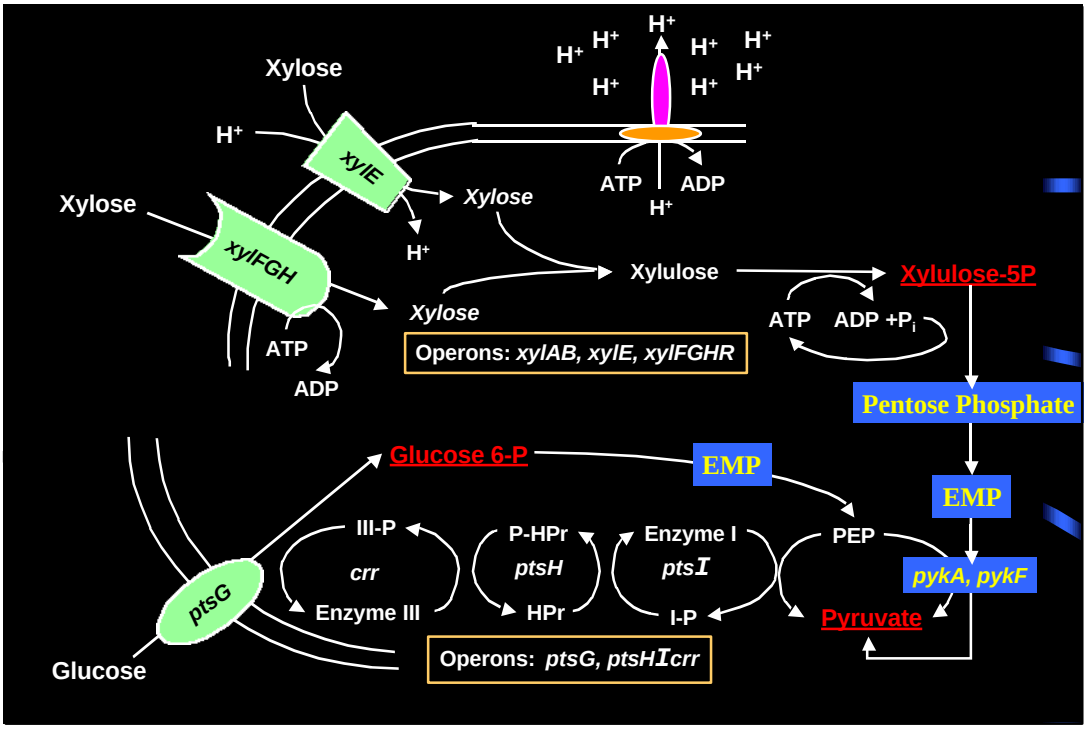

6 Xylose out
- 6 ATP
6 Xylose in
- 6 ATP
6 Xylulose 5-P
- 4 ATP
10 Glyceraldehyde 3-P
10 ATP
10 PEP
10 ATP
10 Pyruvate
16 ATP consumed
20 ATP produced
5 ATP + 5 PEP consumed
15 ATP + 5 PEP produced
5 Glucose out
- 5 PEP
5 Glucose 6-P in
-5 ATP
10 Glyceraldyhyde 3-P
10 ATP
10 PEP
5 ATP
10 Pyruvate
Net: 10 ATP/5 Glucose or 2 ATP/Glucose
ATP yields
Net: 4 ATP/6 Xylose or 0.67 ATP/Xylose

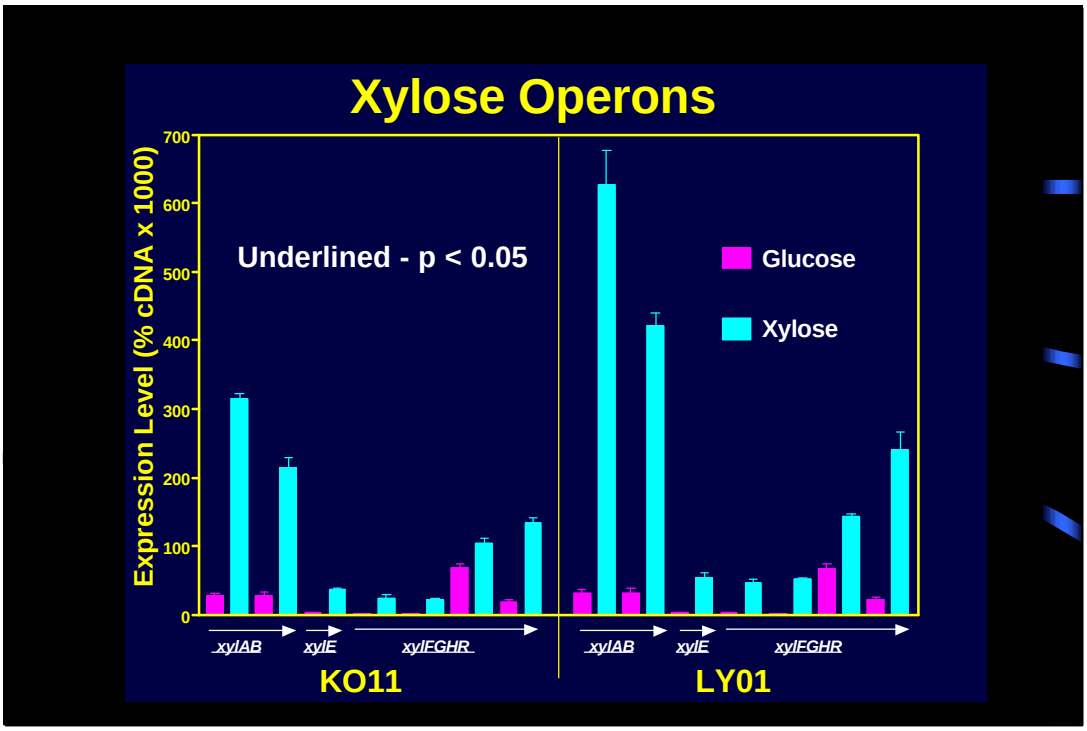

Growth and Flux during Fermentation
(Glucose and Xylose)
Flux (mM / h * g cells) or
Growth Rate (μ x 100)
35
30
25
20
15
10
5
0
G
X
G
X
G
X
G
X
Growth Rate
Flux (pyruvate)
Flux (sugar)
Net ATP

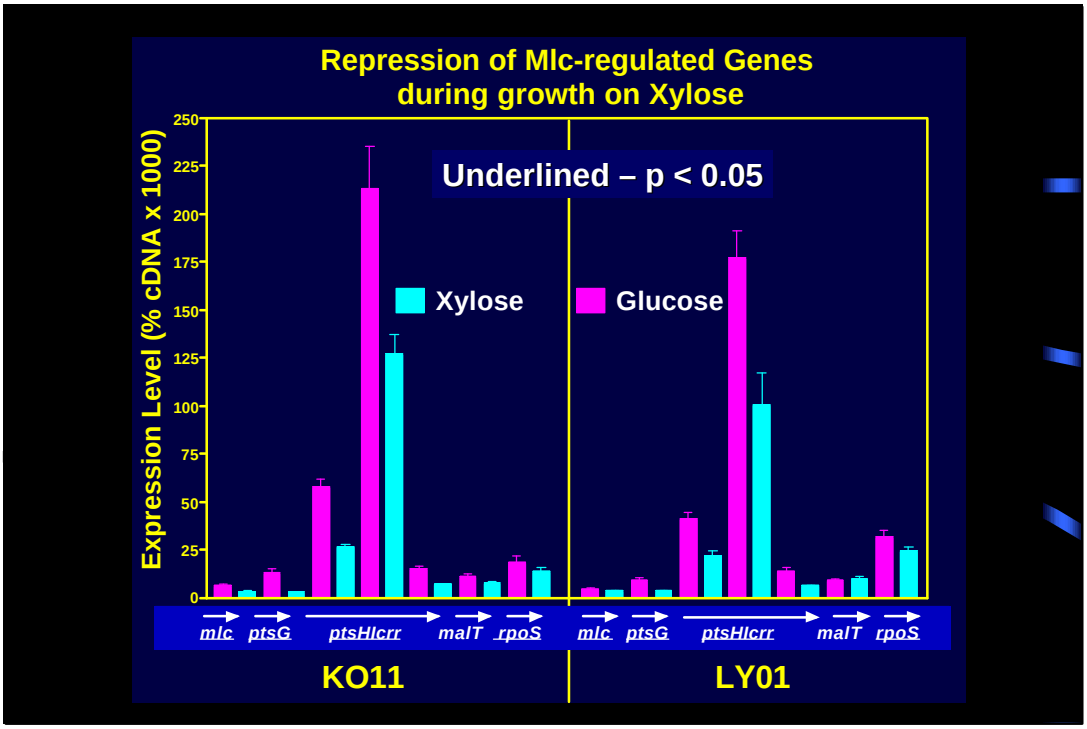

Growth-Rate Related Changes
Genes (% of changed genes, p < 0.05)
100
80
60
40
20
0
Higher with Glu
Higher with Xyl
Amino Acids
Nucleotides
Cofactors
Lipids
Transcription
Translation
Hypothetical
FUNCTIONAL GROUPS

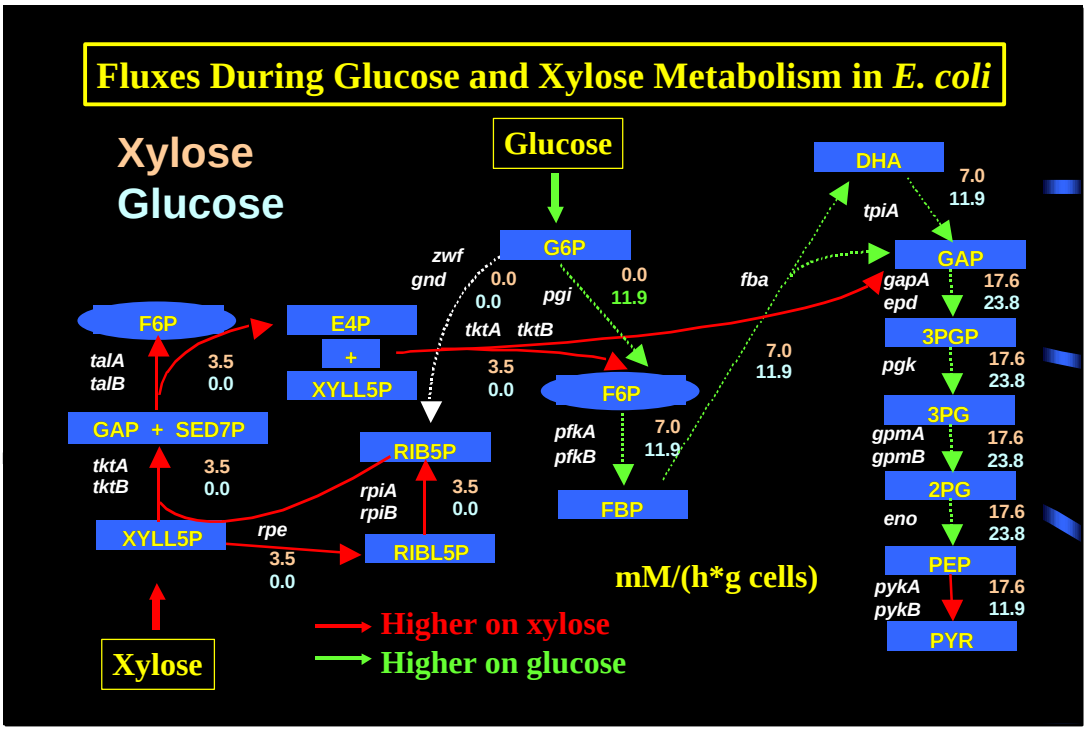

Serine Biosynthesis
Expression Level (% cDNA x 1000)
400
350
300
250
200
150
100
50
0
Underlined - p < 0.05
Glucose
Xylose
serA serB serC-aroA aroC glyA
serA serB serC-aroA aroC glyA
KO11
LY01


"De-repressed" During Growth on Xylose
Genes (% of changed genes, p < 0.05)
100
80
60
40
20
0
Higher with Xyl
Higher with Glu
Transport Carbon Regulation Energy Central Process Phage Putative Structure DNA
FUNCTIONAL GROUPS

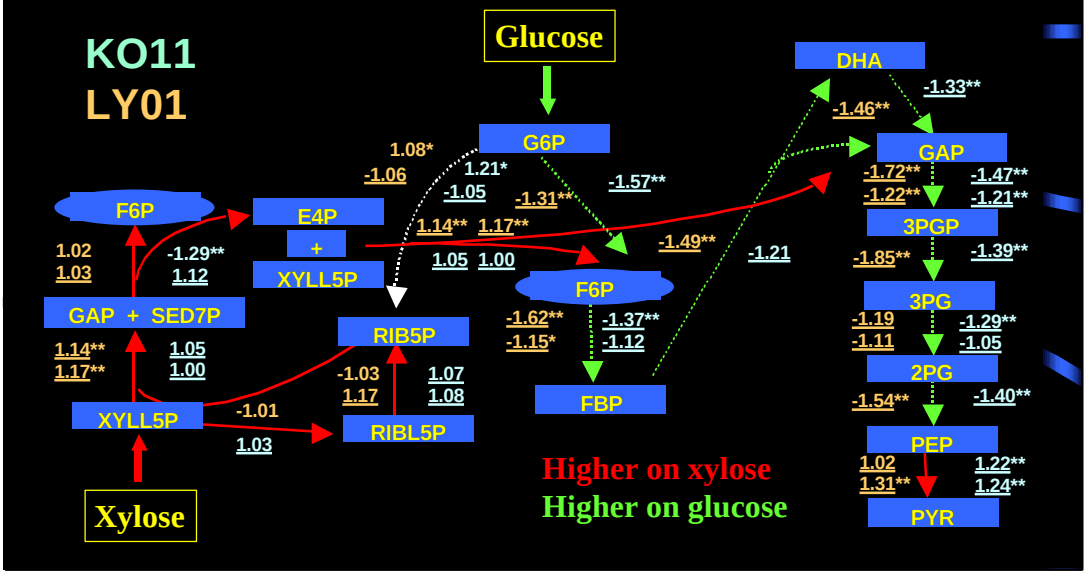

Xylose
H+
xylE
Xylose
xylFGH
Xylose
ATP
ADP
H+
Xylose
Xylulose
Xylulose-5P
ATP
ADP +Pi
Operons: xylAB, xylE, xylFGHR
Pentose Phosphate
EMP
Glucose 6-P
EMP
III-P
crr
Enzyme III
P-HPr
ptsH
HPr
Enzyme I
ptsI
I-P
PEP
pykA, pykF
Pyruvate
ptsG
Glucose
Operons: ptsG, ptsHIcrr


Xylose Operons
Expression Level (% cDNA x 1000)
700
600
500
400
300
200
100
0
Underlined - p < 0.05
Glucose
Xylose
xylAB
xylE
xylFGHR
KO11
LY01


Repression of Mlc-regulated Genes
during growth on Xylose
Expression Level (% cDNA x 1000)
250
225
200
175
150
125
100
75
50
25
0
Underlined – p < 0.05
Xylose
Glucose
mlc
ptsG
ptsHIcrr
malT
rpoS
KO11
LY01


Fluxes During Glucose and Xylose Metabolism in E. coli
Xylose
Glucose
Glucose
G6P
zwf
gnd
0.0
0.0
pgi
0.0
11.9
F6P
talA
talB
3.5
0.0
E4P
+
XYLL5P
tktA
tktB
3.5
0.0
F6P
GAP + SED7P
tktA
tktB
3.5
0.0
RIB5P
rpiA
rpiB
3.5
0.0
XYLL5P
rpe
3.5
0.0
RIBL5P
pfkA
pfkB
7.0
11.9
FBP
fba
DHA
7.0
11.9
tpiA
GAP
gapA
epd
17.6
23.8
3PGP
pgk
17.6
23.8
3PG
gpmA
gpmB
17.6
23.8
2PG
eno
17.6
23.8
PEP
pykA
pykB
17.6
11.9
PYR
7.0
11.9
mM/(h*g cells)
Higher on xylose
Higher on glucose
Xylose

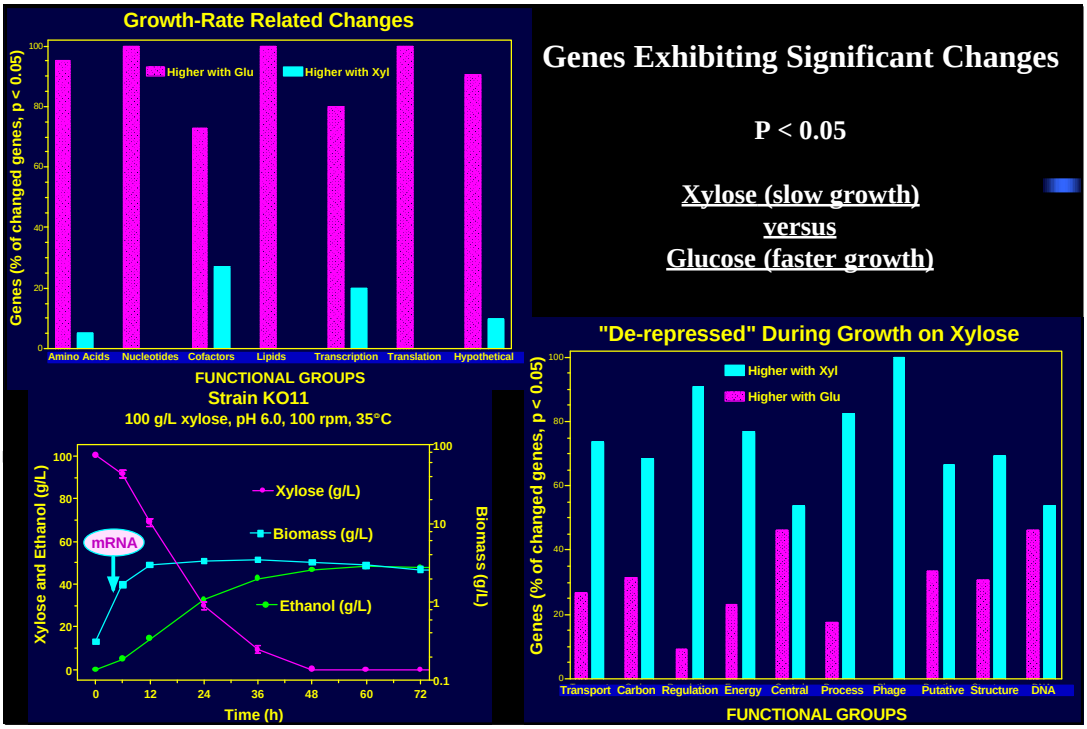
Expression Ratios for Individual Genes During Glucose and Xylose Metabolism in *E. coli*

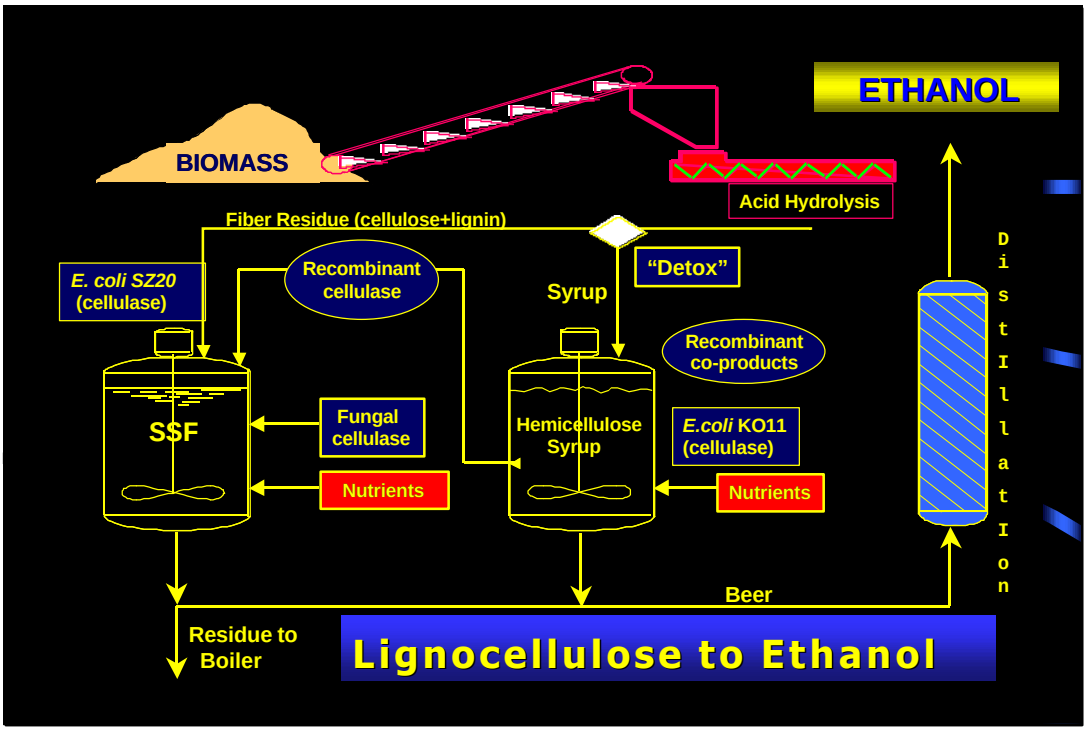


Conclusions

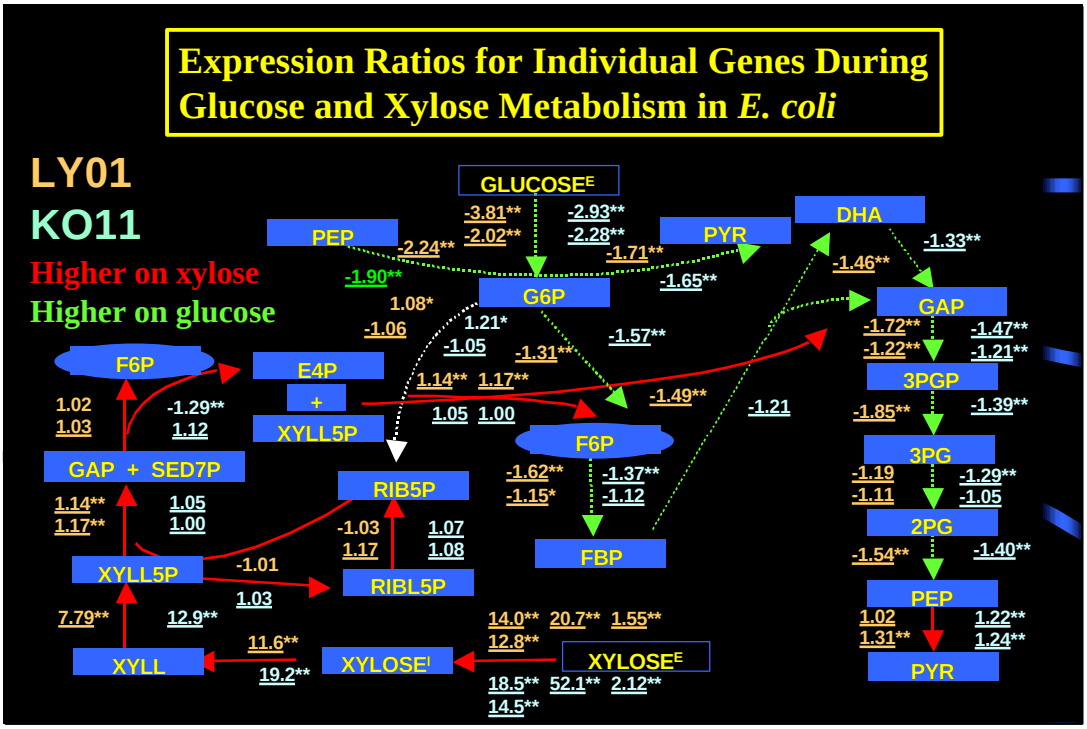
- Reproducibility of Expression Data can exceed that of enzyme assays.
- Expression Data can be a valuable tool to investigate glycolytic flux.
- Cellular regulatory circuits generally do what is expected.
- Embden-Meyerhof-Parnas genes are coordinately regulated. – CsrA?
  EMP gene expression directly related to glycolytic flux.
- Xylose genes are coordinately regulated by Crp/cAMP + XylR.
- Glucose uptake and mannose PTS are coordinately regulated - Mlc.
- Pentose Phosphate genes essentially constant and unresponsive.
- Flux limitation ?? – Xyl genes, Pentose Phosphate, EMP -- ?

Hypotheses can be made for each.


Growth-Rate Related Changes
Genes (% of changed genes, p < 0.05)
Higher with Glu
Higher with Xyl
Amino Acids
Nucleotides
Cofactors
Lipids
Transcription
Translation
Hypothetical
FUNCTIONAL GROUPS
Strain KO11
100 g/L xylose, pH 6.0, 100 rpm, 35°C
Xylose and Ethanol (g/L)
Biomass (g/L)
Xylose (g/L)
Biomass (g/L)
Ethanol (g/L)
mRNA
Time (h)
Genes Exhibiting Significant Changes
P < 0.05
Xylose (slow growth)
versus
Glucose (faster growth)
"De-repressed" During Growth on Xylose
Higher with Xyl
Higher with Glu
Transport
Carbon
Regulation
Energy
Central
Process
Phage
Putative
Structure
DNA
FUNCTIONAL GROUPS


ETHANOL
BIOMASS
Acid Hydrolysis
Fiber Residue (cellulose+lignin)
E. coli SZ20
(cellulase)
Recombinant
cellulase
Syrup
"Detox"
Recombinant
co-products
SSF
Fungal
cellulase
Nutrients
Hemicellulose
Syrup
E.coli KO11
(cellulase)
Nutrients
Distillation
Beer
Residue to
Boiler
Lignocellulose to Ethanol

Expression Ratios for Individual Genes During Glucose and Xylose Metabolism in *E. coli*



Fluxes During Glucose and Xylose Metabolism in *E. coli*