Kinetic Modeling to Investigate the Interactions of the Engineered Pentose Pathway with the Glycolytic (ED) Pathway in *Zymomonas mobilis*

M. Mete Altintas[1], Christina Eddy[2], Min Zhang[2],

James D. McMillan[2] and Dhinakar S. Kompala[1]

1. Department of Chemical Engineering, University of Colorado, Boulder, CO
2. Biotechnology Division for Fuels and Chemicals, NREL, Golden, CO

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Abstract

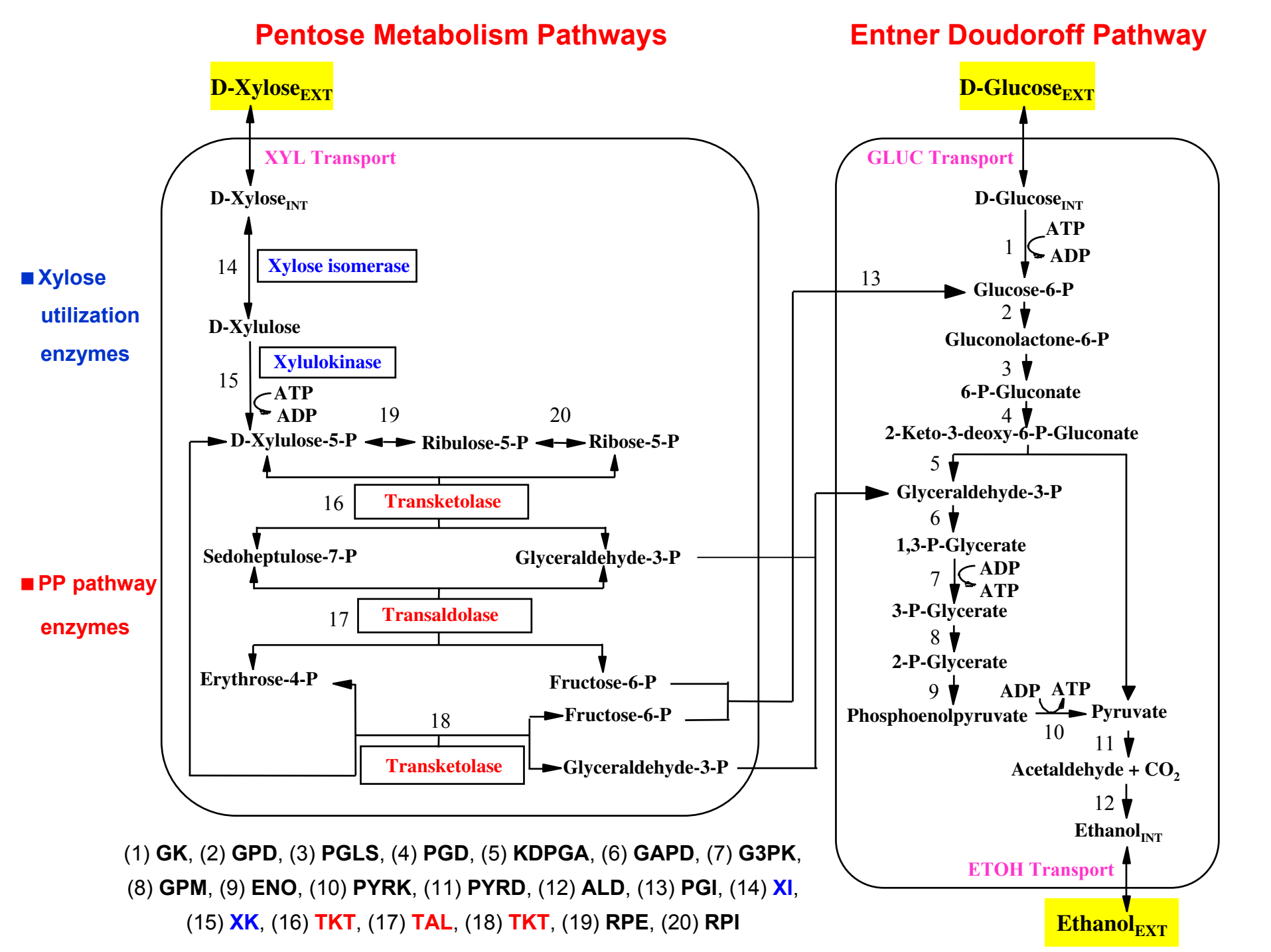
Zymomonas mobilis has engineered with 4 new enzymes to ferment xylose along with glucose and a network of pentose pathway enzymatic reactions interacting with the native glycolytic Entner Doudoroff pathway has been hypothesized.

We are currently investigating this proposed reaction network by developing a kinetic model for all the enzymatic reactions of the pentose phosphate and glycolytic pathways.

Kinetic data on different sugar metabolism rates and enzymatic activity data will be used to refine the model parameters available in the literature and validate the proposed reaction network.

Objectives

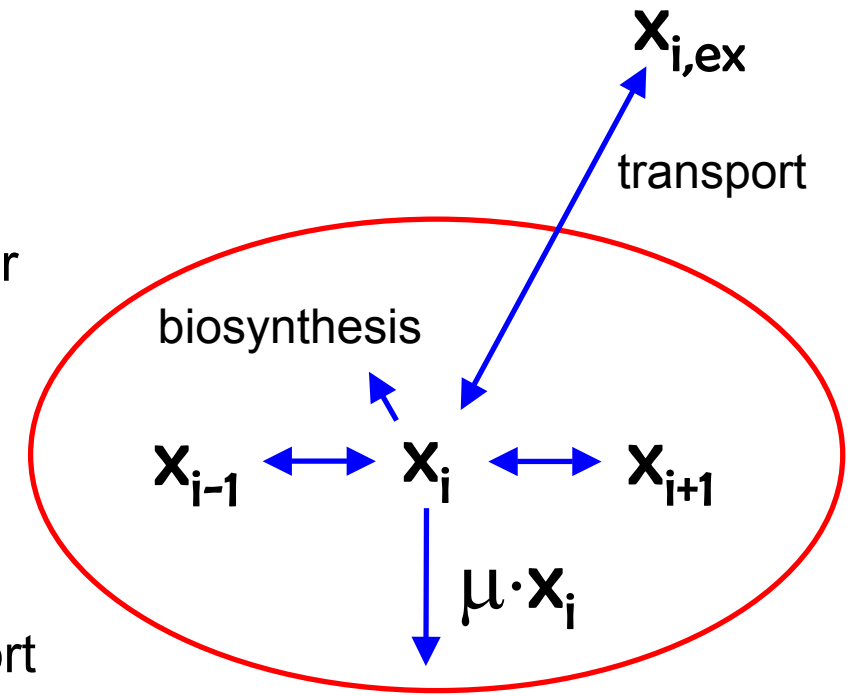
- To investigate the assumed network of enzymatic reactions linking the pentose metabolism and glycolysis pathways.
- To incorporate the non-linear rate expressions for the feedback regulation of enzymatic reactions.
- To find an optimum combination of enzymes needed for maximizing ethanol concentration.


Pentose Metabolism Pathways
Entner Doudoroff Pathway
D-Xylose$_{EXT}$
XYL Transport
D-Xylose$_{INT}$
14
Xylose isomerase
D-Xylulose
15
Xylulokinase
ATP
ADP
19
20
D-Xylulose-5-P
Ribulose-5-P
Ribose-5-P
16
Transketolase
Sedoheptulose-7-P
Glyceraldehyde-3-P
17
Transaldolase
Erythrose-4-P
Fructose-6-P
18
Fructose-6-P
Transketolase
Glyceraldehyde-3-P
D-Glucose$_{EXT}$
GLUC Transport
D-Glucose$_{INT}$
1
ATP
ADP
13
Glucose-6-P
2
Gluconolactone-6-P
3
6-P-Gluconate
4
2-Keto-3-deoxy-6-P-Gluconate
5
Glyceraldehyde-3-P
6
1,3-P-Glycerate
7
ADP
ATP
3-P-Glycerate
8
2-P-Glycerate
9
ADP
ATP
Phosphoenolpyruvate
10
Pyruvate
11
Acetaldehyde + CO_2
12
Ethanol$_{INT}$
ETOH Transport
Ethanol$_{EXT}$
Xylose utilization enzymes
PP pathway enzymes
(1) GK, (2) GPD, (3) PGLS, (4) PGD, (5) KDPGA, (6) GAPD, (7) G3PK,
(8) GPM, (9) ENO, (10) PYRK, (11) PYRD, (12) ALD, (13) PGI, (14) XI,
(15) XK, (16) TKT, (17) TAL, (18) TKT, (19) RPE, (20) RPI

Features of Kinetic Modeling

- The mechanistic rate equations for each of the enzymatic reactions occurring inside the cell mass.

- The rate equations for the transport of major substrates into cells and of major products out of the cells.



The *Zymomonas* System

#	ENZYME	REACTANTS	PRODUCTS	INHIBITORS	MECHANISM
1	Glucokinase	GLUC + ATP	GLUC6P + ADP	GLUC6P	Michaelis-Menten
2	Glucose-6-P dehydrogenase	GLUC6P + NAD(P)	PGL + NAD(P)H	ATP	Michaelis-Menten
3	6-phosphogluconolactonase	PGL	PG	GLUC6P	Michaelis-Menten
4	6-phosphogluconate dehydratase	PG	KDPG	G3P	Michaelis-Menten
5	2-keto-3-deoxy-6-P-gluconate aldolase	KDPG	GAP + PYR		Michaelis-Menten
6	Glyceraldehyde-3-P dehydrogenase	GAP + NAD	DPG + NADH		Michaelis-Menten
7	3-phosphoglycerate kinase	DPG + ADP	G3P + ATP		M.-Menten & Rev.
8	Phosphoglycerate mutase	G3P	G2P		Michaelis-Menten
9	Enolase	G2P	PEP		Michaelis-Menten
10	Pyruvate kinase	PEP + ADP	PYR + ATP		Michaelis-Menten
11	Pyruvate decarboxylase	PYR	ACET		Michaelis-Menten
12	Alcohol dehydrogenase	ACET + NADH	ETOH + NAD		M.-Menten & Rev.
13	Phosphoglucose isomerase	FRUC6P	GLUC6P	PG	Michaelis-Menten
14	Xylose isomerase	XYL	XYLU		Michaelis-Menten
15	Xylulokinase	XYLU + ATP	XYLU5P + ADP		Michaelis-Menten
16	Transketolase	XYLU5P + RIB5P	SED7P + GAP		M.-Menten & Rev.
17	Transaldolase	SED7P + GAP	FRUC6P + E4P		Ping-Pong Bi Bi
18	Transketolase	XYLU5P + E4P	FRUC6P + GAP		M.-Menten & Rev.
19	Ribulose 5-phosphate epimerase	XYLU5P	RIBU5P		M.-Menten & Rev.
20	Ribose 5-phosphate isomerase	RIBU5P	RIB5P		M.-Menten & Rev.

Approach

- The kinetic model developed to describe the *Zymomonas* system is comprised of 25 rate expressions and 25 balance equations.
- All the derivatives of balance equations were set to zero to calculate a steady state. The resulting system of nonlinear equations was solved numerically for the steady state metabolite concentrations.
- The amounts of 5 enzymes (PGI, XI, XK, TKT and TAL) were varied in order to maximize the ethanol concentration. Their sum was limited to 13% so that all (21) enzymes in our system add up to 50% of total cellular protein or less.
- The k_{cat} values reported at varying temperatures are normalized by using the 'glucose consumption vs. temperature' table presented by Scopes and Griffiths-Smith (1986) to estimate the k_{cat} values at 30°C.

Assumptions and Conditions

- The k_{cat} and K_m values used in the model were collected from the literature at varying pH's.
- The concentrations of ATP, ADP, NAD, NADP and NADH were assumed to be constant and equal to their K_m values.
- A constant level of gene expression was assumed, i.e. the enzyme levels that were measured in the wild-type strain were used.
- For the heterologous enzymes, k_{cat} and K_m values from *E. coli* were used.

- Xylose and glucose are constant at 5.0 and 0.1 g/L, respectively.
- The chemostat is operated at a constant dilution rate of 0.05 h^{-1}.
- The cell mass concentration in the bioreactor is constant at 1 g/L.
- The cytoplasmic volume of *Zymomonas* cells growing in the presence of glucose and xylose were taken to be 2.9 ml/g dcm.

Sample Rate Expression

$$\upsilon_1 = \frac{k_{cat} \cdot [E]_T \times \left(\frac{GLUC}{K_{m,GLUC}}\right) \times \left[\frac{ATP}{K_{m,ATP} \times \left(1 + \frac{GLUC6P}{K_{i,GLUC6P}}\right)}\right]}{1 + \frac{GLUC}{K_{m,GLUC}} + \frac{ATP}{K_{m,ATP}} + \frac{GLUC \times ATP}{K_{m,GLUC} \times K_{m,ATP} \times \left(1 + \frac{GLUC6P}{K_{i,GLUC6P}}\right)} + \frac{GLUC6P}{K_{i,GLUC6P}}}$$

2 Sub.s + 2 Prod.s

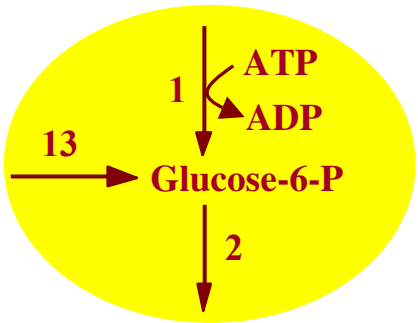
Michaelis-Menten Mech.

Irreversible Rxn.

Competitive product inhibiton term

- K_{cat}, K_m and K_i values are reported in the literature
- $\upsilon_{max} = k_{cat} \cdot [E]_T$
- $[E_T]$ = (g enzyme/g total protein) x (g protein/g dry cell)

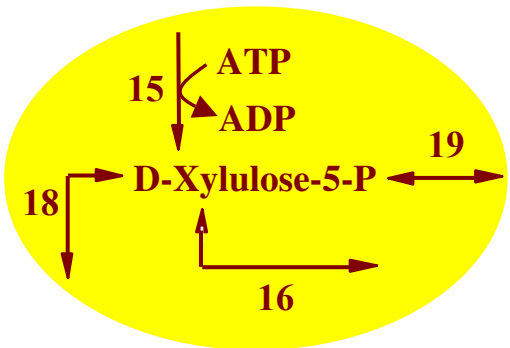
Representative Balance Equations



$$\frac{d[GLUC6P]}{dt} = (\upsilon_1 + \upsilon_{13} - \upsilon_2) - \mu \cdot [GLUC6P]$$

rate expressions

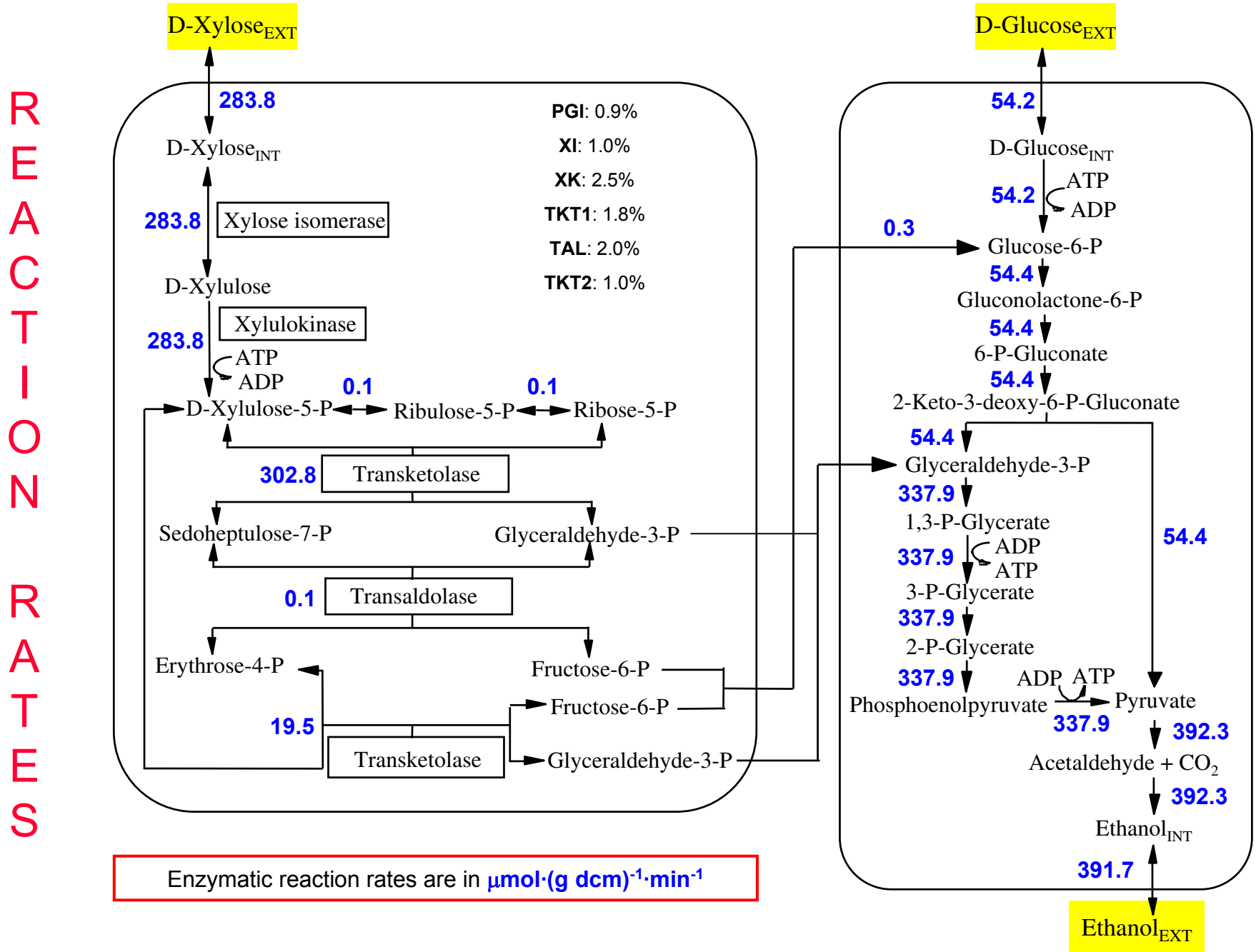
dilution due to growth

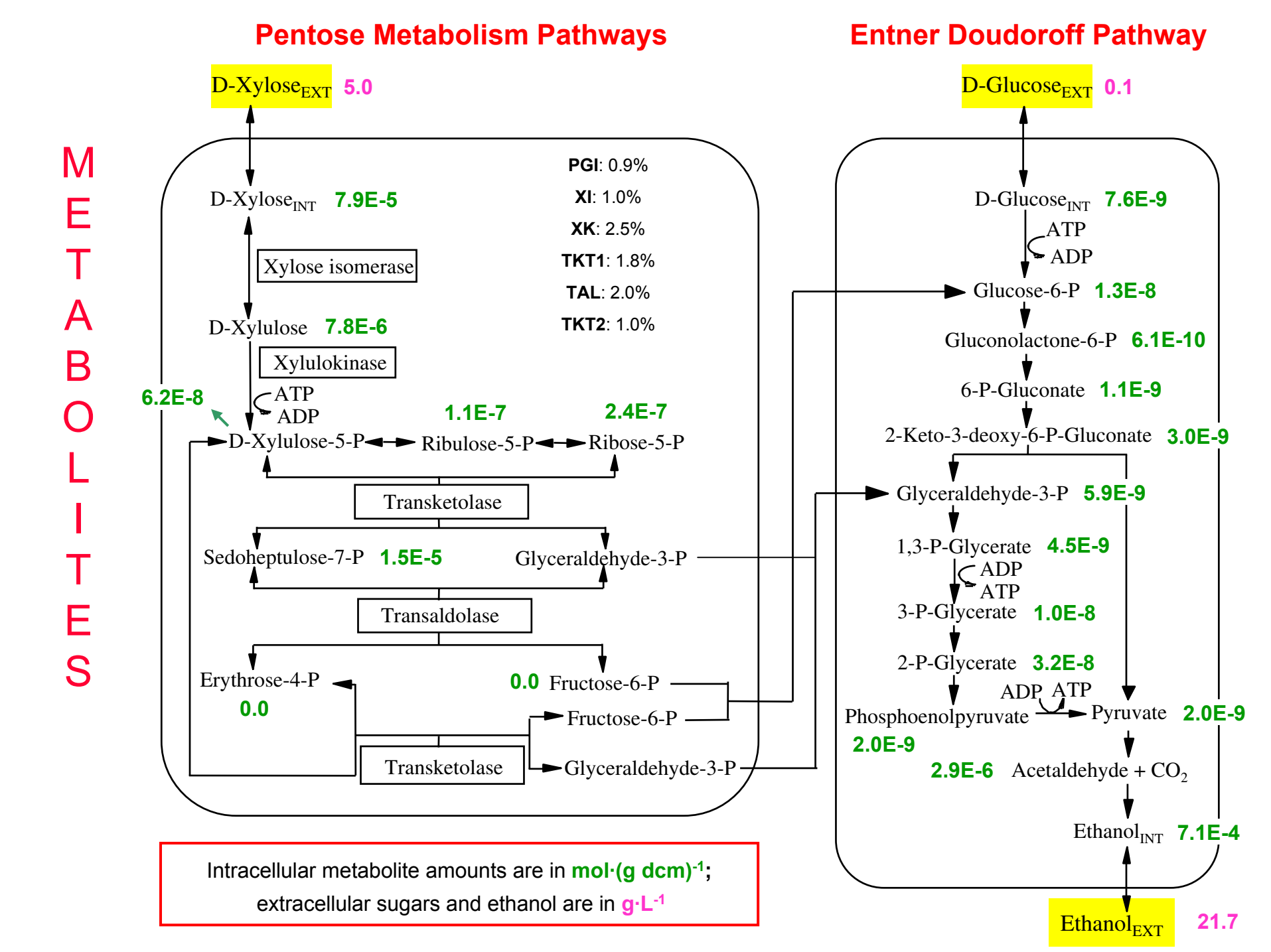


$$\frac{d[XYLU5P]}{dt} = (\upsilon_{15} - \upsilon_{16} - \upsilon_{18} - \upsilon_{19}) - \mu \cdot [XYLU5P]$$

Enzyme Amounts vs Ethanol Concentration

#	Enz.	%	%	%	%	%	%	%
13	PGI	0.9	0.9	0.9	0.9	0.9	0.9	0.9
14	XI	0.1	0.5	1.0	1.0	1.0	1.0	2.5
15	XK	2.5	2.5	2.5	1.0	0.1	2.5	3.5
16	TKT-1	5.5	5.5	5.5	5.5	5.5	1.8	3.0
17	TAL	2.0	2.0	2.0	2.0	2.0	2.0	2.0
18	TKT-2	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Total	12.0	12.4	12.9	11.4	10.5	9.2	12.9
	$ETOH_{ext}$	8.12 g/L	16.67 g/L	21.66 g/L	14.12 g/L	6.80 g/L	21.66 g/L	21.66 g/L


Pentose Metabolism Pathways
Entner Doudoroff Pathway
REACTION RATES
D-Xylose$_{EXT}$
283.8
D-Xylose$_{INT}$
283.8
Xylose isomerase
D-Xylulose
283.8
Xylulokinase
ATP
ADP
D-Xylulose-5-P
0.1
Ribulose-5-P
0.1
Ribose-5-P
302.8
Transketolase
Sedoheptulose-7-P
Glyceraldehyde-3-P
0.1
Transaldolase
Erythrose-4-P
Fructose-6-P
19.5
Fructose-6-P
Transketolase
Glyceraldehyde-3-P
PGI: 0.9%
XI: 1.0%
XK: 2.5%
TKT1: 1.8%
TAL: 2.0%
TKT2: 1.0%
D-Glucose$_{EXT}$
54.2
D-Glucose$_{INT}$
54.2
ATP
ADP
0.3
Glucose-6-P
54.4
Gluconolactone-6-P
54.4
6-P-Gluconate
54.4
2-Keto-3-deoxy-6-P-Gluconate
54.4
Glyceraldehyde-3-P
337.9
1,3-P-Glycerate
54.4
337.9
ADP
ATP
3-P-Glycerate
337.9
2-P-Glycerate
337.9
ADP ATP
Phosphoenolpyruvate
Pyruvate
337.9
392.3
Acetaldehyde + CO_2
392.3
Ethanol$_{INT}$
391.7
Ethanol$_{EXT}$
Enzymatic reaction rates are in μmol·(g dcm)$^{-1}$·min^{-1}


Pentose Metabolism Pathways
Entner Doudoroff Pathway
METABOLITES
D-Xylose$_{EXT}$ 5.0
D-Xylose$_{INT}$ 7.9E-5
Xylose isomerase
D-Xylulose 7.8E-6
Xylulokinase
6.2E-8
ATP
ADP
1.1E-7
2.4E-7
D-Xylulose-5-P
Ribulose-5-P
Ribose-5-P
Transketolase
Sedoheptulose-7-P 1.5E-5
Glyceraldehyde-3-P
Transaldolase
Erythrose-4-P
0.0
0.0 Fructose-6-P
Fructose-6-P
Transketolase
Glyceraldehyde-3-P
PGI: 0.9%
XI: 1.0%
XK: 2.5%
TKT1: 1.8%
TAL: 2.0%
TKT2: 1.0%
D-Glucose$_{EXT}$ 0.1
D-Glucose$_{INT}$ 7.6E-9
ATP
ADP
Glucose-6-P 1.3E-8
Gluconolactone-6-P 6.1E-10
6-P-Gluconate 1.1E-9
2-Keto-3-deoxy-6-P-Gluconate 3.0E-9
Glyceraldehyde-3-P 5.9E-9
1,3-P-Glycerate 4.5E-9
ADP
ATP
3-P-Glycerate 1.0E-8
2-P-Glycerate 3.2E-8
ADP ATP
Phosphoenolpyruvate
Pyruvate 2.0E-9
2.0E-9
2.9E-6 Acetaldehyde + CO_2
Ethanol$_{INT}$ 7.1E-4
Ethanol$_{EXT}$ 21.7
Intracellular metabolite amounts are in mol·(g dcm)$^{-1}$;
extracellular sugars and ethanol are in g·L^{-1}

Results

- The kinetic model simulations result in a steady-state pathway.
- Amongst the 5 enzymes whose amounts were tested in terms of the ethanol production, it was found that TKT, XI and XK were the most important enzymes in terms of their effect on the extracellular ethanol concentration.
- The rates of reactions catalyzed by PGI and TAL are very low.

Results

- Since the reaction rates in the glycolysis pathway are higher than the rates in PP pathway, ‘glyceraldehyde 3-P’ is channeled quickly into the glycolysis pathway.

- Following the flow of ‘glyceraldehyde 3-P’ into the glycolysis pathway, the amounts of ‘erythrose 4-P’ and ‘fructose 6-P’ drops to zero even when TAL and TKT enzymes are available.

- The relatively slow rate of reactions in the PP pathway makes XI and XK enzymes important in terms of their ability to control the reactions immediately following xylose transport into the cell.

Conclusion

- The *in-silico* tools allow us to predict the concentrations of the pentose metabolism enzymes that maximize the ethanol production.
- The model enables us to compare the enzymatic reaction rates in different pathways and estimate the metabolic flux along each reaction.

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