International Journal of Computational Intelligence in Control, 7(1), 2015

ANALYSIS OF ELEMENTARY FLUX MODES FOR RECOMBINANT *SACCHAROMYCES CEREVISIAE* UTILIZING PENTOSE AND HEXOSE TO PRODUCE ETHANOL

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ABSTRACT: Wild-type yeast is in general unable to utilize xylose and arabinose to produce ethanol. In this study, we reconstructed a metabolic pathway network of of *S. cerevisiae* to use glucose, fructose, xylose and arabinose to produce ethanol. The reconstructed stoichiometric model was applied to compute the elementary flux modes. From the set of the elementary flux modes, we analyzed the specific molar yield of ethanol from different sugars. Yeast using hexoses is more efficient than that of pentoses because degradation of xylose and arabinose cause of the accumulation of NADH to give rise cofactor ($NADPH/NAD^+$) unbalance.

Keywords: flux balance analysis, metabolic flux analysis, ethanol fermentation, renewable fuel.

INTRODUCTION

Searching for renewable fuel is the current tendency in recent years [14]. Bioethanol has a number of advantages over conventional fuels and is one of the most developing alternative fuels. It comes from a renewable resource i.e. crops and not from a finite resource and the crops it derives from can grow well in a farm. Another benefit over fossil fuels is the greenhouse gas emissions. Ethanol can be produced from biomass by the hydrolysis and sugar fermentation processes. Biomass wastes contain a complex mixture of carbohydrate polymers from the plant cell walls known as cellulose, hemi-cellulose and lignin. In order to produce sugars from the biomass, the biomass is pre-treated with acids or enzymes in order to reduce the size of the feedstock and to open up the plant structure. Cellulose is hydrolyzed by enzymes into hexose, glucose. Hemicellulose is broken down into pentose, such as xylose and arabinose. Both pentose and hexose are then fermented into ethanol [13].

Yeast, such as *Saccharomyces cerevisiae*, is a useful microbe in industry to produce ethanol. It can utilize hexose, like sugarcane juice or cornstarch, to achieve higher ethanol yield and tolerance. Ethanol fermentation by using glucose is a mature technology. To reduce the operation cost and prevent from worsen food shortage; we have to use biomass wastes, i.e. cellulose and hemi-cellulose, as raw materials to produce ethanol. Wild-type yeast is inefficiently to use pentose to produce ethanol even though it is a good candidate microbe for consuming glucose in ethanol fermentation processes. Although production of ethanol from the fermentation of hexose and pentose has been studied for many years, there are still several bottlenecks for the economical production of fuel ethanol. The fermentation of xylose to ethanol represents the main bottleneck in the production process. Several articles have reported the development of genetically engineered strains that utilize pentose and hexose as substrates in the production of ethanol [1, 4, 10, 15]. Little report has discussed about metabolic flux analysis of *S. cerevisiae* using mixture of pentose and hexose [5]. In this study, we will reconstruct a metabolic pathway network of of *S. cerevisiae* to use glucose, fructose, xylose and arabinose to produce ethanol. The reconstructed network is then to

compute the elementary modes to analyze efficiency of the network. Such information can be provided for microbiologist to efficiently engineer yeast in order to enhance ethanol productivity.

METHOD

The metabolic pathway network of *S. cerevisiae* using glucose and fructose to produce ethanol is well-known [6]. However, using xylose and arabinose as the carbon sources are incomplete so that we reconstructed the network considered these hexose and pentose as the fermented sugars. Both xylose and arabinose uptake pathways are acquired from the literature [2, 7]. To account for cell growth in the network model, we assumed the cellular contents consist of protein, DNA, RNA, lipid and carbohydrates by a fixed ratio of dilution rate at 0.1 h^{-1} [12]. Compartmentation is not considered due to the uncertainty and complexity of the phenomenon [9, 19]. The cofactors, ATP, NAD(P)+ and NAD(P)H, act as an energy currency and are included in the stoichiometric model. For describing maintenance and futile cycle, we assume that ATP and GTP can be interconverted in the network, and the rate reaction is also included in the stoichiometric model [3, 12]. To account for redox balance, NAD(P)+ and NAD(P)H are set to be the internal metabolites. The reconstructed metabolic network consists of 50 metabolites and 53 flux reactions as shown in Figure 1. The detailed information of the network and reactions are shown in appendix A.

The fundamental principle underlying metabolic flux analysis (MFA) is the conservative of mass. The starting point of MFA is the reaction network describing how substrates are converted into products and biomass. A set of measured extracellular rates are used as input calculations [8, 18]. The basis of flux determination is a mass balance specified by the stoichiometry of the biochemical network, and the assumption of pseudo-steady state of intracellular metabolites. The metabolic pathway network can be represented generically using a set of differential equations with the following structure:

$$\frac{d\mathbf{x}}{dt} = \mathbf{N}\mathbf{v} \tag{1}$$

where $\mathbf{x}$ is an n-dimensional vector of metabolite amounts per cell, $\mathbf{v}$ is the vector of r reaction fluxes. The element N_{ij} of the stoichiometric matrix $\mathbf{N}$ is the stoichiometric coefficient that indicates the amount of the i^{th} compound produced per unit flux of the j^{th} reaction. Metabolism usually involves fast reactions and high turnover of substances when compare to regulatory events. Therefore, analysis of metabolic networks is often based on the assumption that, on longer time scales, metabolite concentrations and reaction rates are constant. Applying this quasi (pseudo) steady state assumption to Eq.(1) leads to the fundamental metabolite balancing equation.

$$\mathbf{N}\,\mathbf{v} = \mathbf{0} \tag{2}$$

This homogeneous system of linear equations demands that the production (sum of positive fluxes) and consumption (sum of negative fluxes) of a metabolite must be equal, similar to Kirchhoff's first law for electric circuits. Several methodologies [11] can be applied to analyze the stoichiometric model (2). Some reactions in the model are reversible and some are irreversible, we decompose the flux vector into the sub-vectors $\mathbf{v}^{irr}$ and $\mathbf{v}^{rev}$. To achieve thermodynamic feasibility, the irreversible and reversible fluxes should be constrained as

$$\mathbf{v}^{irr} \geq \mathbf{0} \tag{3}$$

$$\mathbf{v}^{rev,UB} \geq \mathbf{v}^{irr} \geq -\mathbf{v}^{rev,LB} \tag{4}$$

where $\mathbf{v}^{rev,UB}$ and $\mathbf{v}^{rev,LB}$ are the positive upper and lower bounded values. In this study, the elementary flux modes (EFMs) are applied to evaluate the efficiency of the system. Here, we briefly explain the basic properties of EFMs [17].

A set of valid solutions to Eq. (2) subject to the constraints in Eqs. (3) and (4) can be described as a high-dimensional cone that is located in a space where each axis corresponds to a reaction flux. EFMs, as well as extreme pathways [16], use convex analysis to describe this solution space. Every valid flux distribution in a reaction network can be represented as a

nonnegative combination of the convex basis vectors. An EFM is a minimal set of enzymes that could operate at steady state, with all the irreversible reactions used in the appropriate direction [17]. The EFMs have the following three properties.

(I) There is a unique set of elementary modes for a given network.

(II) Each elementary mode consists of the minimum number of reactions that it needs to exist as a functional unit. If any reaction in an elementary mode were removed, the whole elementary mode could not operate as a functional unit. This property has been called 'genetic independence' and 'non-decomposability'.

(III)The elementary modes are the set of all routes through a metabolic network consistent with property II.

The EFMs for biochemical reaction systems as governed by Eqs.(2)-(4) can be detected by the powerful tool, CellNetAnalyzer, which was developed by Klamp and Kamp (http:// www.mpi-magdeburg.mpg.de/projects/ fluxanalyzer). The detailed computational algorithm is described in [17]. Here, we briefly summarize the algorithm as following:

1. Start from a tableau $\mathbf{T}^{(0)}$ containing the transposed stoichiometric matrix, $\mathbf{N}^{T}$, and the identity matrix:

$$\mathbf{T}^{(0)} = \begin{bmatrix} \mathbf{T}_{irr}^{(0)} \\ \mathbf{T}_{rev}^{(0)} \end{bmatrix}_{r\times(n+r)} = \begin{bmatrix} \mathbf{N}_{irr}^{T} & \mathbf{I} & \mathbf{0} \\ \mathbf{N}_{rev}^{T} & \mathbf{0} & \mathbf{I} \end{bmatrix} = \left[\left(\mathbf{N}^{(0)^{T}}\right)_{r\times n} \quad \left(\mathbf{M}^{(0)}\right)_{r\times r} \right]$$

2. From the tableau $\mathbf{T}^{(j)}$, j = 0,1,..., n-1, the elements of which are denoted by $t_{i,k}^{(j)}$, calculate the next tableau $\mathbf{T}^{(j+1)}$ in the following way:

$$\mathbf{T}^{(j+1)} = \left[\mathbf{N}^{(j+1)^{T}} \quad \mathbf{M}^{(j+1)} \right]_{r\times(n+r)}$$

(a) For each row, $\mathbf{m}_i^{(j)}$, of $\mathbf{M}^{(j)} = \left[\left(\mathbf{m}_i^{(j)}\right)_{1\times r} \right]_{r\times r}$, determine the set $S(\mathbf{m}_i^{(j)})$ defined by $S\left(\mathbf{m}_i^{(j)}\right) = \left\{k : m_{i,k}^{(j)} = 0\right\}$.

(b) First, all rows of $\mathbf{T}_{rev}^{(j)}$ with a zero in the $(j+1)$th column go into $\mathbf{T}^{(j+1)}$. Then, new rows formed by allowed linear combinations of pairs of rows of $\mathbf{T}_{rev}^{(j)}$ with non-zero elements in this column consecutively go into $\mathbf{T}^{(j+1)}$ if they fulfill the conditions.

$$t_{i,j+1}^{(j)}\, t_{m,j+1}^{(j)} \neq 0 \tag{5}$$

$$S\left(\mathbf{m}_i^{(j)}\right) \cap S\left(\mathbf{m}_m^{(j)}\right) \not\subset S\left(\mathbf{m}_l^{(j+1)}\right) \tag{6}$$

for all row indices l belonging to the tableau $\mathbf{T}_{rev}^{(j+1)}$ as it has been compiled until that moment. If this tableau does not yet contain any row, condition (6) is taken to be fulfilled. The combination of rows should be done so as to give a zero element in the $(j+1)$th column,

$$\theta = t_{i,j+1}^{(j)}\, \mathbf{t}_m^{(j)} - t_{m,j+1}^{(j)}\, \mathbf{t}_i^{(j)}$$

Relation (6) implies that combination of the rows will lead to a new row containing a set of zeros not yet generated. $\mathbf{T}_{rev}^{(j+1)}$ finally encompasses all rows of $\mathbf{T}_{rev}^{(j)}$ with $t_{i,j+1}^{(j)}$ as well as all the vectors θ.

(c) The first part of $\mathbf{T}_{irr}^{(j+1)}$ is constructed by using all rows of $\mathbf{T}_{irr}^{(j)}$ with $t_{i,j+1}^{(j)} = 0$. Now, transfer into $\mathbf{T}_{irr}^{(j+1)}$ appropriate linear combinations of all pairs of rows, $\mathbf{t}_i^{(j)}$ and $\mathbf{t}_m^{(j)}$, of $\mathbf{T}_{irr}^{(j)}$ containing non-zero elements of opposite sign in the $(j+1)$th column, that is, $t_{i,j+1}^{(j)}\, t_{m,j+1}^{(j)} < 0$ and also satisfying relation (6) for all row indices l belonging to $\mathbf{T}_{irr}^{(j+1)}$ as it has been compiled until that moment. Appropriate combination means addition after scaling to ensure the $(j+1)$th column of the result will be zero, that is, $\phi = \left|t_{i,j+1}^{(j)}\right| \mathbf{t}_m^{(j)} + \left|t_{m,j+1}^{(j)}\right| \mathbf{t}_i^{(j)}$.

(d) Similarly, for all pairs of rows, $\mathbf{t}_i^{(j)}$ and $\mathbf{t}_m^{(j)}$, with $\mathbf{t}_i^{(j)}$ belonging to $\mathbf{T}_{rev}^{(j)}$ and $\mathbf{t}_m^{(j)}$ belonging to $\mathbf{T}_{irr}^{(j)}$ and fulfilling relation (5) and (6) for all row indices l belonging to $\mathbf{T}_{irr}^{(j+1)}$ as it has grown until that moment, calculate the vectors $\tau = \mathrm{sgn}\left(t_{i,j+1}^{(j)}\right)\left(t_{i,j+1}^{(j)}\, \mathbf{t}_m^{(j)} - t_{m,j+1}^{(j)}\, \mathbf{t}_i^{(j)}\right)$ consecutively and append them to

$\mathbf{T}_{irr}^{(j+1)}$. $\mathbf{T}^{(j+1)}$ is obtained by

$$\mathbf{T}^{(j+1)} = \begin{bmatrix} \mathbf{T}_{rev}^{(j+1)} \\ \mathbf{T}_{irr}^{(j+1)} \end{bmatrix}_{r\times(n+r)} = \begin{bmatrix} \mathbf{N}^{(j+1)^T} & \mathbf{M}^{(j+1)} \end{bmatrix}$$

3. The previous phase of the algorithm ends with $\mathbf{T}^{(n)}$. The reversible elementary modes are represented by the rows of $\mathbf{M}_{rev}^{(n)}$ and – $\mathbf{M}_{rev}^{(n)}$, and the irreversible elementary modes are represented by the rows of $\mathbf{M}_{irr}^{(n)}$.

RESULTS AND DISCUSSION

As the mentioned-above section, we have reconstructed the metabolic pathway network of *S. cerevisiae* using glucose, fructose, xylose and arabinose as the carbon sources to produce ethanol. The reconstructed model consisted of 50 metabolites and 53 flux reactions. CellNetAnalyzer are then applied to detect 3401 EFMs from the model. The EFMs can be divided into pathways consuming substrates (pentose or hexose) for product yield and a few futile cycles. They can be regarded as the flexibility of *S. cerevisiae* in anaerobic fermentation, i.e. the cellular metabolic ability in accord with thermodynamics. From the set of EFMs, we can analyze the specific molar yield of ethanol from different sugars. We first considered the pure theoretical yield, i.e. the yeast to use a substrate to produce ethanol without including cell growth. From the set of 3401 EFMS, we found 61 EFMs could use glucose to produce ethanol. The distribution of the specific molar yield for the selected EFMs is shown in Figure 2. Two of the selected EFMs have the maximum specific molar yield of 2 mole ethanol/mole glucose, which is identical to the theoretical value. The overall reaction for the elementary mode as shown in Figure 3 is

$$C_6H_{12}O_6 + 2H^+ + 2H_2PO_4^- + 2ADP \rightarrow 2ATP + 2H_2O + 2C_2H_5OH + 2CO_2$$

This case implies that glucose could convert to ethanol completely. We then inspected the overall EFMs with a cell growth condition. We found out 47 of 3401 EFMs used glucose to produce ethanol with cell growth. One of the selected EFMs has the maximum specific molar yield of 0.81756 mole ethanol/mole glucose, which is less than the theoretical value. The

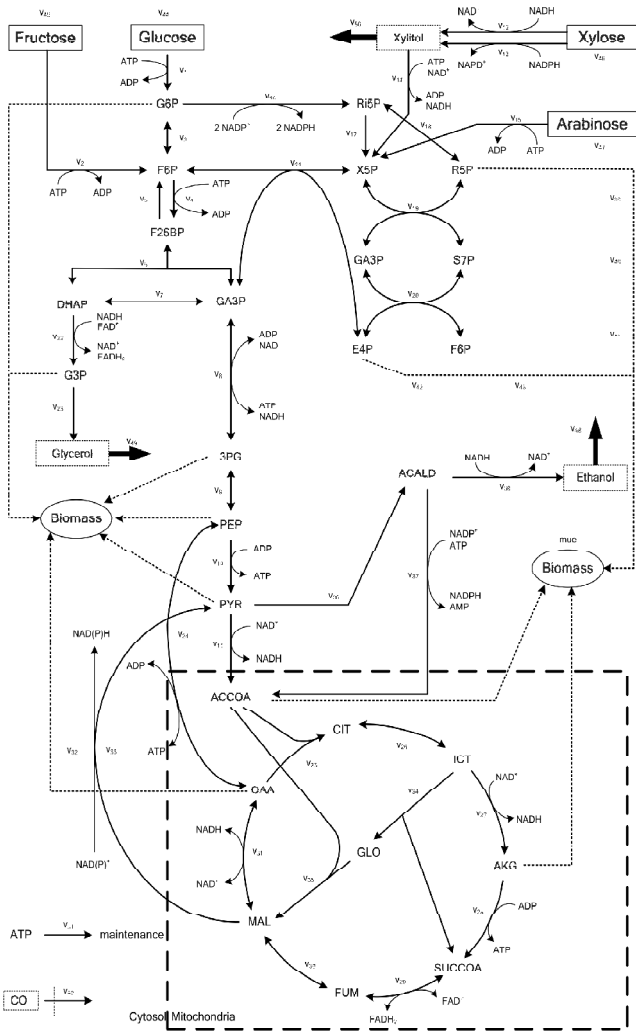

Figure 1: The Metabolic Pathway Network for *Saccharomyces Cerevisiae* after Reconstructing

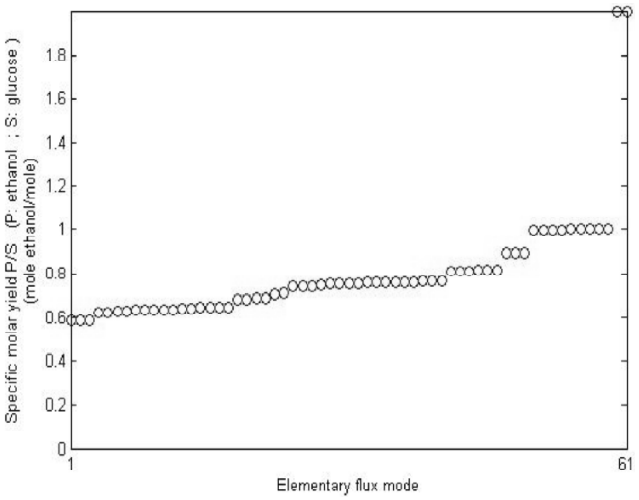

Figure 2: Elementary Flux Mode Distribution of Specific Molar Yield for Yeast without Including Cell Growth to use Glucose to Produce Ethanol

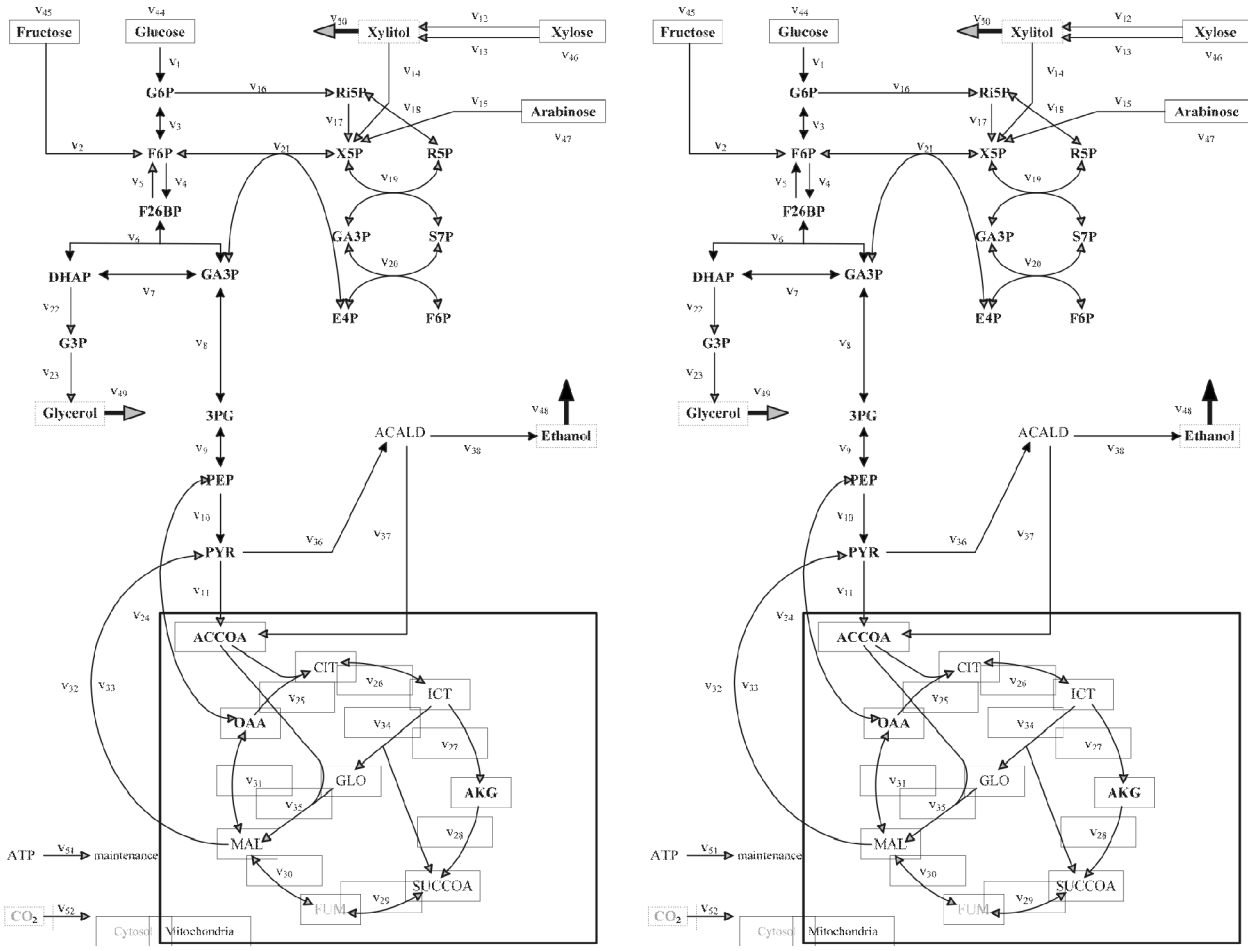

Figure 3: Elementary Modes of the Maximum Specific Molar for Yeast without Including Cell Growth to use Glucose to Produce Ethanol. Black lines Indicate the Reaction Pathways for each Elementary Mode

overall reaction for the elementary mode as shown in Figure 4 is

$$C_6H_{12}O_6 + 0.342H^+ + 0.692H_2O + 0.126\text{phosphate} + 0.000930\text{coenzymeA} + 0.156\text{FAD} + 0.0731\text{ammonium} + 0.00108\text{sufate} + 0.0000730\text{Methyl FH4} + 0.00217\text{otherBiomass} \rightarrow 0.818C_2H_5OH + 0.944\text{glycerol} + 1.472CO_2 + 0.0496\text{diphosphate} + 0.156\text{FADH2}$$

The reaction indicates that glucose is not only consumed to produce ethanol, but also provide for cell growth.

Following similar procedures, we evaluated EFMS and their corresponding specific molar yield of ethanol for using fructose, xylose and arabinose, respectively. Table 1 shows the maximum specific molar yield for using different substrates with/without cell growth. The maximum specific molar yield for using fructose is as same as that for glucose. However, it is inefficient to use xylose and arabinose as observed from the table. The result agrees with the speculations discussed in [7, 20]. The main

Table 1
The Maximal Specific Molar Yield for Different Cases

Individual substrate	*maximal yield mol (mol ethanol)$^{-1}$*	
	Without cell growth	*With cell growth*
glucose	2	0.81756
fructose	2	0.81756
xylose	0.75	0.42113
arabinose	NA	NA
Mixed substrate	*maximal yield mol (mol ethanol)$^{-1}$*	
	Without cell growth	*With cell growth*
Glucose + fructose	2	0.81756
Xylose + arabinose	0.75	0.42113

issue is that xylitol dehydrogenase is applied to catalyze xylitol to form X5P. The reaction is accompanied to accumulate NADH to give rise cofactor (NADPH/NAD$^+$) unbalance. As a result, xylose reductase is applied to convert xylose to xylitol in order to reduce NADH so that the maximum specific yield became 0.42113 mole ethanol/mole xylose. The overall reaction for the elementary mode as shown in Figure 5 is

$$C_5H_{10}O_5 + 0.160H_2O + 0.75H^+ + 0.265\text{phosphate} + 0.0116\text{coenzymeA} + 0.0245\text{FAD} + 0.0366\text{ammonium} + 0.000542\text{sufate} + 0.0000365\text{Methyl FH4} + 0.00109\text{otherBiomass} \rightarrow 0.421C_2H_5OH + 0.228\text{glycerol} + 0.267\text{xylitol} + 0.789CO_2 + 0.0245\text{FADH2} + 0.227\text{ATP}$$

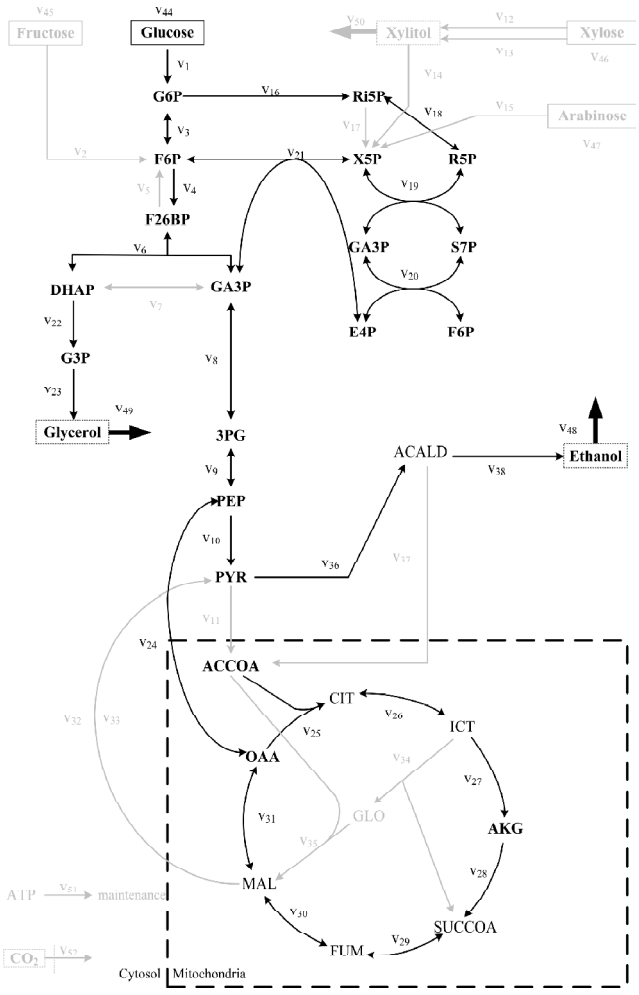

Figure 4: The Best Elementary mode for Yeast with Cell Growth to use Glucose to Produce Ethanol. Black Lines Indicate the Reaction Pathways for the Elementary Mode

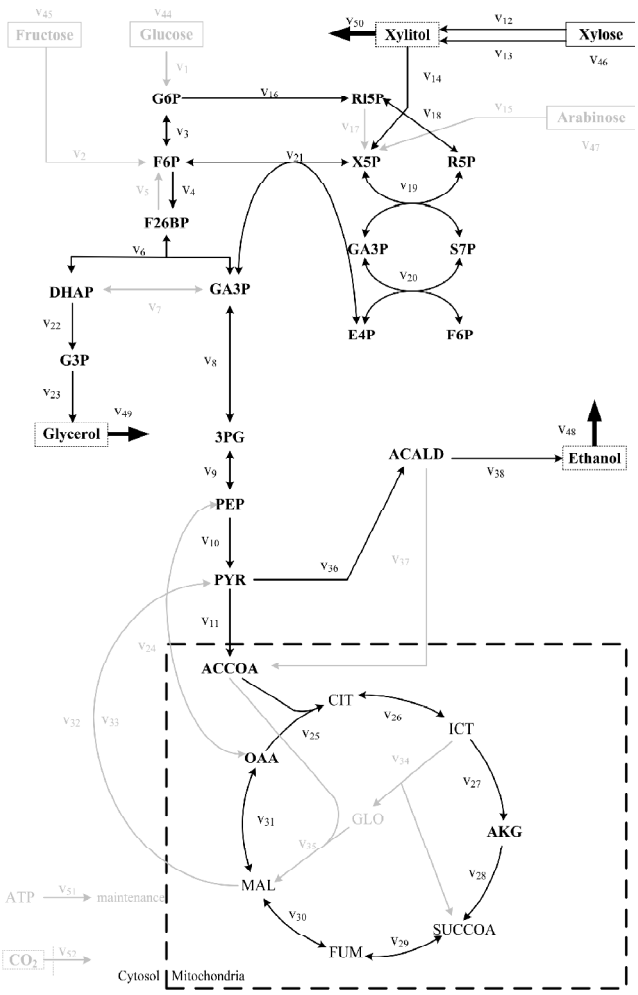

Figure 5: The Best Elementary Mode for Yeast with Cell Growth to use xylose to Produce Ethanol. Black Lines Indicate the Reaction Pathways for the Elementary Mode

However, the yeast is incapable of using arabinose only to produce ethanol due to absent reductases to balance NADPH so that the specific molar yield is not available.

We next analyzed the pathway for the yeast to use sugar mixtures to produce ethanol. The yeast could use hexoses, glucose and fructose, so we obtained the maximum specific molar yield of 0.81756. The EFM was shown in Figure 6. In contrast, the yeast is inefficient to use penoses, xylose and arabinose due to cofactor (NADPH/NAD$^+$) unbalance. Arabinose is unable to be utilized alone as discussed in the mentioned-above. However, it can be consumed with accompanying xylose existed to produce ethanol because NADPA is balanced by

the pathway from xylose to xylitol. The EFM was shown in Figure 7. From EMFs in Figures 6 and 7, we found the pathways for hexoses and pentoses to form ethanol could be separated as the mutual and individual owned enzymes, as summarized in Table 2. The information could provide for a microbiologist to engineer the yeast efficiently.

CONCLUSIONS

Using hexose and pentose simultaneously to produce ethanol is a challenged research in renewable fuels. In this study, we reconstructed a metabolic pathway network of *S. cerevisiae* to use glucose, fructose, xylose and arabinose to produce ethanol. The elementary flux modes were computed through the reconstructed

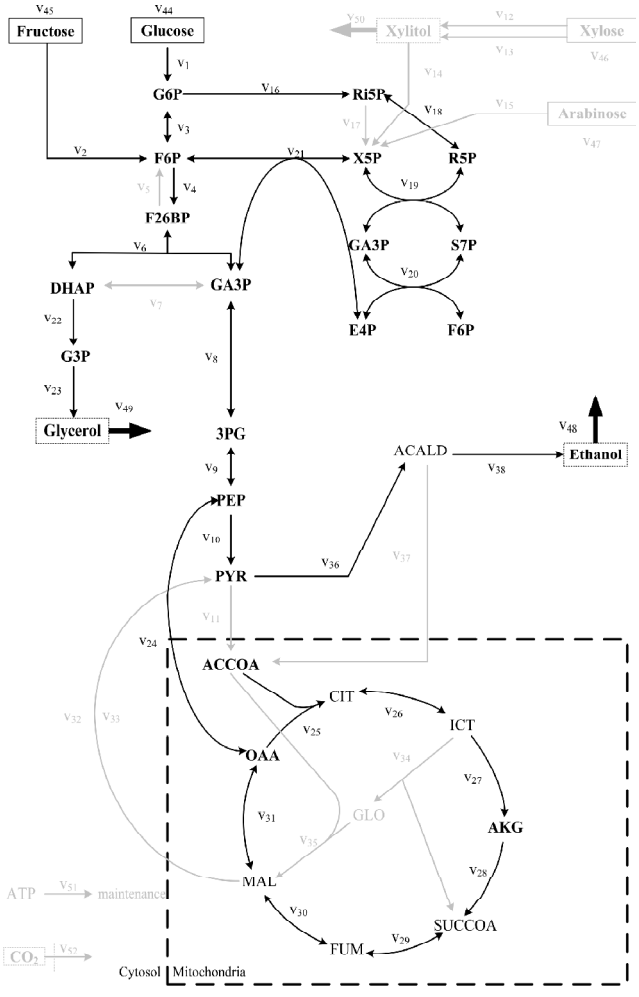

Figure 6: The Best Elementary Mode for Yeast with Cell Growth to use Fructose and Glucose to Produce Ethanol. Black Lines Indicate the Reaction Pathways for the Elementary Mode

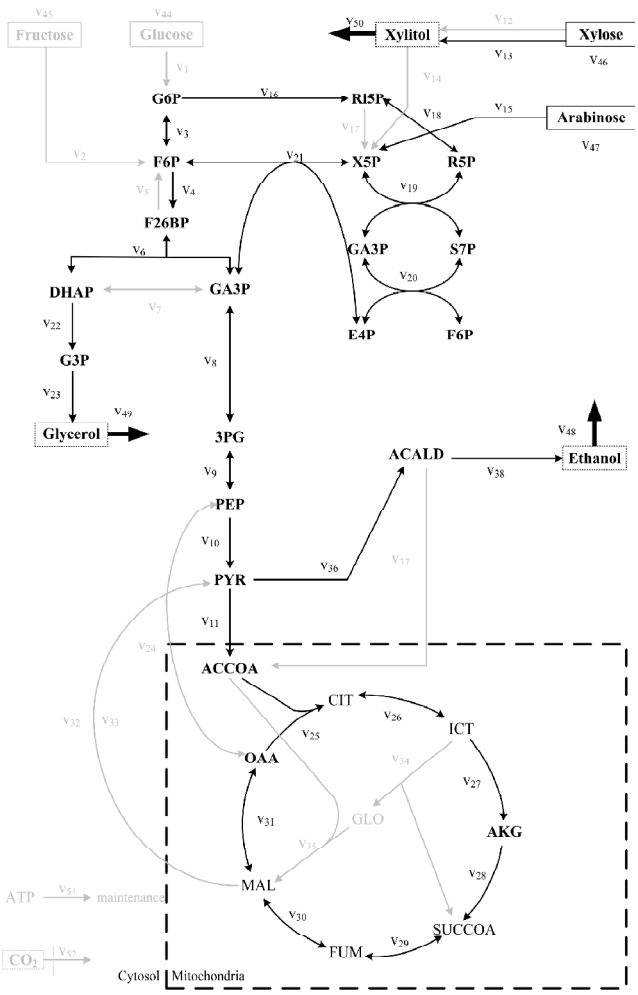

Figure 7: The Best Elementary Mode for Yeast with Cell Growth to use Xylose and Arabinose to Produce Ethanol. Black Lines Indicate the Reaction Pathways for the Elementary Mode

stoichiometric model in order to analyze efficiency of the network. Such information could be provided for microbiologist to efficiently engineer yeast in order to enhance ethanol productivity.

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Table 2
The Mutual and Individual Enzymes in Pathways for Hexoses and Pentoses to form Ethanol

	Hexoses	*Pentoses*
Individual enzymes	Hexokinase	glucose 6-phosphate isomerase
	phosphoenolpyruvate carboxykinase	xylose reductase
	aldehyde dehydrogenase	xylitol dehydrogenase
	acetyl-CoA synthetase	xylulokinase
		arabinose isomerase ribulokinase
		ribulose 5-phosphate 4-epimerase
Mutual enzymes	6-phosphofructo-2-kinase	succinate dehydrogenase
	fructose-bisphosphate aldolase	fumarase
	glyceraldehyde-3-phosphate dehydrogenase	malate dehydrogenase
	phosphoglycerate kinase	pyruvate decarboxylase
	phosphoglycerate mutase	alcohol dehydrogenase
	enolase	glutamate dehydrogenase
	pyruvate kinase	glutamine synthetase
	pyruvate dehydrogenase	aspartate transaminase
	glucose 6-phosphate dehydrogenase	phosphoserine transaminase
	phosphogluconate dehydrogenase	phosphoglycerate dehydrogenase
	ribose-5-phosphate isomerase	phosphoserine phosphatase
	transketolase	adenylosuccinate lyase
	transaldolase	phosphoribosylaminoimidazolesuccinocarboxamide synthase
	glycerol-3-phosphate dehydrogenase	phosphoribosylaminoimidazole carboxylase
	glycerol-3-phosphatase	phosphoribosylaminoimidazole synthase
	citrate synthase	phosphoribosylformylglycinamidine synthase
	aconitase	phosphoribosylglycinamide formyltransferase
	Isocitrate dehydrogenase	phosphoribosylglycinamide synthaseglutamine phosphoribosyldiphosphate amidotransferase
	oxoglutarate dehydrogenase	phosphoribosylpyrophosphate synthetase
	succinate-CoAligase	methenyltetrahydrofolate cyclohydrolase
		glycine hydroxymethyltransferase
		methylenetetrahydrofolate dehydrogenase

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APPENDIX

The tables below are the total metabolites and the reactions in the recombinant *Saccharomyces cerevisiae*.

A. The Metabolites

metabolite name	*abbreviation*
Proton	H+
Phosphate	pi
Diphosphate	ppi
Water	H2O
Carbondioxide	CO2
FAD	FAD+
FADH2	FADH2
coenzyme A	CoA
Ammonium	NH4
Sulfate	SO4
nicotinamide adenine dinucleotide phosphate – reduced	NADPH
nicotinamide adenine dinucleotide	NAD+
adenosine triphosphate	ATP
Xylose	XYL
xylulose 5-phosphate	X5P
glucose 6-phosphate	G6P
ribose 5-phosphate	Ri5P
ribose 5-phosphate	R5P
glyceraldehyde 3-phosphate	GA3P
sedoheptulose 7-phosphate	S7P
erythrose 4-phosphate	E4P
fructose 6-phosphate	F6P
Glucose	GLUC
fructose 6-phosphate	FRU
dihydroxyacetone phosphate	DHAP
Glycerol	GLY
3-phosphoglycerate	3PG
Phosphoenolpyruvate	PEP
Pyruvate	PYR
acetyl-CoA	AcCoA
Oxaloacetate	OAA
Citrate	CIT
Isocitrate	ICT
2-oxoglutarate	AKG
Succinate	SUC
Malate	MAL
Glyoxylate	GLO
Acetaldehyde	ACALD
Arabinose	ARA
Ethanol	ETH
Glutamine	GLN
Asparagines	ASP
Serine	SER
Glutamine	GLU
5-amino-1-(5-phospho-ribosyl)imidazole-4-carboxamide	AICAR
Fumarate	FUM
Methyl FH4	MEFH4
fructose 2,6-bisphosphate	F26BP
glycerol 3-phosphate	G3P
Xylitol	XYLI

B. The reactions

symbol	*protein*	*enzyme name*	*reaction equation*
Glycolysis			
v_1	Hxk(1,2)	hexokinase	1 ATP + 1 GLUC → 1 H+ + 1 G6P
v_2	Hxk(1,2)	hexokinase	1 ATP + 1 FRU → 1 H+ + 1 F6P
v_3	Pgi1	glucose 6-phosphate isomerase	1 G6P ↔ 1 F6P
v_4	Pfk(26,27)	6-phosphofructo-2-kinase	1 ATP + 1 F6P → 1 H+ + 1 F16BP
v_5	Fbp26	fructose-2,6-bisphosphate 2-phosphatase	1 H2O + 1 F26BP → 1 pi + 1 F6P
v_6	Fba1	fructose-bisphosphate aldolase	1 F26BP ↔ 1 GA3P + 1 DHAP
v_7	Tpi1	triose-phosphate isomerase	1 DHAP ↔ 1 GA3P
v_8	Tdh(1-3)	glyceraldehyde-3-phosphate dehydrogenase phosphoglycerate kinase	1 pi + 1 NAD+ + 1 GA3P ↔ 1 H+ + 1 ATP + 1 3PG
v_9	Gpm(1-3) Eno(1,2)	phosphoglycerate mutase enolase	1 3PG ↔ 1 H2O + 1 PEP
v_{10}	Cdc19 Pyk2	pyruvate kinase	1 H+ + 1 PEP ↔ 1 ATP + 1 PYR
Pentose phosphate pathway			
v_{11}	PdE(1-3)	pyruvate dehydrogenase	1 CoA + 1 NAD+ + 1 PYR → 1 CO2 + 1 AcCoA
v_{12}	Xor	xylose reductase	1 H+ + 1 XYL → 1 NAD+ + 1 XYLI
v_{13}	Xor	xylose reductase	1 H+ + 1 NADPH + 1 XYL → 1 XYLI
v_{14}	Dxd Xks	xylitol dehydrogenase xylulokinase	1 NAD+ + 1 ATP + 1 XYLI → 2 H+ + 1 X5P
v_{15}	AraA AraB AraD	arabinose isomerase ribulokinase ribulose 5-phosphate 4-epimerase	1 ATP + 1 ARA → 1 X5P
v_{16}	Zwf1 Sol(1-4) Gnd(1,2)	glucose 6-phosphate dehydrogenase phosphogluconate dehydrogenase	1 H2O + 1 G6P → 2 H+ + 1 CO2 + 2 NADPH + 1 Ri5P
v_{17}	Rpe1	ribulose 5-phosphate 3-epimerase	1 Ri5P → 1 X5P
v_{18}	Rki1	ribose-5-phosphate isomerase	1 Ri5P → 1 R5P
v_{19}	Tkt(1,2)	transketolase	1 X5P + 1 R5P ↔ 1 GA3P + 1 S7P
v_{20}	Tal(1,2)	transaldolase	1 X5P + 1 E4P ↔ 1 GA3P + 1 F6P
Glycerolipid metabolism			
v_{21}	Tkt(1,2)	transketolase	1 X5P + 1 E4P ↔ 1 GA3P + 1 F6P
v_{22}	Gpd1	glycerol-3-phosphate dehydrogenase	1 H+ + 1 DHAP +1 FAD+ → 1 NAD+ + 1 G3P + 1 FADH2
v_{23}	Hor2 Rhr2	glycerol-3-phosphatase	1 H2O + 1 G3P → 1 pi + 1 GLY
v_{24}	Pck1	phosphoenolpyruvate carboxykinase	1 ATP + 1 OAA ↔ 1 CO2 + 1 PEP
v_{25}	Cit(1,3)	citrate synthase	1 H2O + 1 AcCoA + 1 OAA → 1 H+ + 1 CoA + 1 CIT
v_{26}	Aco1	aconitase	1 CIT ↔ 1 ICT
v_{27}	Idh(1,2)	Isocitrate dehydrogenase	1 NAD+ + 1 ICT → 1 CO2 + 1 AKG
v_{28}	Kgd(1,2) PdE3	oxoglutarate dehydrogenase	1 H+ + 1 pi + 1 NAD+ + 1 AKG → 1 CO2 + 1 ATP + 1 SUC
v_{29}	Sdh(1-4)	succinate-CoAligase succinate dehydrogenase	1 H2O + 1 FAD+ + 1 SUC ↔ 1 FADH2 + 1 MAL + 1 FUM
v_{30}	Fum1	fumarase	1 H2O + 1 FUM ↔ 1 MAL

v_{31}	Mdh(1,2)	malate dehydrogenase	1 NAD+ + 1 MAL ↔ 1 H+ + 1 OAA
v_{32}	Mae1	malic enzyme	1 MAL → 1 CO2 + 1 NADPH + 1 PYR
Anaplerotic reactions			
v_{33}	Mae2	malic enzyme	1 NAD+ + 1 MAL → 1 CO2 + 1 PYR
v_{34}	Icl1	isocitrate lyase	1 ICT → 1 SUC + 1 GLO
v_{35}	Dal1 Mis1	malate synthase	1 H2O + 1 AcCoA + 1 GLO → 1 H+ + 1 CoA + 1 MAL
Pyruvate metabolism			
v_{36}	Pde(1,5,6)	pyruvate decarboxylase	1 H+ + 1 PYR → 1 CO2 + 1 ACALD
v_{37}	Acs(1,2)	aldehyde dehydrogenase acetyl-CoA synthetase	1 H2O + 1 CoA + 1 ATP + 1 ACALD → 2 H+ + 1 ppi + 1 NADPH + 1 AcCoA
v_{38}	Adh (1,2,4,5)	alcohol dehydrogenase	1 H+ + 1 ACALD → 1 NAD+ + 1 ETH
Biomass synthesis			
v_{39}	Gdh(1,3)	glutamate dehydrogenase	1 H2O + 1 GLU ↔ 1 H+ + 1 NH4 + 1 NADPH + 1 AKG
v_{40}	Gin1	glutamine synthetase	1 NH4 + 1 ATP + 1 GLU ↔ 1 H+ + 1 pi + 1 GLN
v_{41}	Aat(1,2)	aspartate transaminase	1 AKG + 1 ASP ↔ 1 OAA + 1 GLU
		phosphoserine transaminase	
v_{42}	Ser1	phosphoglycerate	
	Ser(3,33)	dehydrogenase	1 H2O + 1 NAD+ + 1 3PG + 1 GLU ↔ 1 H+ + 1 pi + 1
	Ser2	phosphoserine phosphatase	AKG + 1 SER
v_{43}	Ade(1-8)	adenylosuccinate lyase	
	Shm2	phosphoribosylaminoimid	2 H2O + 1 CO2 + 1 NAD+ + 5 ATP + 1 R5P + 1 GLN + 1
	Prs(1-5)	azolesuccinocarboxamide	ASP + 1 SER ↔ 8 H+ + 5 pi + 1 GLU + 1 AICAR +
		synthase	1 FUM
		phosphoribosylaminoimid	
		azole carboxylase	
		phosphoribosylaminoimid	
		azole synthase	
		phosphoribosylformylglyci	
		namidine synthase	
		phosphoribosylglycinamide	
		formyltransferase	
		phosphoribosylglycinamide	
		synthaseglutamine	
		phosphoribosyldiphosphate	
		Amidotransferase	
		phosphoribosylpyrophosphate	
		synthetase	
		methenyltetrahydrofolate cyclohydrolase	
		glycine	
		hydroxymethyltransferase	
		methylenetetrahydrofolate	
		dehydrogenase	
Other fluxes			
v_{44}	–	–	Uptake → GLUC
v_{45}	–	–	Uptake → XYL
v_{46}	–	–	Uptake → FRU
v_{47}	–	–	Uptake → ARA
v_{48}	–	–	ETH → Excrete
v_{49}	–	–	GLY → Excrete
v_{50}	–	–	XYLT → Excrete
v_{51}	–	–	ATP → Maintenance
v_{52}	–	–	CO2 → CO2EX
mue	–	–	Growth rate

The cofactor pair(ex. ATP, ADP) is shown on one side for each reaction only.