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 **x** is an n-dimensional vector of metabolite amounts per cell, **v** is the vector of r reaction fluxes. The element N_{ij} of the stoichiometric matrix **N** is the stoichiometric coefficient that indicates the amount of the ith compound produced per unit flux of the jth 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

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

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

where $\mathbf{v}^{\text{rev},\text{UB}}$ and $\mathbf{v}^{\text{rev},\text{LB}}$ 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 'nondecomposability'.
- (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:

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

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:

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

- (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.

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

$$S(\mathbf{m}_{i}^{(j)}) \cap S(\mathbf{m}_{m}^{(j)}) \not\subset S(\mathbf{m}_{l}^{(j+1)})$$
(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 $\mathbf{t}_{i,j+1}^{(j)} = 0$. 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, $\phi = |t_{i,j+1}^{(j)}| \mathbf{t}_m^{(j)} + |t_{m,j+1}^{(j)}| \mathbf{t}_i^{(j)}$.

(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 $\tau = \operatorname{sgn}(t_{i,j+1}^{(j)})(t_{i,j+1}^{(j)} \mathbf{t}_{m}^{(j)} - t_{m,j+1}^{(j)} \mathbf{t}_{i}^{(j)})$ 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

 $\mathrm{C_6H_{12}O_6}$ + 2H⁺+2H₂ PO₄ +2ADP →2ATP+2H₂O +2C₂ H₅ OH + 2CO₂

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

 $C_6H_{12}O_6 + 0.342H^+ + 0.692H_2O +$

- 0.126phosphate + 0.000930coenzymeA
- + 0.156FAD + 0.0731ammonium
- + 0.00108sufate + 0.0000730Methyl FH4
- + 0.00217 other Biomass \rightarrow 0.818C₂H₅OH
- + 0.944glycerol + 1.472CO₂ + 0.0496diphosphate + 0.156FADH2

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

Table 1 The Maximal Specific Molar Yield for **Different Cases**

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

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 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

 $\begin{array}{l} C_5 H_{10} O_5 + 0.160 H_2 O + 0.75 H^+ + \\ 0.265 phosphate + 0.0116 coenzymeA \\ + 0.0245 FAD + 0.0366 ammonium \\ + 0.000542 sufate + 0.0000365 Methyl \\ FH4 + 0.00109 other Biomass \\ \rightarrow 0.421 C_2 H_5 OH + 0.228 glycerol \\ + 0.267 xylitol + 0.789 CO_2 \\ + 0.0245 FAD H2 + 0.227 ATP \end{array}$



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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	Hexoses	Pentoses
Individual enzymes	Hexokinase phosphoenolpyruvate carboxykinase aldehyde dehydrogenase acetyl-CoA synthetase	glucose 6-phosphate isomerase xylose reductase xylitol dehydrogenase 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

 Table 2

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

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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	Н+
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	АТР
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

symbol protein reaction equation enzyme name Glycolysis Hxk(1,2)hexokinase $1 \text{ ATP} + 1 \text{ GLUC} \rightarrow 1 \text{ H} + 1 \text{ G6P}$ \mathbf{v}_1 Hxk(1,2) hexokinase $1 \text{ ATP} + 1 \text{ FRU} \rightarrow 1 \text{ H} + + 1 \text{ F6P}$ \mathbf{v}_2 glucose 6-phosphate isomerase $1 \text{ G6P} \leftrightarrow 1 \text{ F6P}$ Pgi1 \mathbf{v}_3 Pfk(26,27) 6-phosphofructo-2-kinase $1 \text{ ATP} + 1 \text{ F6P} \rightarrow 1 \text{ H} + 1 \text{ F16BP}$ v_4 v_5 Fbp26 fructose-2,6-bisphosphate $1 \text{ H2O} + 1 \text{ F26BP} \rightarrow 1 \text{ pi} + 1 \text{ F6P}$ 2-phosphatase Fba1 fructose-bisphosphate aldolase $1 \text{ F26BP} \leftrightarrow 1 \text{ GA3P} + 1 \text{ DHAP}$ \mathbf{v}_{6} Tpi1 triose-phosphate isomerase 1 DHAP \leftrightarrow 1 GA3P **v**₇ Tdh(1-3) glyceraldehyde-3-phosphate 1 pi + 1 NAD+ + 1 GA3P \leftrightarrow 1 H+ + 1 ATP + 1 3PG v_8 dehydrogenase phosphoglycerate kinase Gpm(1-3)phosphoglycerate mutase $1 3PG \leftrightarrow 1 H2O + 1 PEP$ v_9 Eno(1,2)enolase Cdc19 pyruvate kinase $1 \text{ H} + 1 \text{ PEP} \leftrightarrow 1 \text{ ATP} + 1 \text{ PYR}$ **V**₁₀ Pyk2 Pentose phosphate pathway 1 CoA + 1 NAD+ + 1 PYR \rightarrow 1 CO2 + 1 AcCoA PdE(1-3) pyruvate dehydrogenase **v**₁₁ Xor xvlose reductase $1 \text{ H} + 1 \text{ XYL} \rightarrow 1 \text{ NAD} + 1 \text{ XYLI}$ v_{12} xvlose reductase Xor $1 \text{ H}++1 \text{ NADPH}+1 \text{ XYL} \rightarrow 1 \text{ XYLI}$ v_{13} Dxd xylitol dehydrogenase 1 NAD+ + 1 ATP + 1 XYLI \rightarrow 2 H+ + 1 X5P v_{14} Xks xvlulokinase AraA arabinose isomerase v_{15} AraB ribulokinase $1 \text{ ATP} + 1 \text{ ARA} \rightarrow 1 \text{ X5P}$ AraD ribulose 5-phosphate 4-epimerase Zwf1 glucose 6-phosphate v_{16} Sol(1-4) dehydrogenase $1 \text{ H2O} + 1 \text{ G6P} \rightarrow 2 \text{ H} + 1 \text{ CO2} + 2 \text{ NADPH} + 1 \text{ Ri5P}$ Gnd(1,2) phosphogluconate dehydrogenase Rpe1 ribulose 5-phosphate $1 \operatorname{Ri5P} \rightarrow 1 \operatorname{X5P}$ **v**₁₇ 3-epimerase Rki1 ribose-5-phosphate $1 \operatorname{Ri5P} \rightarrow 1 \operatorname{R5P}$ **V**₁₈ isomerase Tkt(1,2)transketolase $1 \text{ X5P} + 1 \text{ R5P} \leftrightarrow 1 \text{ GA3P} + 1 \text{ S7P}$ v_{19} Tal(1,2) transaldolase $1 \text{ X5P} + 1 \text{ E4P} \leftrightarrow 1 \text{ GA3P} + 1 \text{ F6P}$ v_{20} **Glycerolipid metabolism** transketolase $1 \text{ X5P} + 1 \text{ E4P} \leftrightarrow 1 \text{ GA3P} + 1 \text{ F6P}$ Tkt(1,2) $v_{21}^{}$ Gpd1 glycerol-3-phosphate 1 H+ + 1 DHAP +1 FAD+ \rightarrow 1 NAD+ + 1 G3P + 1 v_{22} dehydrogenase FADH2 Hor2 v_{23} Rhr2 glycerol-3-phosphatase 1 H2O + 1 G3P \rightarrow 1 pi + 1 GLY Pck1 phosphoenolpyruvate $1 \text{ ATP} + 1 \text{ OAA} \leftrightarrow 1 \text{ CO2} + 1 \text{ PEP}$ v_{24} carboxykinase Cit(1,3) citrate synthase $1 \text{ H2O} + 1 \text{ AcCoA} + 1 \text{ OAA} \rightarrow 1 \text{ H} + 1 \text{ CoA} + 1 \text{ CIT}$ v_{25} Aco1 aconitase $1 \text{ CIT} \leftrightarrow 1 \text{ ICT}$ \mathbf{v}_{26} 1 NAD+ + 1 ICT \rightarrow 1 CO2 + 1 AKG Isocitrate dehydrogenase Idh(1,2) v_{27} Kgd(1.2) oxoglutarate 1 H+ + 1 pi + 1 NAD+ + 1 AKG \rightarrow 1 CO2 + 1 ATP + v_{28} PdE3 dehydrogenase 1 SUC Sdh(1-4) succinate-CoAligase $1 \text{ H2O} + 1 \text{ FAD} + + 1 \text{ SUC} \leftrightarrow 1 \text{ FADH2} + 1 \text{ MAL} + 1 \text{ FUM}$ v_{29} succinate dehydrogenase Fum1 fumarase $1 \text{ H2O} + 1 \text{ FUM} \leftrightarrow 1 \text{ MAL}$ v_{30}

B. The reactions

v_{31}	Mdh(1,2)	malate dehydrogenase	1 NAD+ + 1 MAL \leftrightarrow 1 H+ + 1 OAA
\mathbf{v}_{32}	Mae1	malic enzyme	$1 \ \mathrm{MAL} \rightarrow 1 \ \mathrm{CO2}$ + 1 NADPH + 1 PYR
Anaple	erotic reaction	IS	
V ₃₃	Mae2	malic enzyme	1 NAD+ + 1 MAL \rightarrow 1 CO2 + 1 PYR
V ₃₄	Icl1	isocitrate lyase	$1 \text{ ICT} \rightarrow 1 \text{ SUC} + 1 \text{ GLO}$
V ₃₅	Dal1	malate synthase	1 H2O + 1 AcCoA + 1 GLO \rightarrow 1 H+ + 1 CoA + 1
	Mis1		MAL
Pyruva	ate metabolisn	n	
V.26	Pde(1,5,6)	pyruvate decarboxylase	1 H + + 1 PYR \rightarrow 1 CO2 + 1 ACALD
V ₂₇	Acs(1,2)	aldehyde dehydrogenase	1 H2O + 1 CoA + 1 ATP + 1 ACALD \rightarrow 2 H+ + 1 ppi + 1
57		acetyl-CoA synthetase	NADPH + 1 AcCoA
V ₃₈	Adh (1,2,4,5)	alcohol dehydrogenase	1 H+ + 1 ACALD \rightarrow 1 NAD+ + 1 ETH
Bioma	ss synthesis		
V ₂₀	Gdh(1,3)	glutamate dehydrogenase	$1 \text{ H2O} + 1 \text{ GLU} \leftrightarrow 1 \text{ H} + + 1 \text{ NH4} + 1 \text{ NADPH} + 1 \text{ AKG}$
V.0	Gin1	glutamine synthetase	$1 \text{ NH4} + 1 \text{ ATP} + 1 \text{ GLU} \leftrightarrow 1 \text{ H} + + 1 \text{ pi} + 1 \text{ GLN}$
V.1	Aat(1,2)	aspartate transaminase	$1 \text{ AKG} + 1 \text{ ASP} \leftrightarrow 1 \text{ OAA} + 1 \text{ GLU}$
41		phosphoserine	
		transaminase	
V42	Ser1	phosphoglycerate	
	Ser(3,33)	dehydrogenase	1 H2O + 1 NAD+ + 1 3PG + 1 GLU \leftrightarrow 1 H+ + 1 pi + 1
	Ser2	phosphoserine	AKG + 1 SER
		phosphatase	
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	$ASP + 1 SER \leftrightarrow 8 H+ + 5 pi + 1 GLU + 1 AICAR +$
		synthase	1 FUM
		phosphoribosylaminoimid	
		azole carboxylase	
		phosphoribosylaminoimid	
		azole synthase	
		phosphoribosylformylglyci	
		namume synthase	
		formultron afore ao	
		nhosphorihosylglycinamide	
		synthaseglutamine	
		phosphoribosyldiphosphate	
		Amidotransferase	
		phosphoribosylpyrophosphate	
		synthetase	
methenvltetrah		methenyltetrahydrofolate cyclo	hydrolase
		glycine	
		hydroxymethyltransferase	
		methylenetetrahydrofolate	
		dehydrogenase	
Other	fluxes		
V.,	_	_	Uptake \rightarrow GLUC
V44 V45	_	_	$Uptake \rightarrow XYL$
v46	_	_	$Uptake \rightarrow FRU$
v_{47}	_	_	$\text{Uptake} \rightarrow \text{ARA}$
v_{48}	_	-	$ETH \rightarrow Excrete$
v_{49}	_	-	$GLY \rightarrow Excrete$
v_{50}	_	-	$XYLT \rightarrow Excrete$
v ₅₁	_	-	ATP \rightarrow Maintenance
V 52	_	-	$OU2 \rightarrow OU2EA$
mue	_	-	GIUWHI TALE

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