Stoichiometry in Metabolic Reactions

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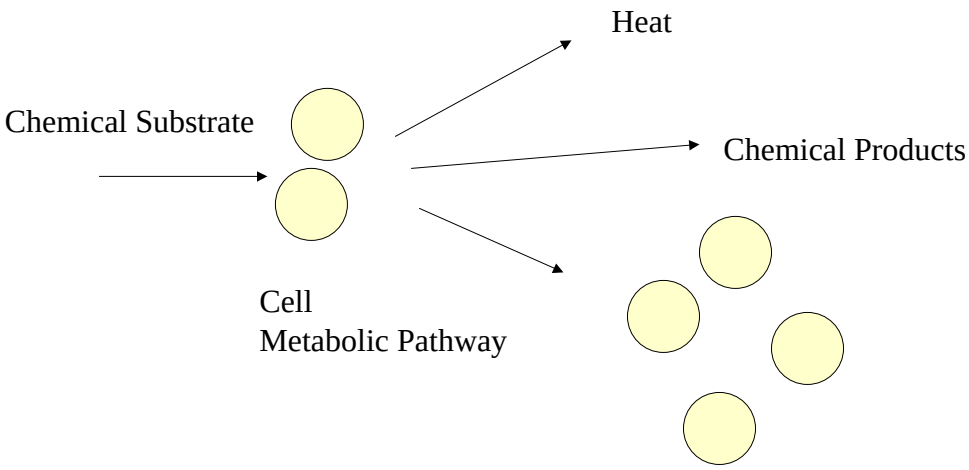
Chemical Species Flow into and out of the System(Fig.3.2, p.164)



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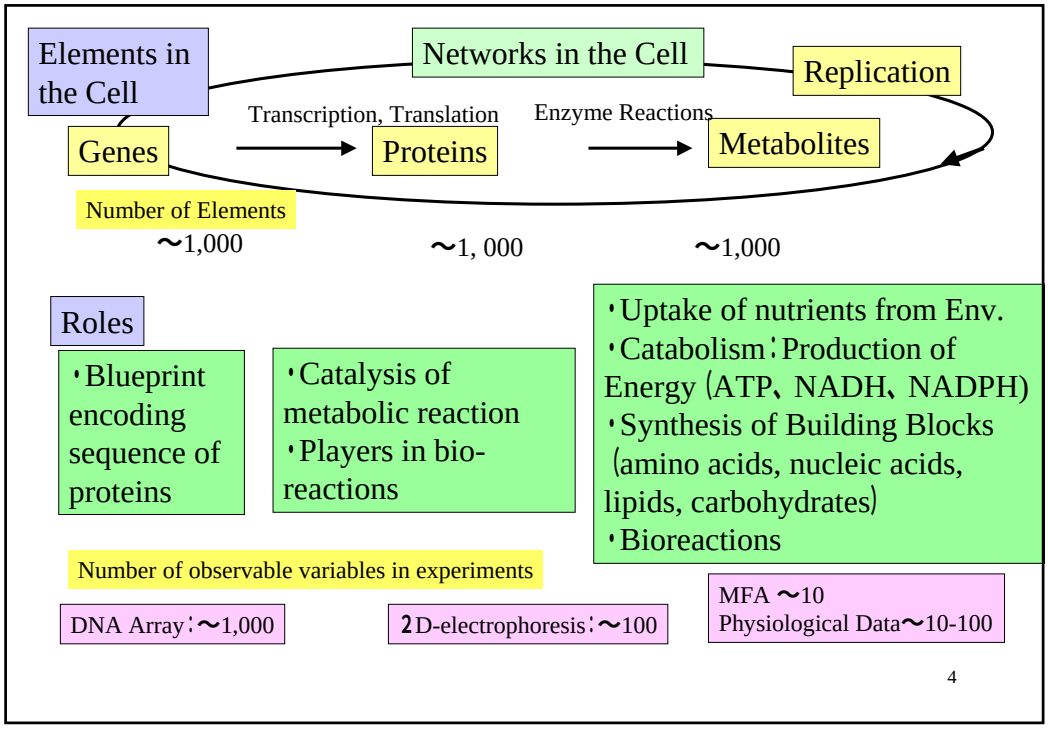

Table 3.1 (p.168) Molecular composition of Bacterium

Component	Wt %	MW	Number of Molecules
Entire cell	100		
Water	80	18	$4x10^{10}$
Dry cell	20		
Protein			
Ribosomal	1.5	$4x10^{4}$	$3x10^{5}$
Non Ribosomal	10	$5x10^{4}$	$1.8x10^{6}$
RNA			
ribosomal (16S)	1.0	$6x10^{5}$	$1.5x10^{4}$
ribosomal (23S)	1.0	$1.2x10^{6}$	$1.5x10^{4}$
t-RNA	1.0	$2.5x10^{4}$	$3.5x10^{5}$
mRNA	1.0	$1x10^{6}$	$9x10^{5}$
DNA	1.0	$4.5x10^{9}$	2
Polysaccarides			
Lipids	1.0	$1x10^{3}$	$9x10^{6}$
Small Molecules	0.5	$4x10^{2}$	$1.2x10^{7}$

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Elemental Composition of Microorganisms

Microorganism		%C	%H	%O	%N	ash%
Yeast	(Lab strain)	38	6	35	9	12
	(Brew. Strain)	46	7	33	10	4.3
C.glutamicum		48	7	26	11	6

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Elemental Composition Balance of the Cell (p.167)

$$\alpha CH_lO_m + \beta NH_3 + \gamma O_2 \rightarrow CH_aO_bN_c + \delta CH_pO_qN_r + \varepsilon H_2O + \kappa CO_2$$

C-source Cell product

CH_lO_m: elemental composition of carbon source (l, m: constants)

$CH_aO_bN_c$: elemental composition of the cell (a, b, c: constants)

$CH_pO_qN_r$: elemental composition of the extracellular product (p, q, r: constants)

Quiz: Make elemental composition balance.

α.....κ: Time variant parameters

7

Answer

C: $\alpha = 1 + \delta + \kappa$

H: $l\alpha + 3\beta = a + p\delta + 2\varepsilon$ (1)

O: $m\alpha + 2\gamma = b + q\delta + \varepsilon + 2\kappa$

N: $\beta = c + r\delta$

Quiz 2
Change Eq. (1) to Matrix and vector form.

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$$\alpha CH_lO_m + \beta NH_3 + \gamma O_2 \rightarrow CH_aO_bN_c + \delta CH_pO_qN_r + \varepsilon H_2O + \kappa CO_2$$

Answer 2

$$\begin{bmatrix} 1 & 0 & 0 & -1 & 0 & -1 \\ l & 3 & 0 & -p & -2 & 0 \\ m & 0 & 2 & -q & -1 & -2 \\ 0 & 1 & 0 & -r & 0 & 0 \end{bmatrix} \begin{bmatrix} \alpha \\ \beta \\ \gamma \\ \delta \\ \varepsilon \\ \kappa \end{bmatrix} = \begin{bmatrix} 1 \\ a \\ b \\ c \end{bmatrix}$$

Quiz 3
How to determine all the reaction rates ?

9

$$\alpha CH_lO_m + \beta NH_3 + \gamma O_2 \rightarrow CH_aO_bN_c + \delta CH_pO_qN_r + \varepsilon H_2O + \kappa CO_2$$

Answer 3

$$\begin{bmatrix} 1 & 0 & 0 & -1 & 0 & -1 \\ l & 3 & 0 & -p & -2 & 0 \\ m & 0 & 2 & -q & -1 & -2 \\ 0 & 1 & 0 & -r & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} \alpha \\ \beta \\ \gamma \\ \delta \\ \varepsilon \\ \kappa \end{bmatrix} = \begin{bmatrix} 1 \\ a \\ b \\ c \\ R_S \\ R_{NH3} \end{bmatrix}$$

$Ar_c=r_m$

$r_c=A^{-1}r_m$

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Elemental Composition Balance of the Cell

$$\alpha CH_lO_m + \beta NH_3 + \gamma O_2 \rightarrow CH_aO_bN_c + \delta CH_pO_qN_r + \varepsilon H_2O + \kappa CO_2$$

C-source Cell product

Reaction Rate (mol/h)
Rs: substrate consumption (uptake) rate
R_{NH3}: ammonia consumption (uptake) rate
Ro: Oxygen consumption (uptake) rate
Rcell: Cell growth rate
Rp: Product formation rate
Rh: H_2O production rate
Rc: CO_2 production rate

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$$\alpha CH_lO_m + \beta NH_3 + \gamma O_2 \rightarrow CH_aO_bN_c + \delta CH_pO_qN_r + \varepsilon H_2O + \kappa CO_2$$

Reaction Rate (mol/h)
Rs: substrate consumption (uptake) rate
R_{NH3}: ammonia consumption (uptake) rate
Ro: Oxygen consumption (uptake) rate
Rcell: Cell growth rate
Rp: Product formation rate
Rh: H_2O production rate
Rc: CO_2 production rate

Stoichiometric Relation

Ex. $\alpha : 1 = Rs : Rcell$

$$RQ = \frac{RCO2}{RO2} = \frac{\kappa}{\gamma}$$ Respiratory Quotient

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$\alpha.....\kappa$: Six unknown reaction rates

How to know All the reaction rate?

(1)Measure some Reaction Rate.

(2) Determine other reaction rates with Linear Constraints.

C: $\alpha = 1 + \delta + \kappa$

H: $l\alpha + 3\beta = a + p\delta + 2\varepsilon$

O: $m\alpha + 2\gamma = b + q\delta + \varepsilon + 2\kappa$

N: $\beta = c + r\delta$

Two more reaction rates are necessary !
Rcell, Rn: for example should be measured.

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Metabolic Coupling

ATP and NAD^+

ATP: energy currency metabolite in the cell

NADH: Electron transport
NADPH: Electron transport

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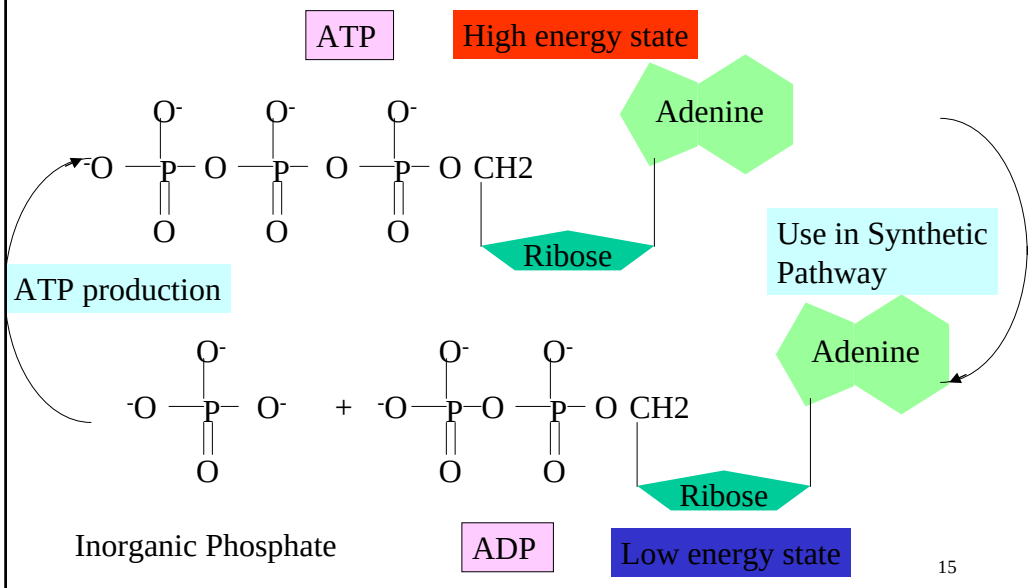
(Currency Metabolites ATP)

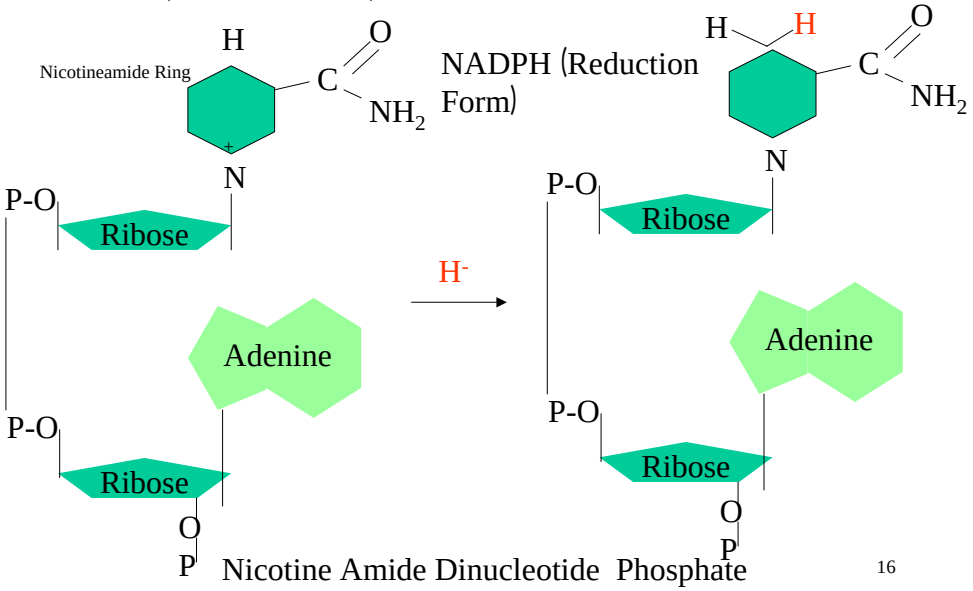


15

(Currency Metabolites NADPH)

Electron Transfer

NADP+ (Oxidation form)



Nicotine Amide Dinucleotide Phosphate

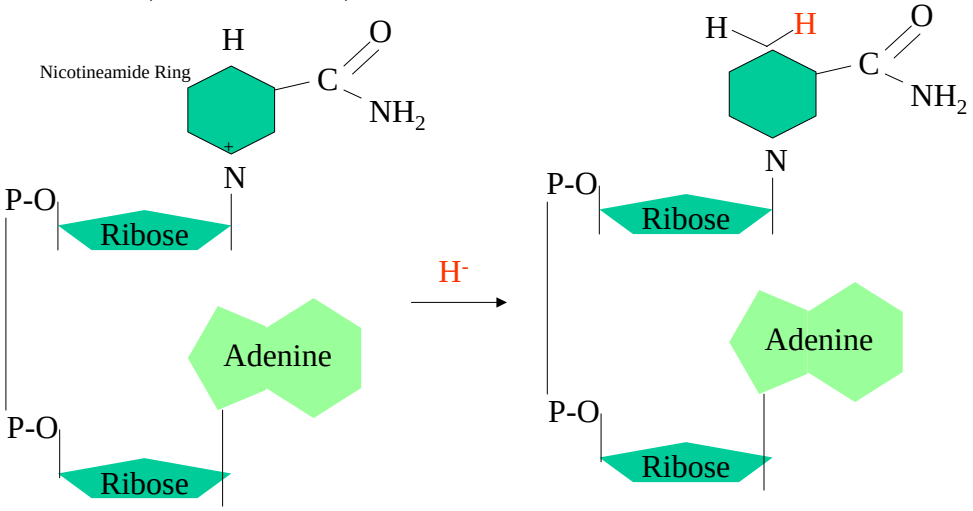
16

(Currency Metabolites: NADH)

Electron transport

NAD+ (oxidation form)

NADH (reduction form



Nicotine Amide Dinucleotide

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Energy Generation (1)

Direct formation of ATP from substrate level

$ADP + Pi \rightarrow ATP + H_2O$

(ex) Pyruvate kinase

PEP+ADP-> Pyr +ATP

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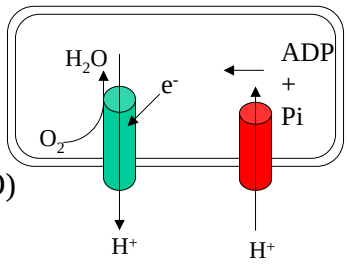
Energy Generation (2)

Oxidative phosphorylation

$2\ NADH + O_2 \rightarrow NAD + 2H_2O$ (2 electrons, 4 protons)

$2\ (P/O)\ (ADP + Pi + H^+) \rightarrow 2(P/O)(ATP + H_2O)$

P/O: Oxidative phosphorylation ratio (P/O)
Ideally (P/O): 3 (Actually 1-2)



deleted based on copyright concern.

Electron Transport Chain and Oxidative phosphorylation In Eukaryotes

Metabolic Eng by Greg Stephanopouls et al. (1998)

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ATP Consumption in Biosynthesis

$$\alpha CH_lO_m + \beta NH_3 + \gamma O_2 \rightarrow CH_aO_bN_c + \delta CH_pO_qN_r + \varepsilon H_2O + \kappa CO_2$$

C-source Cell product

$1/Y_{ATP}(ATP) \rightarrow 1/Y_{ATP}(ADP)$

Y_{ATP}: Gram cell produced per mole ATP consumed.

m_{ATP}: consumption of ATP for cell maintenance

Many other yield are shown in (p. 173-174)

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Many yields

$$Y_{X/S} = \frac{Cell\ \mathrm{Pr}oduced}{SubstrateConsumed} = \frac{1}{\alpha}$$

$$Y_{X/O} = \frac{Cell\ \mathrm{Pr}oduced}{O_2Consumed} = \frac{1}{\gamma}$$

$$Y_{P/S} = \frac{\mathrm{Pr}oduct\ \mathrm{Pr}oduced}{SubstrateConsumed} = \frac{\delta}{\alpha}$$

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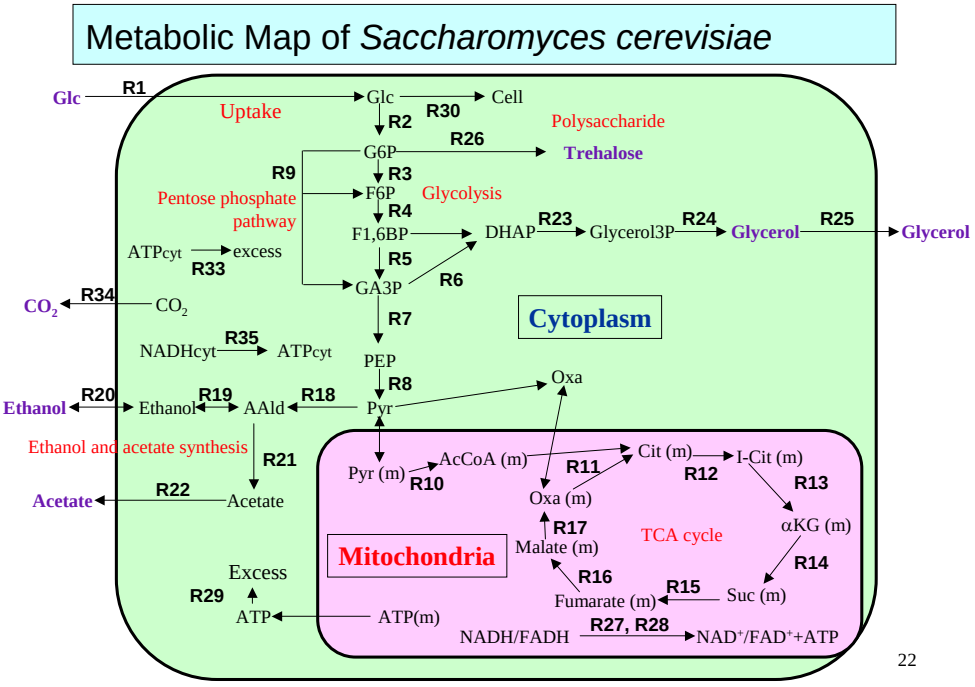
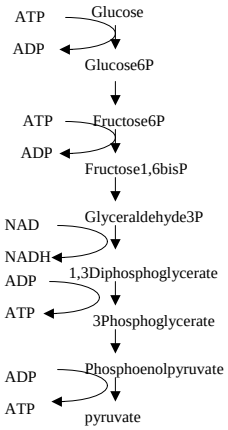


22



Question 1

Figure shows a metabolic pathway of glycolysis. Make one stoichiometric equation, summarizing up from glucose to pyruvate. Answer number of correct equation.

1. Gluc+2ADP+2NAD=2PYR+2ATP+2NADH
2. Gluc+ADP+NAD=2PYR+ATP+NADH
3. Gluc+NAD=2PYR+NADH

Fig. A metabolic pathway of glycolysis.

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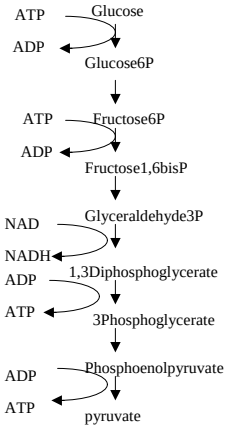

Stoichiometric Equation of Each Metabolic Reaction

Gluc+ATP=Gluc6P+ADP

Gluc6P=F6P

F6P+ATP=F1,6BP+ADP

F1,6BP=2(G3P)

(G3P)+NAD=(1,3PG)+NADH

(1,3PG)+ADP=(3PG)+ATP

(3PG)=PEP

PEP+ADP=PYR+ATP

Fig. A metabolic pathway of glycolysis.

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Answer of Q1

1. **Gluc+2ADP+2NAD=2PYR+2ATP+2NADH**

Gluc+ATP=Gluc6P+ADP

Gluc6P=F6P

F6P+ATP=F1,6BP+ADP

F1,6BP=2(G3P)

2(G3P)+2NAD=2(1,3PG)+2NADH

2(1,3PG)+2ADP=2(3PG)+2ATP

2(3PG)=2PEP

2PEP+2ADP=2PYR+2ATP

Gluc+(4-2)ADP+2NAD=2PYR+2ATP+(4-2)NADH

Correct Answer: (1)

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Metabolic Reactions in *S. cerevisiae*

(Glycolysis)

R1: Glc_ext ----------> Glct

R2: Glc + ATP ------> G6P

R3: G6P --------------> F6P

R4: F6P + ATP------> F1,6P

R5: F1,6P ------------> DHAP + GA3P

R6: GA3P -----------> DHAP

R7: GA3P -----------> PEP + ATP + NADH

R8: PEP --------------> Pyr+ ATP

(Penotose phosphate pathway)

R9: G6P ------------> 2/3 F6P + 1/3 GAP + 1 CO_2 + 2 NADPH

(TCA cycle)

R10: Py----------> AcCoA + CO_2 +NADH

R11: AcCoA + Oxa ---------> Cit

R12: Cit--------------> IsoCit

R13: IsoCit ----------> αKG + CO_2 + NADH

R14: αKG ---------> Suc + ATP + CO_2 + NADH

R15: Suc ------------> Fumarate + FADH

R16: Fumarate -------> Malate

R17: Malate ----------> Oxa + NADH

(Ethanol Synthesis)

R18: Pyr ----------> AcAld + CO_2

R19: AcAld + NADH----------> EtOH

R20: EtOH ----------> EtOH_ext

(Acetate Syntesis)

R21: AcAld ----------> Acetate

R22: Acetate ----------> Acetate_ext

(Glycerol synthesis)

R23: DHAP + NADH ----------> Glycerol3P

R24: Glycerol3P ----------> Glycerol

R25: Glycerol ----------> Glycerol_ext

(Trehalose Synthesis)

R26: 2 G6P ----------> Trehalose

(Oxidative phosphorelation)

R27: NADH ----------> 3 ATP

R28: FADH ----------> 2 ATP

(Excess ATP)

R29: ATP ----------> excess

(Cell growth)

R30: (α/6) Glc + NH_3 + (MW / Y_{ATP}) ATP ----------> Cell

Macroscopic Stoichiometry (Over all reaction)

Glycolysis(R1-R8)

Gluc_ext------→ 2 Pyr +2ATP +2NADH

TCA cycle(R10-R17)

Pyr--------→3CO2 +FADH+4NADH

Glycolysis(R1-R8) + TCA cycle(R10-R17) +Oxidative phosphorylation(R27-R28)

Gluc_ext--------→ 6CO2+ 38ATP (1)

Ethanol Synthesis (R1-R8) +(R18-R20)

Gluc_ext--------→ 2CO2 +2EtOH_ext +2ATP (2)

Acetate Synthesis (R1-R8)+(R18, R21, R2)

Gluc_ext--------→ 2CO2 +2Acetate_ext +10ATP (3)

Glycerol Synthesis (R1-R6, R23-R25)

Gluc_ext + 2ATP + 2NADH --------→ 2Glycerol_ext (4)

Trehalose Synthesis

2Gluc_ext+2ATP--------→ Trehalose (5)

(Maintenance)

ATP ----------> excess (6)

(Cell growth)

(α/6) Glc + NH_3 + (MW / Y_{ATP}) ATP -------> Cell (7)

Linear combination of Eqs. (1)-(7)

$$r_{gluc}=aR_{cell} + b\,R_{et}+cR_{ace}+dR_{gly}+eR_{tre}+f\,R_{CO2}+g\,r_{mATP}$$

Home Work

Confirm stoichiometric equations, based on reactions (1)-(30) in *S. cerevisiae.*

Glycolysis(R1-R8) + TCA cycle(R10-R17) +Oxidative phosphorylation(R27-R28)

Gluc_ext--------→ 6CO2+ 38ATP (1)

Ethanol Synthesis (R1-R8) +(R18-R20)

Gluc_ext--------→ 2CO2 +2EtOH_ext +2ATP (2)

Acetate Synthesis (R1-R8)+(R18, R21, R2)

Gluc_ext--------→ 2CO2 +2Acetate_ext +10ATP (3)

Discuss which pathway is the best for ATP generation.

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