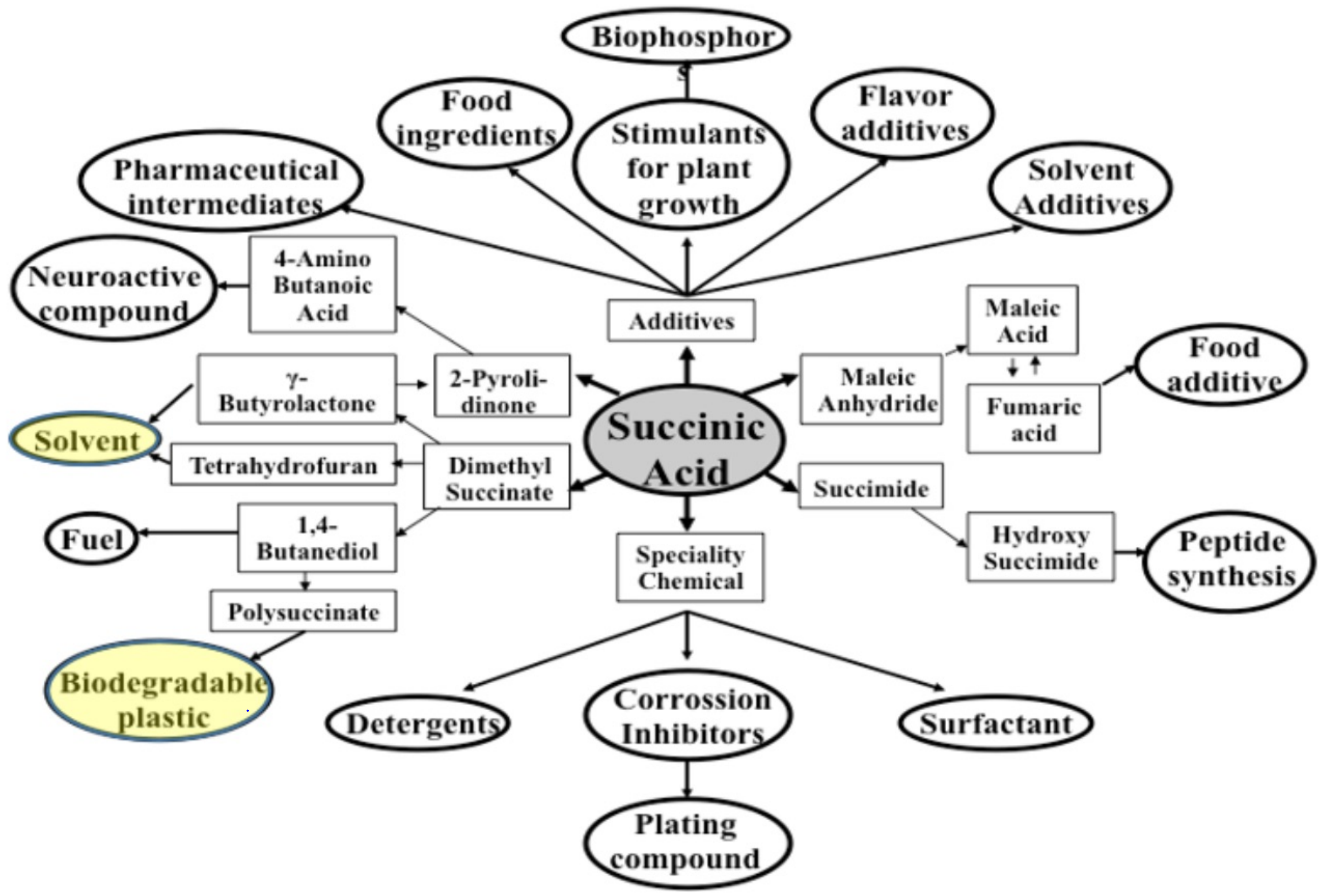
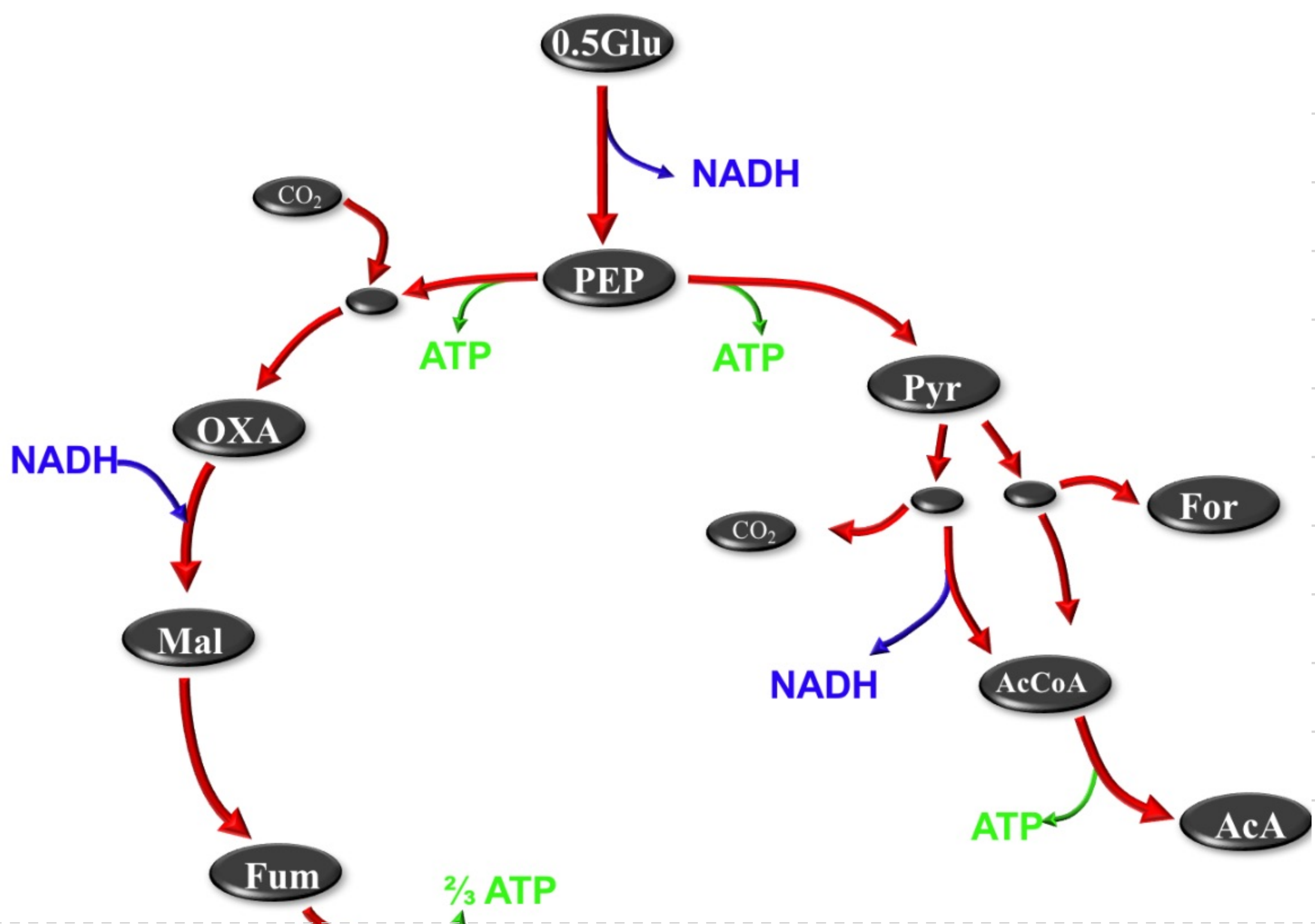
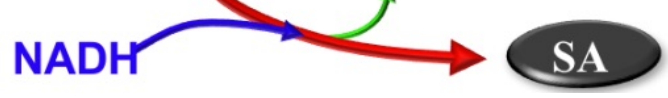
Tutorial 5

For this tutorial we'll consider the bacterium of tut 1. *Actinobacillus succinogenes* is a natural succinic acid producer under anaerobic conditions. Microbial production of succinic acid (or biosuccinic acid) as bulk chemical has taken off during the past decade and numerous organisms, natural and modified, are considered for commercial use. The diagram below show some of the applications of succinic acid, with the bioplastic and solvent applications having the largest bulk scale potential.



The central carbon metabolism of *A. succinogenes* is given below. You will note the reverse of the TCA cycle is used up to succinic acid (SA) and that oxaloacetate (OXA) is formed by carboxylation of phosphoenolpyruvate (PEP) and not from pyruvate like in eukaryotes. The PEP carboxylation step is also associated with the formation of ATP via the specialized enzyme PEP carboxykinase. Also note that pyruvate is oxidised via the pyruvate dehydrogenase as well as formate lyase route. All NADH and ATP is given on a molar basis of substrate except for the NADH in glycolysis where half a mole of glucose was used as indicated in the metabolic map.



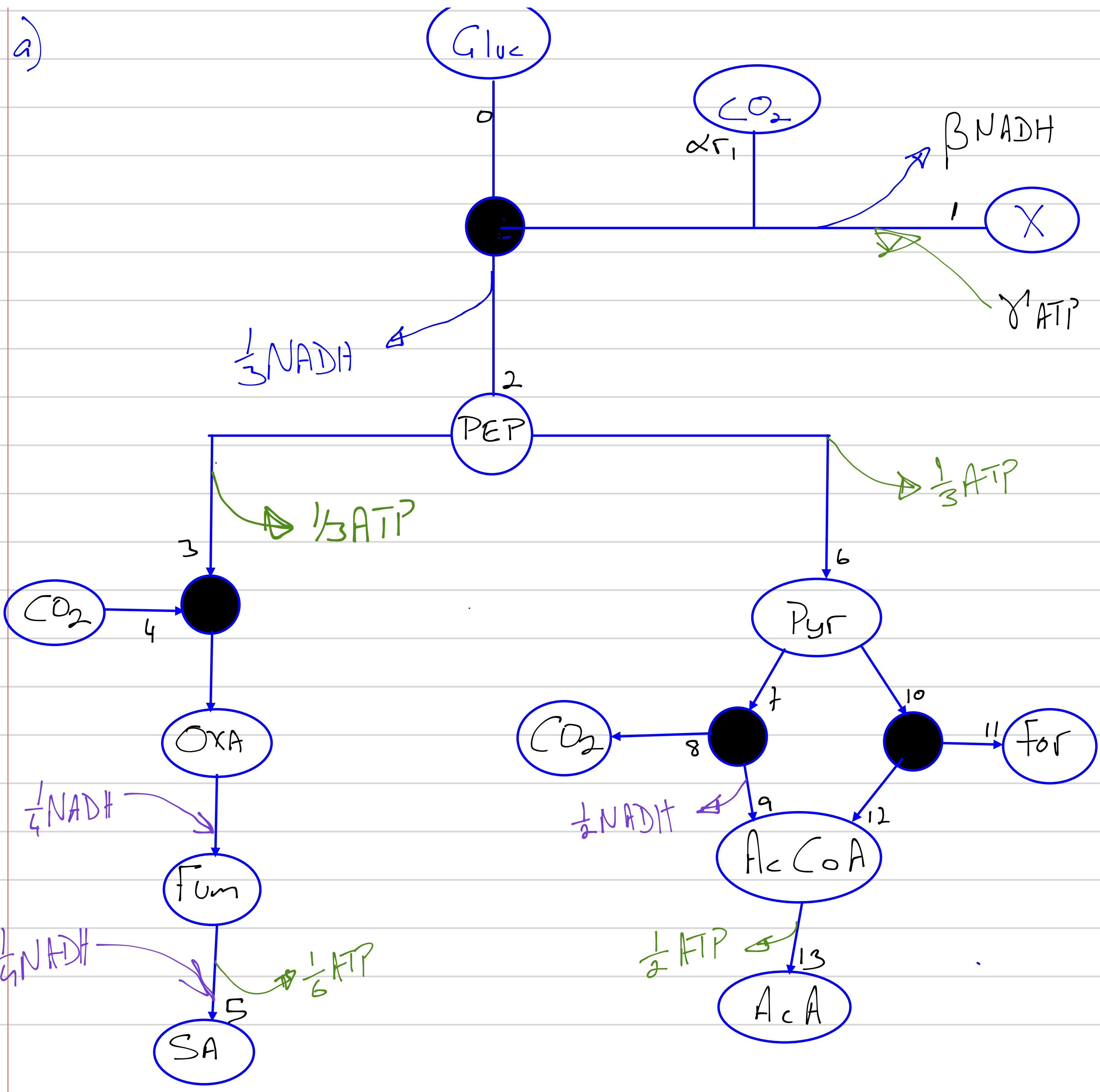


The following physiological parameters are known:

α	γ	μ	θ
$\frac{\text{cmol } CO_2}{\text{cmol X}}$	$\frac{\text{mol ATP}}{\text{cmol X}}$	$\frac{1}{\text{h}}$	$\frac{\text{mol ATP}}{\text{cmol X}\cdot\text{h}}$
0.12	1.8	0.15	0.05

The biomass formula is given by $CH_{1.9}O_{0.45}N_{0.23}$

a) Set up the cmol pathway map and include the formation of biomass. Determine the value of β. [0.085]



Anabolism:

$Y_{XS} CH_2O + Y_{XN} NH_3 + \gamma ATP \rightarrow X + \alpha CO_2 + \beta NADH + Y_{SW} H_2O$

DoR:

$CH_2O = 4$

$NH_3 = 0$

$X = CH_{1,9}O_{0,45}N_{0,23} = 4 + 1,9 - 2(0,45) - 3(0,23) = 4,31$

$NADH = 2$

$$
\begin{array}{c}
\\ C \\ DoR \\ N \\ Basis\,1 \\ Basis\,2
\end{array}
\begin{array}{c}
\begin{array}{ccccc} r_{xs} & r_{xN} & r_x & r_c & r_{NADH} \end{array} \\
\begin{bmatrix}
1 & 0 & 1 & 1 & 0 \\
4 & 0 & 4,31 & 0 & 2 \\
0 & 1 & 0,23 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 1 & 0
\end{bmatrix}
\end{array}
\times
\begin{bmatrix}
-r_{xs} \\ -r_{xN} \\ r_x \\ r_{xc} \\ r_{NADH}
\end{bmatrix}
=
\begin{bmatrix}
0 \\ 0 \\ 0 \\ 1 \\ 0,12
\end{bmatrix}
$$

$r_{NADH} = \beta = 0,085$

b) Set up the flux model and show that there is a single degree of freedom.

Node Balance

1. $r_0 = (1+\alpha) r_1 + r_2$
2. $r_2 = r_3 + r_6$
3. $r_3 + r_4 = r_5$
4. $r_6 = r_7 + r_{10}$
5. $r_7 = r_8 + r_9$
6. $r_{10} = r_{11} + r_{12}$
7. $r_9 + r_{12} = r_{13}$

Stoichiometric Balance

8. $r_3 = 3/4\, r_5$
9. $r_9 = 2/3\, r_7$
10. $r_{11} = 1/3\, r_{10}$

NADH Balance

(11) $\beta r_1 + \frac{1}{3}r_2 - \frac{1}{2}r_5 + \frac{1}{2}r_9 = 0$

ATP Balance

(12) $-\gamma r_1 + \frac{1}{3}r_3 + \frac{1}{6}r_5 + \frac{1}{3}r_6 + \frac{1}{2}r_{13} = 0$

Basis

(13) $r_X = r_1 = \mu$

We have 14 unknowns and 13 equations, where:

$\beta = 0{,}085$

$\alpha = 0{,}12$

$\gamma = 1{,}8$

$\mu = 0{,}15$

$\theta = 0{,}05$

Therefore we need 1 more specification as there is one degree of freedom.

c) Assume zero pyruvate dehydrogenase action (your single specification) and determine all the glucose based yield coefficients on a cmol basis.

[$Y_{SX} = 0.216$, $Y_{SSA} = 0.542$, $Y_{SAA} = 0.234$, $Y_{SF} = 0.117$].

Specification

(14) $r_7 = 0$

(Just look at excretion products)

$Y_{SX} = \frac{r_1}{r_0} = \frac{0{,}150}{0{,}695} = 0{,}216 \frac{\text{cmol X}}{\text{cmol Glu}}$

$Y_{SSA} = \frac{r_3}{r_0} = \frac{0{,}377}{0{,}695} = 0{,}542 \frac{\text{cmol SA}}{\text{cmol Glu}}$

$$Y_{SAA} = \frac{r_{13}}{r_0} = \frac{0,163}{0,695} = 0,234 \frac{\text{cmol AA}}{\text{cmol Glc}}$$

$$Y_{SF} = \frac{r_{11}}{r_0} = \frac{0,081}{0,695} = 0,117 \frac{\text{cmol for}}{\text{cmol Glc}}$$

d) Determine the CO_2 production/consumption rate (c). [$-r_{CO_2} = 0.0762 \frac{\text{mol } CO_2}{\text{cmol X·h}}$]

Assuming $r_7 = 0$ from previous questions:

$$-r_{CO_2} = -\left[\alpha r_1 - \tfrac{1}{4} r_5\right]$$
$$= -\left[0,12(0,15) - 0,25(0,377)\right]$$
$$= 0,0762 \frac{\text{mol } CO_2}{\text{cmol X h}}$$

e) Test your answer for mass balance consistancy by performing an elemental balance (chapter 3).

$$CH_rO + NH_3 + CO_2 \rightarrow X + \overset{SA}{CH_{1,5}O} + \overset{AA}{CH_2O} + \overset{f}{CH_2O_2} + H_2O$$

Not getting consistency

8 components

4 elemental balances, 1 basis

3 Specifications $\rightarrow$ $r_{SX} = 0,216$

$r_{SSA} = 0,542$

$r_{SAA} = 0,234$

CO_2, and Formate not balancing

f) Assume zero formate formation (only pyruvate dehydrogenase) and determine the yield coefficients.

[$Y_{SX} = 0.211$, $Y_{SSA} = 0.703$, $Y_{SAA} = 0.158$].

Used same matrix as before, just changed
$r_7 = 0$, to $r_P = 0$

$\therefore$ $Y_{SX} = 0{,}211$
$Y_{SSA} = 0{,}703$
$Y_{SAA} = 0{,}158$

g) Why is the SA yield in (f) higher than in (c)?

NADH is produced for oxydative phosphorylation, generating more ATP, therefore higher yield SA

h) The organism does not grow under high acid conditions. Determine the cmol based succinic acid yield on glucose under these conditions when pyruvate oxidation occurs via the pyruvate dehydrogenase pathway. [$Y_{SAA} = 0.89$]

$\mu = 0 \Rightarrow Y_{SSA} = 0{,}106$
$r_{XSA} = 0{,}075$

i) For the conditions in (h), what is rate of succinic acid production (r_{SA})? How does this rate compare to the r_{SA} when growth occurs (low acid conditions).

[$r_{SA} = 0.075 \frac{\text{cmol SA}}{\text{cmol X}\cdot\text{h}}$, **15%**]

$r_{SA} = 0{,}424$