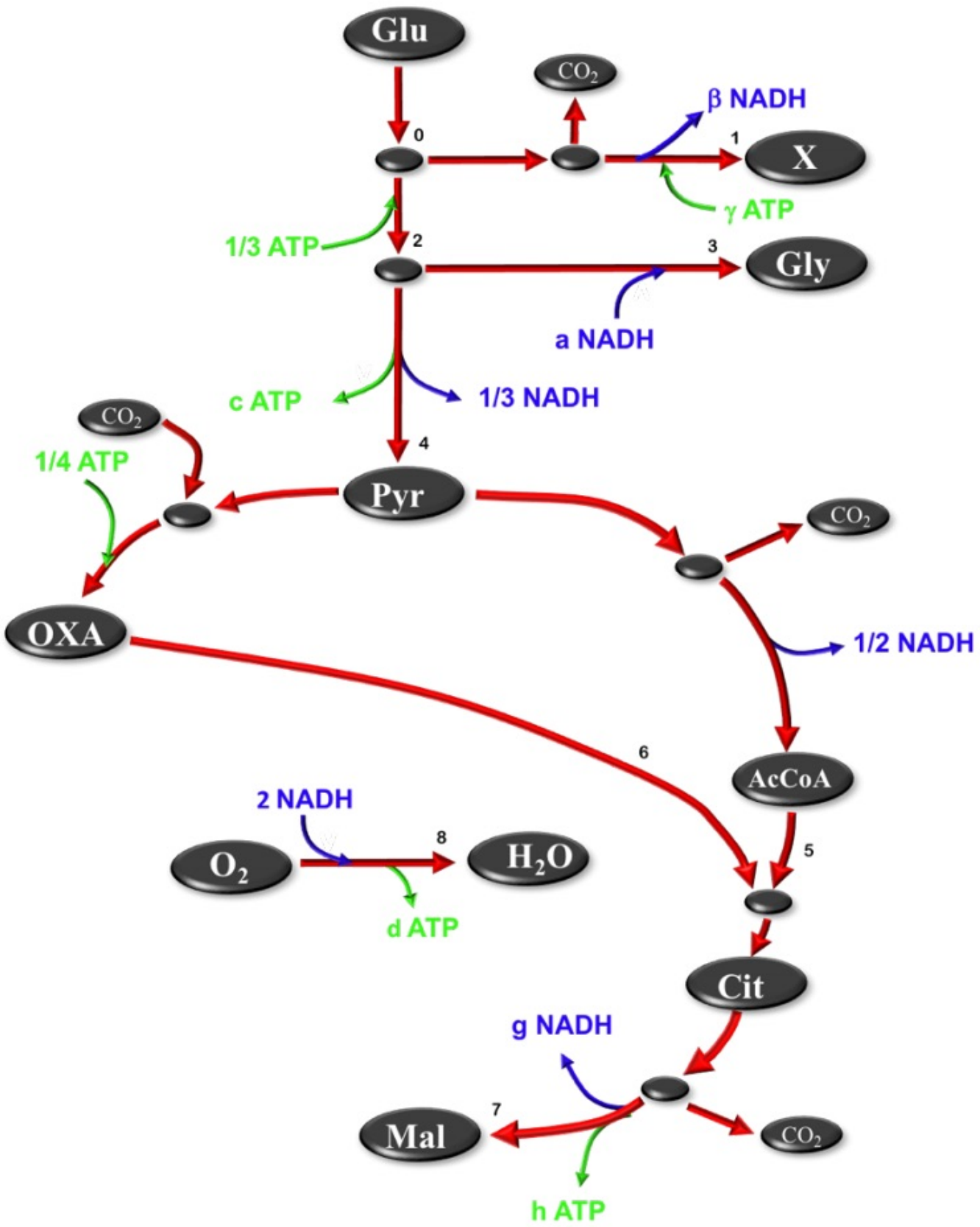
*Aspergillus oryzae* produces glycerol ($C_3H_8O_3$), biomass ($CH_{1.75}O_{0.45}N_{0.23}$) and malic acid ($C_4H_6O_5$) under aerobic conditions. Take the (P/O) ratio for NADH as 1.6 and assume that $FADH_2$=NADH. The metabolism is given by the following cmol based map:




The following is known about the physiology of the microbe:

| $\alpha$ | $\gamma$ | $\mu$ | $\theta$ |
|---|---|---|---|
| $\frac{cmol\ CO_2}{cmol\ X}$ | $\frac{mol\ ATP}{cmol\ X}$ | $\frac{1}{h}$ | $\frac{mol\ ATP}{cmol\ X \cdot h}$ |
| 0.12 | 2.2 | 0.15 | 0.1 |

1. What is the value of a? [1]

1/3

2. What is the value of β? [3]

Anabolic reaction:

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

$\alpha = 0{,}12 \dfrac{C\text{-mol } CO_2}{C\text{-mol } X}$ (given)

DOR: $CH_2O = 4 + 2(1) - 2(1) = 4$

$NH_3 = 0$

$CH_{1{,}75}O_{0{,}45}N_{0{,}23} = 4 + 1{,}75 - 2(0{,}45) - 3(0{,}23) = 4{,}16$

$$\begin{array}{c} \\ C \\ DOR. \\ N \\ Basis \\ rate \end{array} \begin{array}{c} \begin{array}{ccccc} S & N & X & C & NADH \end{array} \\ \begin{bmatrix} 1 & 0 & 1 & 1 & 0 \\ 4 & 0 & 4{,}16 & 0 & 2 \\ 0 & 1 & 0{,}23 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \end{bmatrix} \end{array} \times \begin{bmatrix} -r_{XS} \\ -r_{XN} \\ r_X \\ r_{XC} \\ r_{NADH} \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 1 \\ 0{,}12 \end{bmatrix}$$

$r_{NADH} = \beta = 0{,}16$

3. What is the value of c? [2]

2/3

From glucose to pyruvate (glycolysis), there are a net 2mol ATP produced per mol Glucose.

The cmol balance around the pyruvate node is given as:

$$r_4 = \delta\, r_6 + \varepsilon\, r_5$$

$r_4' = \frac{3}{4} r_6$

$r_5 = \frac{1}{3} r_4''$

$r_4'' = \frac{3}{2} r_5$

$r_4 = r_4' + r_4''$

$= \frac{3}{4} r_6 + \frac{3}{2} r_5$

4. What is the value of $\delta$? [1]

$\delta = 3/4$ →

5. What is the value of $\varepsilon$? [1]

$\varepsilon = 3/2$ →

6. What is the value of g? [2]

$\frac{3\ NADH}{mol\ Malate} \Big| \frac{1\ mol\ Mal}{4\ cmol\ Mal} = 3/4\ NADH = g$ → (include $FADH_2$!)

7. What is the value of h? [1]

$\frac{1\ mol\ ATP}{mol\ Mal} \Big| \frac{mol\ M.1}{4\ cmol\ Mal} = 1/4\ ATP = h$

Consider GTP as an ATP for the 4-carbon Malate

8. Determine κ: $r_6 + r_5 = \kappa r_7$ [1]

$$r_7 = \frac{4}{6}(r_6 + r_5)$$

$$r_6 + r_5 = \frac{3}{2} r_7$$

$$k = 3/2$$

The 'adapted' flux model is given in the attached Excel and Python files (see semester test 1 files under tests). Note that it represents **seven** equations, with the last equation representing the energy balance. All equations are equal to zero except the last equation that is equal to θ.

9. Determine the mass based yield of malic acid on glucose in *g/g* if the oxygen rate is known to be 0.13 *mol $O_2$/(cmol X.h)*. [3]

$$\begin{bmatrix} 7 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 8 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 9 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \times \begin{bmatrix} r_6 \\ r_7 \\ r_8 \end{bmatrix} = \begin{bmatrix} 0,11 \\ 0,15 \\ 0,13 \end{bmatrix}$$

$$r_7 = r_{M,1} = 0,280 \frac{cmol\,M,1}{cmol\,Gl.h} \left| \frac{Cmol\,Gl}{32\,Gl} \right| \frac{33,5\,g\,M_a)}{cmol\,M,1} = 0,313\ g\,M_a l / gGl$$

10. Determine the rate of $CO_2$ formation for the conditions in question 9. Give answer in *mol $CO_2$/(cmol X.h)*. [2]

$$CO_2\ formed = \alpha r_1 + \frac{1}{2} r_5 + \frac{1}{2} r_7 - \frac{1}{4} r_6$$

$$= 0,12(0,150) + \frac{1}{2}(0,111) + \frac{1}{2}(0,222) - \frac{1}{4}(0,122)$$

$$= 0,129 \frac{mol\,CO_2\ produced}{cmol\,X.h}$$

11. Determine the rate of water formation/depletion for the conditions in question 9. Give answer in *mol $H_2O$/(cmol X.h)*. [4]

MA Gly

$CH_2O + NH_3 + O_2 \rightarrow CH_{1.75}O_{0.45}N_{0.23} + CH_{\frac{3}{2}}O_{\frac{5}{4}} + CH_{\frac{8}{3}}O + CO_2 + H_2O$

Do mass balance (see spreadsheet)

for additional equations, use: $\mu = 0{,}15$ 1/h

$O_2$ uptake = 0,13 mol $O_2$ / cmol X.h

$CO_2$ production = 0,129 mol $CO_2$ / cmol X.h

Malate production = 0,222 mol Mal / cmol X.h

12. Determine the oxygen rate that will result in the formation of zero glycerol. Give your answer in *mol $O_2$/(cmol X.h)*. [3]

$r_{O_2} = r_8 = 0{,}113$ mol $O_2$ / cmol X.h →

{ make $r_3 = 0$ and just read result }

13. What is the maximum possible yield of malic acid on glucose. Give your answer in *g/g*. [3]

Set $\mu = r_1 = 0$ and $r_3 = 0$ ✓✓

$r_7 = 0{,}034$ cmol Mal / cmol X.h | cmol X.h / 0,052 cmol Gluc | 133,5 g Mal / 30 g Gluc = 0,744 g Mal / g Gluc →

14. For the condition in question 13, how many moles of oxygen ($O_2$) are consumed per **mole** of glucose? [2]

$r_8 = 0{,}5$ mol $O_2$ / cmol Gluc | 6 cmol Gluc / mol Gluc = 3 mol $O_2$ / mol Gluc →

15. Determine $Y_{XO}$ in mol/cmol that will result in an equi**molar** formation rate of glycerol and malic acid. Growth occurs at the normal rate. [4]

$r_3 = r_7$

$r_3 - r_7 = 0$ (eq 8)

$\mu = 0{,}15$

$Y_{XO} = \frac{r_8}{r_1} = 0{,}124 \frac{mol\,O_2}{cmol\,X.h} \times \frac{L_1}{0{,}150\ l} = 0{,}829 \frac{mol\,O_2}{cmol\,X}$