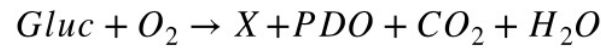


The **aerobic** 1,3 propanediol (PDO) example discussed in [tutorial 3](#) will be covered in more detail. Ignore all values given before.

You are a bioprocess engineer working at a PDO factory. Your boss is a capitalist to the bone. He knows how to get the maximum out of people and micro-organisms. He keeps on asking that he wants an online measurement of the rate at which each bug excretes PDO. He says: "It is all about measuring productivity in real time".

The overall reaction is given by:



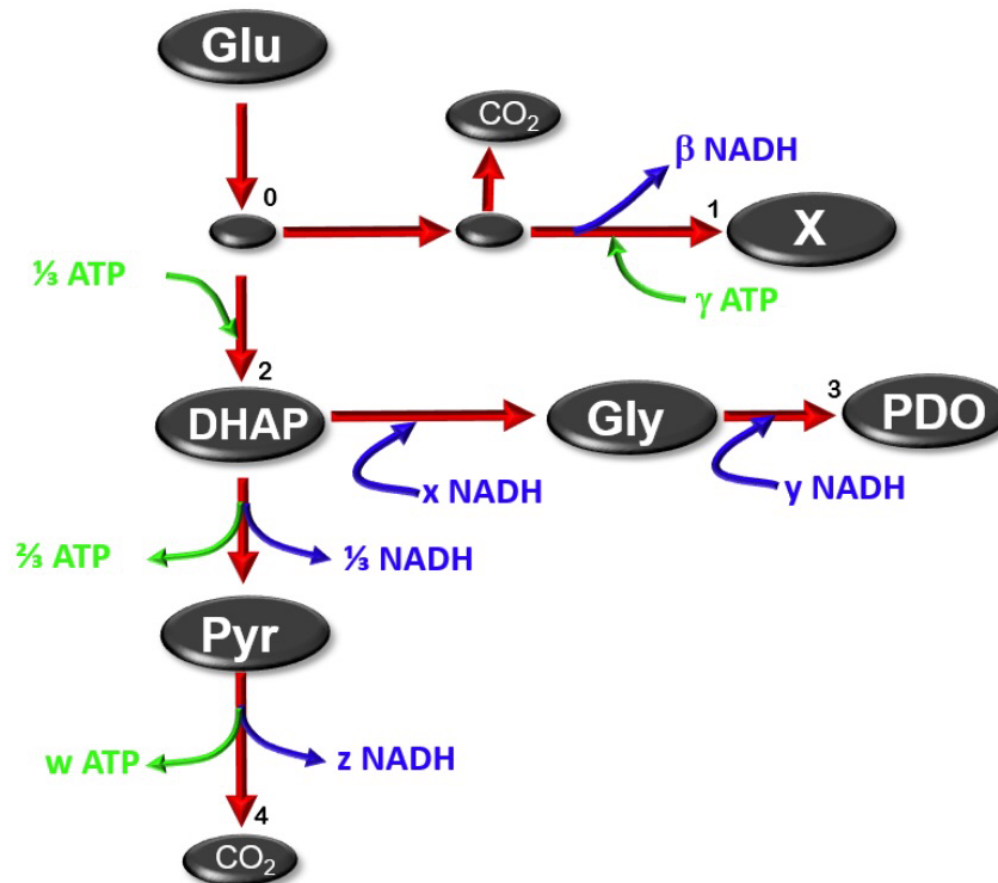
You are able to measure the concentration of (dry) biomass in gram biomass per liter (C_X) with online spectrophotometry. You can also measure the online rate of oxygen uptake per liquid volume of fermenter (r_O^v). Given known C_X you can determine the rate of oxygen uptake per gram of biomass.

In addition you can use the change in biomass concentration measurement against time to predict the fractional increase in biomass as time progress (μ). This value can be measured online by using biomass measurements from a few minutes before.

So in short you have an online measurement of r_X and r_O . The normal operating point of your fermenter is at $r_X = 0.15 \frac{1}{h}$ and $r_O^v = 219 \frac{mmol}{L \cdot h}$. You further know that $C_X = 30 \frac{g}{L}$. The biomass has a formula of $CH_{1.91}O_{0.48}N_{0.22}$

a) Use a mass balance to predict the number your boss wants (r_{PDO} in $\frac{g PDO}{g X \cdot h}$). Any issues?

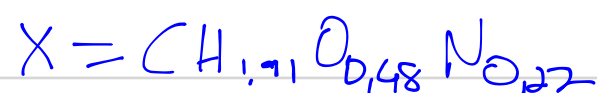
In desperation you visit the physiology department of the biorefinery. The people over there tell you that that can provide you with basic physiological properties of your modified *E.Coli*. The physiologists provide you with a limited cmol metabolic pathway map.



They also tell you that when the organism grows it requires more or less 100 mmol ATP per gram of biomass produced. They also know that this strain generates anabolic CO_2 when it grows. So in addition to making CO_2 with respiration, it makes additional CO_2 when making biomass. To be exact it makes a mol of CO_2 for every 10 cmols of biomass formed. That tell you that the ATP for cell maintenance is very small, almost negligible compared to the ATP required for growth.

$$a) \quad r_X = 0.15 \frac{1}{h}$$

$$r_O^v = 219 \frac{mmol}{L \cdot h}$$



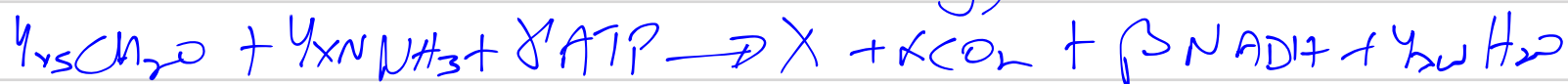
$$C_X = 30 \frac{g}{L}$$

$$r_0 = \frac{r_0^v}{C_x} = \frac{219 \text{ mmol}}{\text{L} \cdot \text{h}} \times \frac{\text{L}}{30 \text{ g}} \left| \frac{24,67 \text{ g}}{\text{mmol} \times 1000 \text{ mmol}} \right|$$

$$= \frac{0,18 \text{ mmol}}{\text{mmol} \cdot \text{h}} \rightarrow$$

* Can't do normal mass balance matrix \rightarrow missing an equation

b) Biomass Production: (Fermentative Pathway)



$$\text{DoR: } CH_2O = 4 + 2(1) - 2(1) = 4$$

$$NH_3 = -3 + 3 = 0$$

$$X = C_{H_{91}O_{0,48}N_{0,22}} = 4 + 1,91(1) - 2(0,48) - 3(0,22) = 4,29$$

$$CO_2 = 4 - 2(2) = 0$$

	S	N	X	C	NADH
C:	-1	0	1	1	0
DoR	-4	0	4,29	0	2
N	0	-1	0,22	0	0
Basis	0	0	1	0	0
NADH	0	0	0	1	0

$$\times \begin{bmatrix} -r_s \\ -r_N \\ r_X \\ r_C \\ r_{NADH} \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 1 \\ \alpha \end{bmatrix}$$

$$-r_s = 1,1$$

$$-r_N = 0,22$$

$$r_X = 1$$

$$r_C = 0,1$$

$$\boxed{r_{NADH} = 0,055 = \beta}$$

$\gamma = 2,5$ if aerobic conditions

$\gamma = 1,8$ if anaerobic conditions

$$x = \frac{1}{3} \text{ NADH}$$

$$y = \frac{1}{3} \text{ NADH}$$

$$w = \frac{1}{3} \text{ ATP}$$

$$z = \frac{5}{3} \text{ NADH} \quad \left(\text{ } \begin{array}{c} \text{PYR} \end{array} \rightarrow \begin{array}{c} 4 \\ \text{CO}_2 \end{array} \right) \text{ represents the breakdown of pyruvate and 1 TCA cycle which produces 4 NADH and 1 FADH}_2 \text{ [FADH}_2 \text{ treated as NADH]} \text{ therefore total of 5 NADH per mol Pyruvate}$$

b) Complete the unknown numbers on the metabolic map

c) Use the information from the physiologists to predict r_{PDO} . $\left[2.654 \frac{\text{g PDO}}{\text{g X} \cdot \text{h}} \right]$

d) Use the glucose consumption rate determined in (c) in the mass balance from (a) as an additional specification. Carefully compare the results from the mass balance to the results from the flux model (c). What do you see? What does this mean?

$$\beta = 0,055 \quad (\text{calculated above})$$

$$\gamma \approx 2,5 \quad (\text{ATP from aerobic catabolism})$$

$$x = y = w = \frac{1}{3} \quad (\text{from map})$$

$$z = \frac{5}{3}$$

$$\gamma' = \frac{\text{mol ATP}}{\text{cmol X}} \quad (\text{given in text})$$

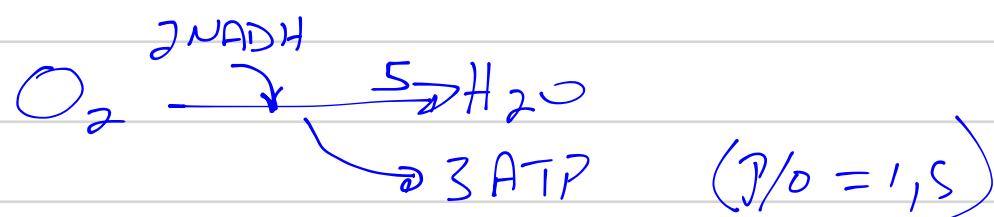
$$\therefore \frac{100 \text{ mmol ATP}}{\text{g X}} \left| \frac{\text{mol}}{1000 \text{ mmol}} \right| \frac{24,67 \text{ g}}{\text{cmol X}} = 2,47 \frac{\text{mol ATP}}{\text{cmol X}}$$

$$\Rightarrow \gamma' = 2,47$$

c) Given, $\alpha = 0,1$

$\theta \approx 0$ (assumed negligible)

Add oxidative phosphorylation flux to given map:



Node Balance:

$$\textcircled{1} \quad r_0 = (1+\alpha)r_1 + r_2$$

$$\textcircled{2} \quad r_2 = r_3 + r_4$$

3 unknowns (r_0, r_1, r_2)

2 unknowns (r_3, r_4)

NADH Balance

$$\textcircled{3} \quad \beta r_1 - \frac{2}{3}r_3 + 2r_4 - 2r_5 = 0$$

ATP Balance

$$\textcircled{4} \quad -8r_1 - \frac{1}{3}r_2 + r_4 + 3r_5 = 0$$

Basis

$$\textcircled{5} \quad \mu = r_1 = 0,15$$

Rates

$$\textcircled{6} \quad r_5 = 0,18 \quad (\text{oxygen uptake})$$

$$\begin{array}{l} \text{eq 1} \\ \text{eq 2} \\ \text{eq 3} \\ \text{eq 4} \\ \text{eq 5} \\ \text{eq 6} \end{array} \begin{bmatrix} r_0 & r_1 & r_2 & r_3 & r_4 & r_5 \\ -1 & (1+\alpha) & 1 & 0 & 0 & 0 \\ 0 & 0 & -1 & 1 & 1 & 0 \\ 0 & \beta & 0 & -2/3 & 2 & -2 \\ 0 & -8 & -1/3 & 0 & 1 & 3 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \times \begin{bmatrix} r_0 \\ r_1 \\ r_2 \\ r_3 \\ r_4 \\ r_5 \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0,15 \\ 0,18 \end{bmatrix}$$

$$r_0 = 3,782$$

$$r_3 = 2,581$$

$$r_1 = 0,150$$

$$r_4 = 1,036$$

$$r_2 = 3,617$$

$$r_5 = 0,180$$

$$r_{PDO} = r_3 = \frac{2,581 \text{ mol PDO}}{\text{mol X} \cdot \text{h}} \left| \frac{\text{mol X}}{24,675 \text{ g X}} \right| \frac{25,335 \text{ PDO}}{\text{mol PDO}} = 2,65 \frac{\text{g PDO}}{\text{g X} \cdot \text{h}}$$

d) $r_s = r_0 = 3,782 \frac{\text{mol Glc}}{\text{mol X} \cdot \text{hr}}$

$$\begin{array}{l} \text{C} \\ \text{H} \\ \text{O} \\ \text{N} \\ \text{rate 1} \\ \text{Basis} \\ \text{rate 2} \end{array} \begin{bmatrix} r_s & r_N & r_0 & r_x & r_c & r_w & r_{PDO} \\ 1 & 0 & 0 & 1 & 1 & 0 & 1 \\ 2 & 3 & 0 & 1,91 & 0 & 2 & 8/3 \\ 0 & 0 & 2 & 0,48 & 2 & 1 & 2/3 \\ 0 & 1 & 0 & 0,22 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 \end{bmatrix} \times \begin{bmatrix} -r_s \\ -r_N \\ -r_0 \\ r_x \\ r_c \\ r_w \\ r_{PDO} \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ -3,782 \\ 0,15 \\ -0,18 \end{bmatrix}$$

$$-r_s = -3,782$$

$$r_c = 1,036$$

$$-r_w = -0,033$$

$$r_w = 0,247$$

$$-r_0 = 0,180$$

$$r_{PDO} = 2,581$$

$$r_x = 0,150$$

Mass balance and flux model balance are the same.

$$r_{O_{max}} = \frac{511 \text{ mmol}}{\text{L} \cdot \text{h}} \left| \frac{\text{L}}{30,5 \text{ g X}} \right| \frac{24,675 \text{ g X}}{\text{mol X}} \left| \frac{\text{mol}}{1000 \text{ mmol}} \right| = 0,42 \frac{\text{mol O}}{\text{mol X} \cdot \text{h}}$$

You go to your boss and show him the nice and fixed relationships between the measurements and brag about your ability to predict the online PDO rate. His answer is this:

"So if I understand you correctly, if one rate increases the other rates will increase in proportion."

"Exactly sir" you reply.

"So which of these rates do we have direct control over?" he asks

You go back to the process control room and realise that the oxygen supply to the fermenter can be increased by increasing the oxygen flow-rate through the fermenter. You tell this to your boss and he replies:

"Well then open the f**ing valve!"

You do this and realise that all the rates do increase proportionally. Upto a maximum of $511 \frac{\text{mmol}}{\text{L} \cdot \text{h}}$ when $C_X = 30 \frac{\text{g}}{\text{L}}$. Further increasing the oxygen flow rate through the fermenter does not increase the oxygen uptake by the organism.

f) Determine r_{PDO} at this condition. [$6.19 \frac{\text{g PDO}}{\text{g X} \cdot \text{h}}$]

h) How fast does the organism grow at this condition. Try and think about how fast this is. [$0.35 \frac{1}{\text{h}}$]

f) changing Γ_0 to $0,284 \text{ mol O}_2 / \text{mol X} \cdot \text{h}$ results in $6,19 \frac{\text{g PDO}}{\text{g X} \cdot \text{h}}$

h) How fast does the organism grow at this condition. Try and think about how fast this is. [$0.35 \frac{1}{\text{h}}$]

What have you learned from this tutorial?

$$i) \frac{\text{kmol ATP}}{\text{g X} \cdot \text{h}} \bigg| \frac{\text{mol}}{1000 \text{ mmol}} \bigg| \frac{24,675 \text{ X}}{\text{mol}} = 0,0987 \frac{\text{mol ATP}}{\text{mol X} \cdot \text{h}} = \theta$$

Updating spreadsheet, $\Gamma_3 = \Gamma_{PDO} = 1,693 = 1,745 \frac{\text{g PDO}}{\text{g X} \cdot \text{h}}$