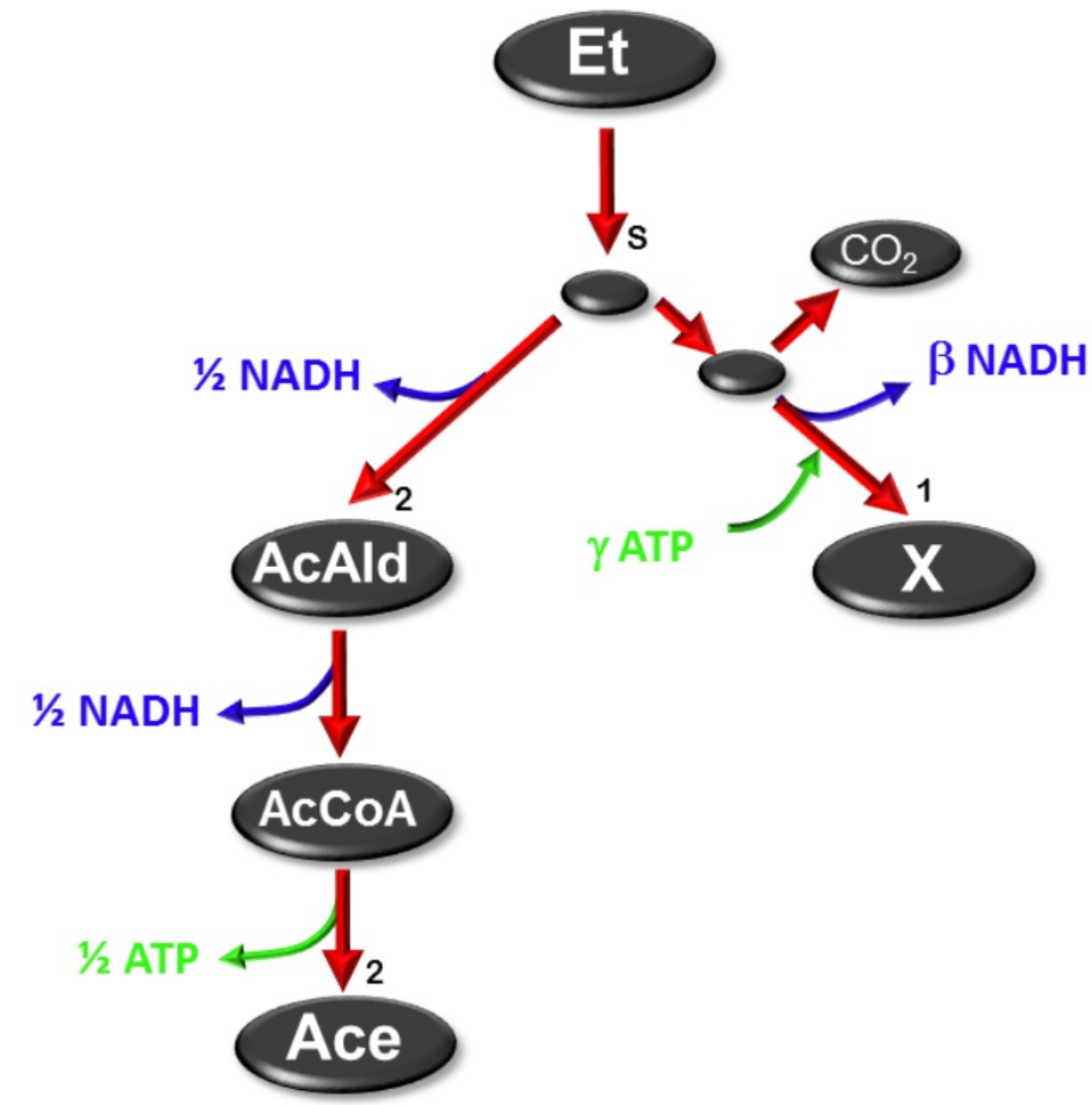
Tutorial 6

The bacteria genus *Acetobacter* produces acetic acid by using ethanol as substrate. Most commercial vinegar (acetic acid) is produced via the **aerobic** conversion of ethanol.

The metabolic pathway of the process is given by the following:



The following physiological parameters are known:

α	γ	μ	θ	P/O
$\frac{\text{cmol } CO_2}{\text{cmol X}}$	$\frac{\text{mol ATP}}{\text{cmol X}}$	$\frac{1}{\text{h}}$	$\frac{\text{mol ATP}}{\text{cmol X·h}}$	$\frac{\text{mol ATP}}{\text{mol NADH}}$
0.08	2.4	0.3	0	1.6

The biomass formula is given by $CH_{1.8}O_{0.5}N_{0.2}$

a) Determine the mass based yield of acetic acid on ethanol. [0.26 g/g]

EtOH X C W

$$CH_3O_{1/2} + NH_3 + \gamma ATP \rightarrow CH_{1.8}O_{0.5}N_{0.2} + \alpha CO_2 + \beta NADH + H_2O$$

	S	N	X	C	NADH			
C	1	0	1	1	0	$-r_S$	0	
DOR	6	0	4,2	0	2	$-r_N$	0	
N	0	1	0,2	0	0	r_X	0	
Basis	0	0	1	0	0	r_C	1	
Rate	0	0	0	1	0	r_{NADH}	$\alpha = 0{,}08$	

$\Rightarrow r_{NADH} = \beta = 1{,}14$

Flux equations:

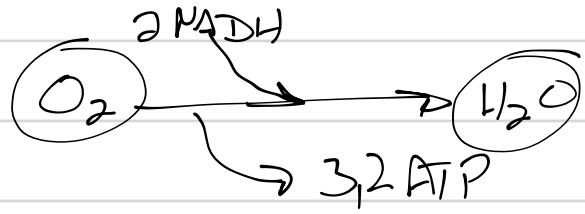
① $r_S = (1+\alpha)r_1 + r_2$

② $\beta r_1 + r_2 - 2r_3$ (NADH balance)

③ $-\gamma r_1 + \frac{1}{2}r_2 + 3{,}2r_3 = 0$ (ATP balance)

④ $r_1 = \mu = 0{,}3$ specification

where



where $P/O = 1{,}6$

Plug above in a flux matrix where $\gamma = -2{,}4$ and $\beta = 1{,}14$

$$Y_{SO} = \frac{r_2}{r_S} = 0{,}09229 \frac{\text{cmol Ace}}{\text{cmol X·h}} \left| \frac{\text{cmol X·h}}{0{,}406\,\text{cmol S}} \right| \frac{30\,\text{g Ace}}{\text{cmol Ace}} \left| \frac{\text{cmol EtOH}}{23\,\text{g EtOH}} \right. = 0{,}264\,\frac{\text{g Ace}}{\text{g EtOH}}$$

b) Will the value in (a) change if the growth rate (μ) was $0.4\frac{1}{\text{h}}$?

No, Y_{SO} is unchanged.

c) What is the rate of ethanol consumption in (a)? [0.406 $\frac{\text{cmol Et}}{\text{cmol X}\cdot\text{h}}$]

Read off the matrix for a), $r_S = 0{,}406 \frac{\text{cmol Et}}{\text{cmol X}\cdot\text{h}}$

d) What will happen to the bacterium under anaerobic conditions?

All the ethanol is used to make biomass.

e) If the θ value becomes 0.15 $\frac{\text{mol ATP}}{\text{cmol X}\cdot\text{h}}$, what will the answer in (a) become and why? [0.420 g/g]

$$Y_{SA} = \frac{r_2}{r_S} = \frac{0{,}322 \text{ cmol Ac}}{\text{cmol EtOH}} \left| \frac{32 \text{ g Ac}}{\text{cmol Ac}} \right| \frac{\text{cmol Et}}{23 \text{ g Et}} = 0{,}420 \text{ g Ace/g EtOH}$$

By increasing θ to 0,15 and keeping μ at 0,3, there is a bigger surplus left ATP for maintenance i.e. more ATP was produced, therefore more Ace. (Ac increases proportionally)

f) What fraction of ATP is spent on maintenance in (e)? [17.2%]

ATP is produced along the way to Ace. This ATP production is only spent on maintenance and growth.

maintenance, $\theta = 0,15$

growth, $\gamma r_1 = 2,4(0,3) = 0,72$

Produced $= 0,15 + 0,72 = 0,87$

Percentage on maintenance $= \frac{0,15}{0,87} \times 100\% = 17,24\%$ →

Total ATP produced can also be calculated from:

$$\frac{1}{2} r_4 + 3,2 r_8 = \frac{1}{2}(0,11537) + 3,2(0,2470) = 0,187$$ →

g) What fraction of acetic acid is spent on maintenance in (e)? [46.5% cmol basis]

From e), $r_2 = 0,154 \frac{\text{cmol Ace}}{\text{cmol X.h}}$

To see the rate of acetic acid production only, set $\mu = 0$

$\therefore r_2 = 0,0714 \frac{\text{cmol Ace}}{\text{cmol X.h}}$

$\therefore$ Percentage Acetic acid spent on maintenance only:

$$\frac{0,0714}{0,154} \times 100\% = 46,47\%$$ →

h) What is the maximum mass based yield of acetic acid on ethanol and what will you do to achieve this? [1.304 g/g]

$$Y_{SA} = \frac{r_{xA}}{r_{xs}} = \frac{r_A}{r_s} = 0{,}0714 \frac{cmol\ Ace}{cmol\ X.h} \times \frac{cmol\ X.h}{0{,}0714\ cmol\ EtOH} = 1{,}00 \frac{cmol\ Ace}{cmol\ EtOH}$$

$$1{,}00 \frac{cmol\ Ace}{cmol\ EtOH} \left| \frac{cmol\ EtOH}{23\ g\ EtOH} \right| \frac{30\ g\ Ace}{c_1 mol\ Ace} = 1{,}304\ g\ Ace / g\ EtOH$$