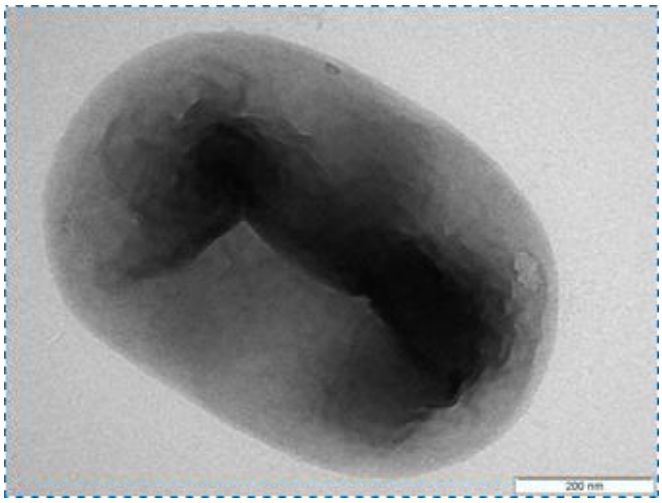
CBI 310 tutorials

Contents

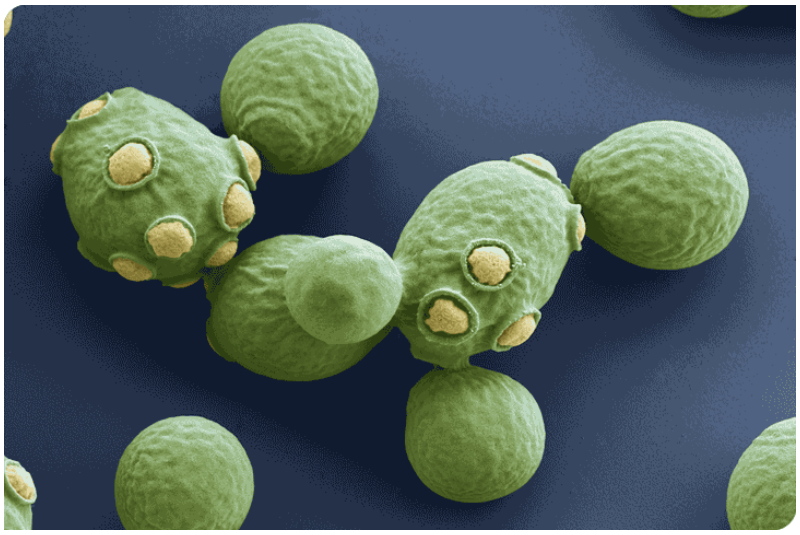
Tutorial 1



The rumen bacterium *Actinobacillus succinogenes* consumes glucose anaerobically to produce succinic, acetic and formic acid. Ignore biomass formation for this question. Carbon dioxide can be a reagent or product in this reaction, while water is also formed as product. It was determined from a fermentation run that the succinic acid yield on glucose is 0.68 g SA/(g Gluc) and the formic acid yield 0.15 g FA/(g Gluc).

a) Determine the mass based yield of acetic acid on glucose. [0.346 g/g]
b) Is CO_2 formed as product or used as reagent? If determined to be a reagent in what enzymatic step was the CO_2 incorporated. Determine the moles of CO2 formed/used per mol of glucose used. [0.81 mol/mol]
c) In an new experiment zero formic acid formed, while the cmol yield of acetic acid was determined to be 0.2 cmol AA/(cmol Gluc). What is the mass based succinic acid yield? [0.9]
d) Will ATP be generated or consumed in the overall reaction?

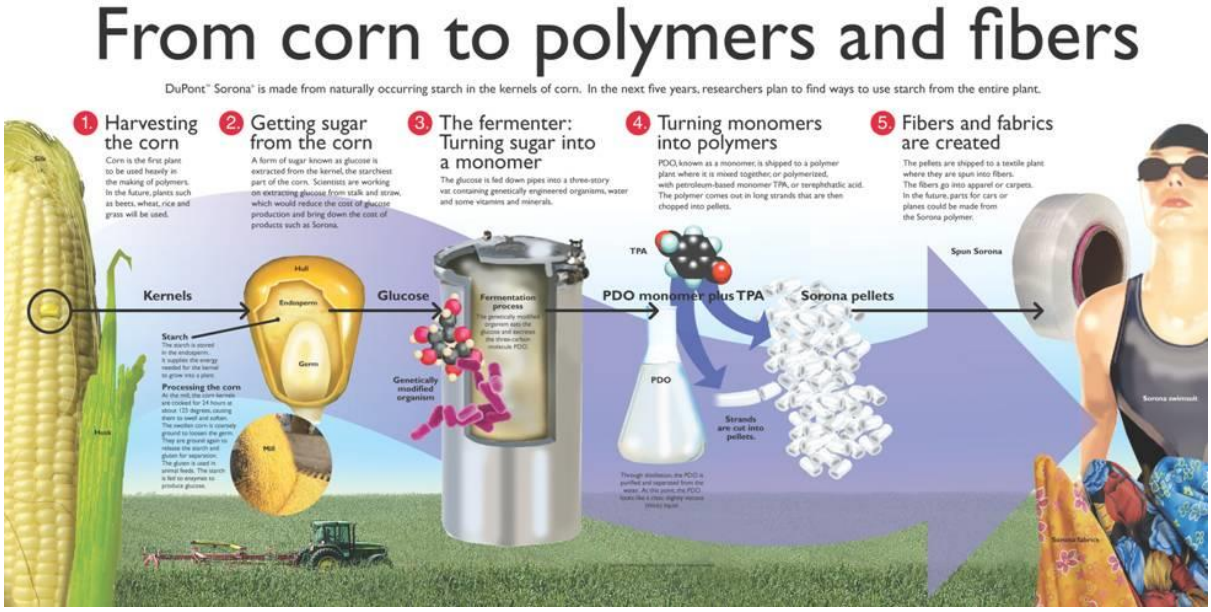
Tutorial 2



The baking yeast *Saccharomyces cerevisiae* is produced commercially on a large scale to provide bakers around the world with the 'magic stuff' that makes dough rise. Using the generic biomass formula for the yeast cells, write down the overall equation for their production using glucose, ammonia and oxygen as reagents and cells, CO_2 and H_2O as products**.**

a) What is the mass based yield of biomass on glucose is zero oxygen is used? [0.78 g/g]
b) Will the reaction in (a) be feasible, give reasons.
c) If oxygen is introduced into the system (@ 0.38 mol O_2/cmol glucose), what will be the biomass yield and why the change from (a)? [0.48 g/g]
d) All the oxygen consumed is via the process of oxidative phosphorylation. Use this to determine the moles of ATP generated per cmol of biomass (X) formed for the scenario in (c). Assume a $(P/O)_{NADH}$ of 1.7 and a $(P/O)_{FADH}$ of 1.2. [2.51 mol ATP/(cmol X)

Tutorial 3



The production of 1,3 propanediol (PDO) by the company DuPont is one of the success stories of bioproduction of polymer intermediates on a bulk scale. Read on the polymer SORONA for some background on the process and final product. PDO is a natural metabolic product when *Klebsiella pneumonica* grows on glycerol as substrate. DuPont opted for glucose as feedstock and engineered *Escherichia coli* to aerobically convert glucose into PDO.

Write out the overall stoichiometry of the reaction using glucose, ammonia and oxygen as feed. Assume that apart from PDO, no by-products are formed except water and CO_2. It is further given that biomass can be described by $CH_{1.91}O_{0.48}N_{0.22}$. The following yields are given:

Y_{SX} = 0.0822 g/g

Y_{SO} = -0.00267 mol O_2/g

a) Determine the mass based yield of PDO on glucose(Y_{SP}). [0.51 g/g]
b) Perform the calculation in (a) by doing a degree of reduction (DOR) balance. Prove to yourself that the balance is a linear algebraic equation with Y_{SP} the only unknown.
c) The DOR balance effectively replaces the hydrogen and oxygen balance (while the water yield Y_{SW} becomes redundant). The carbon balance however remains the same as before. Show from the carbon balance that the CO_2 yield Y_{SC} can solved after Y_{SP} was obtained from the DOR balance. [Y_{SC}=0.29 cmol/cmol]
d) Given the O_2 consumption, what is the estimated energy expenditure for building biomass (use only respiration ATP). Assume a $(P/O)_{NADH}$ and $(P/O)_{FADH}$ of 1.5. [2.9 mol ATP/cmol X].

Have a look at the pathway map from glucose to PDO in the figure below. Only consider the synthetic route (right side) since the production via the natural route (aspartate intermediate) is negligible. Note the NADH and ATP required to get to PDO. You might have noticed from (c) that the

DOR of PDO is 5.33, much higher than glucose (DOR=4). The addition of biological hydrogen (NADH) is the reason for the DOR increase. Also note that only the first section of glycolysis was employed up to dihydroxyacetone phosphate and that ATP was consumed in this section.

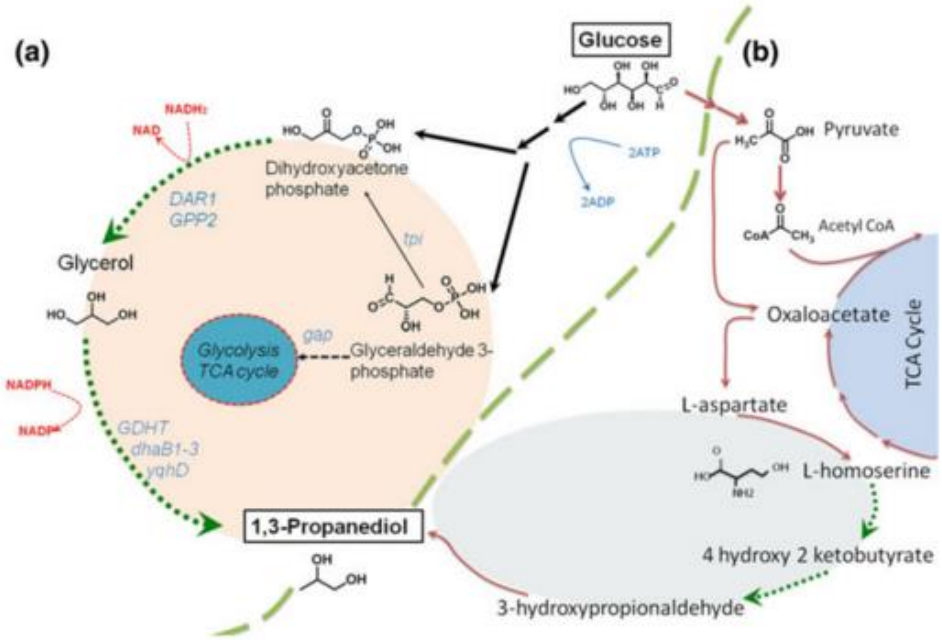


Fig. 7 Engineered *E. coli* strains for the production of 1,3-PDO from glucose. **a** Glycerol-dependent synthetic pathway [95] and **b** non-glycerol-dependent pathway [96]. *Dotted arrows* indicate introduced synthetic pathway steps

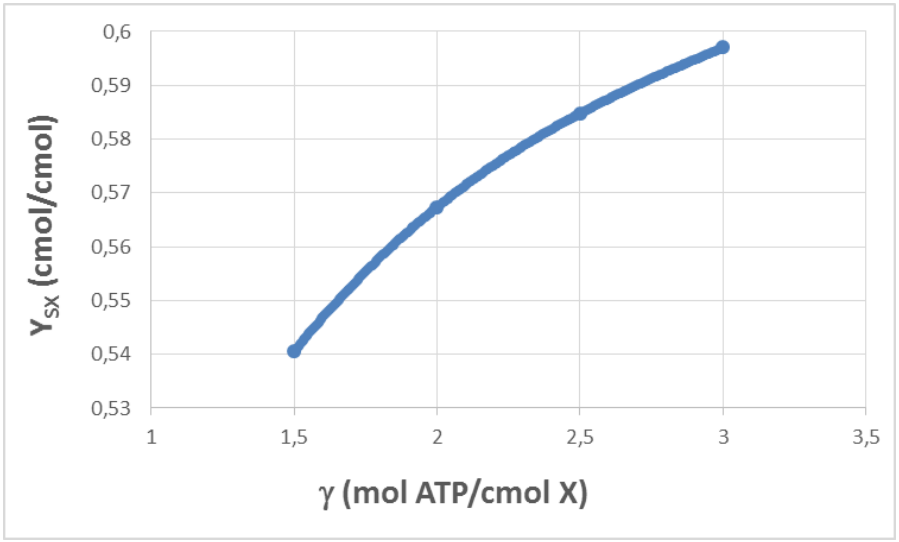
e) Without performing calculations (we'll do this in the next chapter) re-evaluate the answer determined in (d). Will the number be higher or lower given the ATP and NADH requirement in making PDO?

Tutorial 4

Take the overall equation for making biomass (see section 4.6 under part 3). Set up a matrix in sympy and solve (symbolically) for all the yield coefficients as a function of α.

a) Use the generic formula for biomass. [$\beta=2\alpha-0.1$]
b) Use the following biomass composition: $CH_{1.91}O_{0.53}N_{0.18}$. [$\beta=2\alpha-0.155$]
c) Perform a DOR balance on (a) and obtain the answer by hand.

Tutorial 5

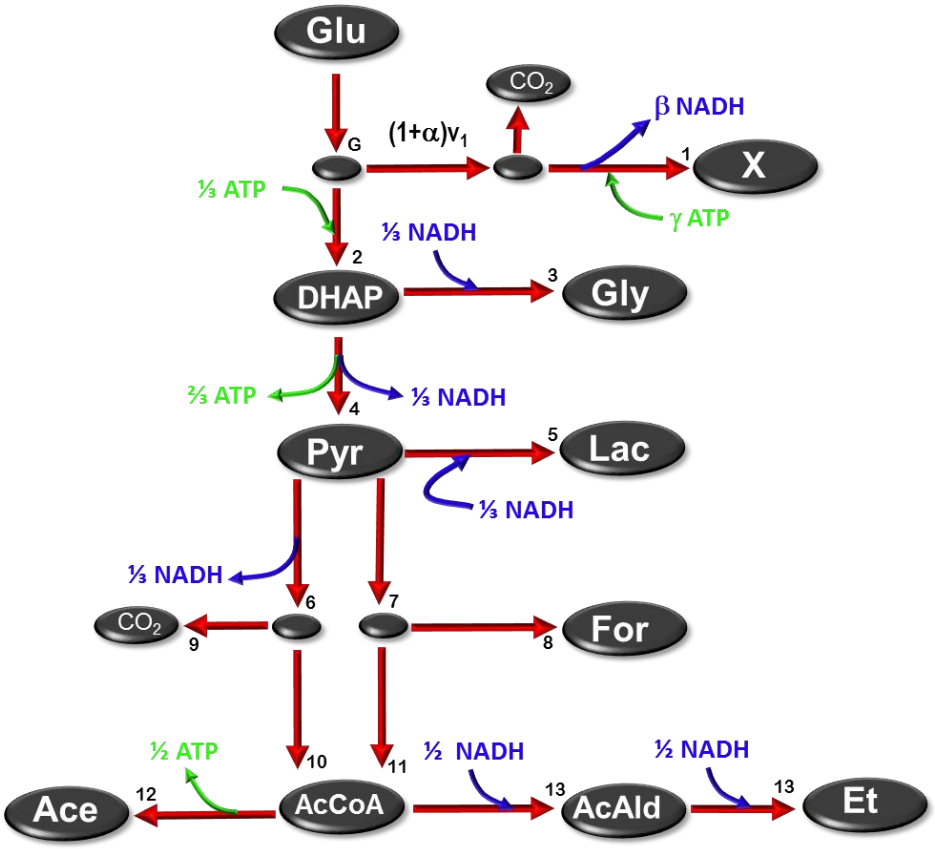


In this tutorial we look at anaerobic ethanol fermentation. We'll evaluate the effect of **γ** (or $\mathbf{Y_{XATP}}$) on the overall yield coefficients by balancing ATP between the anabolic and catabolic half reactions.

a) Assume a redox neutral anabolic reaction (β=O) while using the standard formula for biomass ($CH_{1.8}O_{0.5}N_{0.2}$). What is the value of α?
b) Write out the overall catabolic reaction by including a numerical value of Y_{SATP} (moles ATP per cmol S). Examine the metabolic pathway of forming ethanol in *Saccharomyces cerevisiae* in order to calculate Y_{SATP}.
c) Take a γ value of 2 mol ATP/(cmol X) for the anabolic reaction. Multiply the yield coefficients of the catabolic reaction to balance the ATP. After this normalise the two 'half' reactions so that the sum of the two reactions results in an overall equation where Y_{SS}=1 (glucose yield coefficient). [Y_{SX}=0.142 cmol/cmol in overall equation].
d) Check the coefficients of the overall equation by performing an overall mass balance (you will have to use one of your answers as specification in order to check). The answers should be exactly the same.
e) What will be the mass based ethanol yield on glucose if γ is 1.6 mol ATP/(cmol X)? [0.419g/g]

Tutorial 6

Consider the fermentative pathways discussed in the notes given by the following cmol flux diagram:



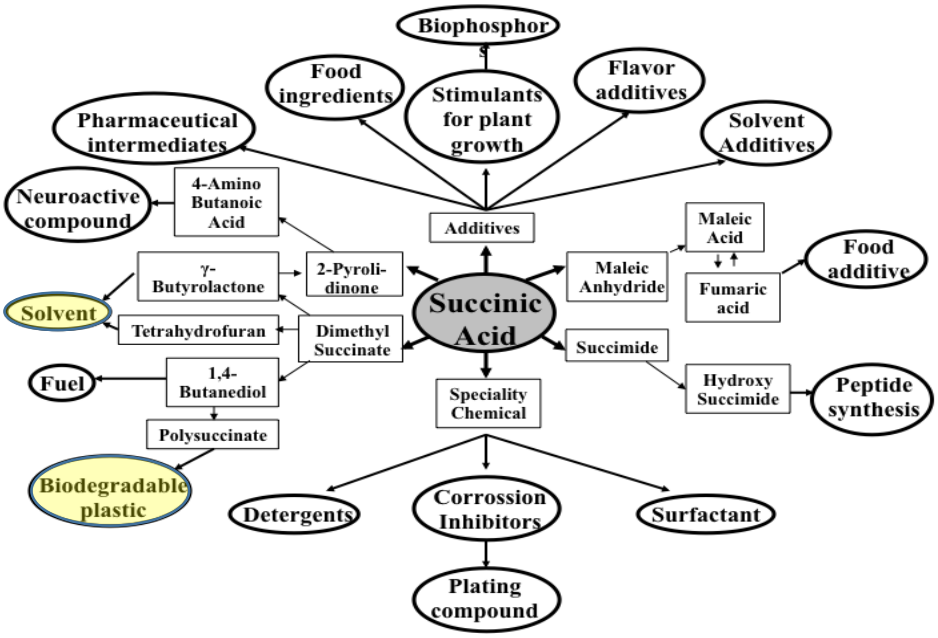
Use the flux numbering given in this diagram and set up the 13x13 stoichiometric matrix and corresponding C vector. Use the standard biomass formula and an α value of 0.1.

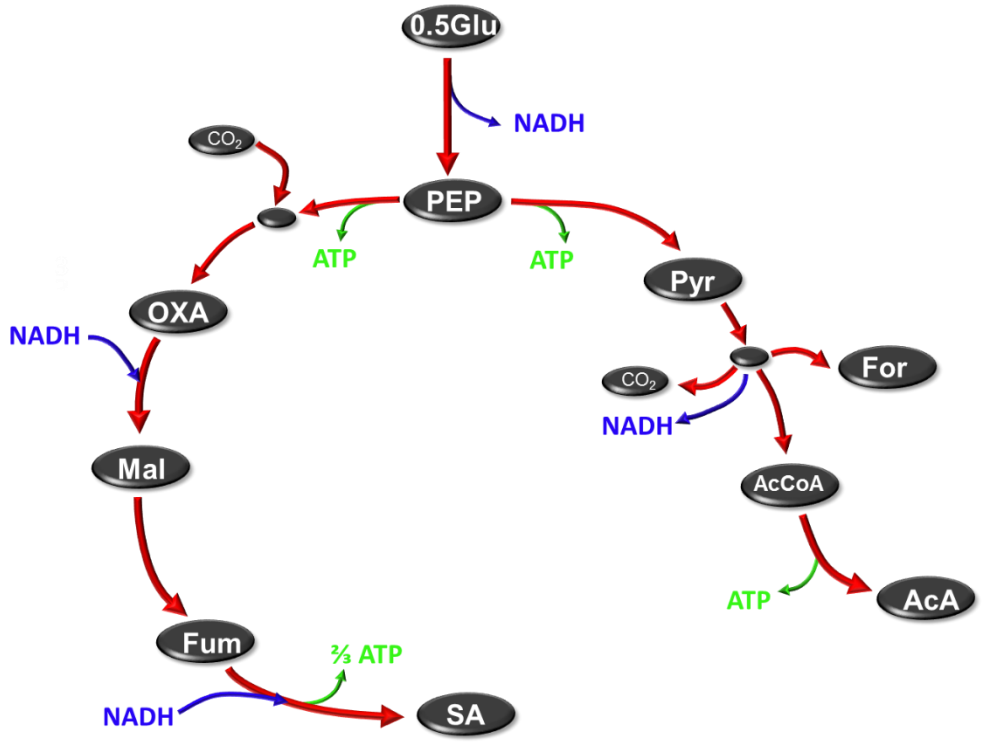
a) Confirm the answers from the example in the notes, by specifying zero ethanol excretion, zero pyruvate dehydrogenase (PDH) flux and a lactic acid yield of 0.28 cmol lactate/(cmol gluc).
b) Solve the flux model by specifying zero acetic, formic and lactic acid excretion. [Y_{SX}=0.141, Y_{SG}=0.042, Y_{SE}=0.535]
c) Solve the flux model by specifying zero glycerol and lactic acid excretion. Allow for equal PDH and pyruvate formate lyase (PFL) flux. [Y_{SX}=0.181, Y_{SE}=0.419, Y_{SF}=0.134, Y_{SA}=0.116]
d) Given yield specifications for glycerol, lactic acid and acetic acid, prove the following linear relationship:

$$\mathbf{Y_{SX} = 0.231Y_{SA} - 0.308Y_{SG} + 0.154}$$

Tutorial 7

For this tutorial we'll consider the bacterium of tut 1, *Actinobacillus succinogenes. A. 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 by the following:

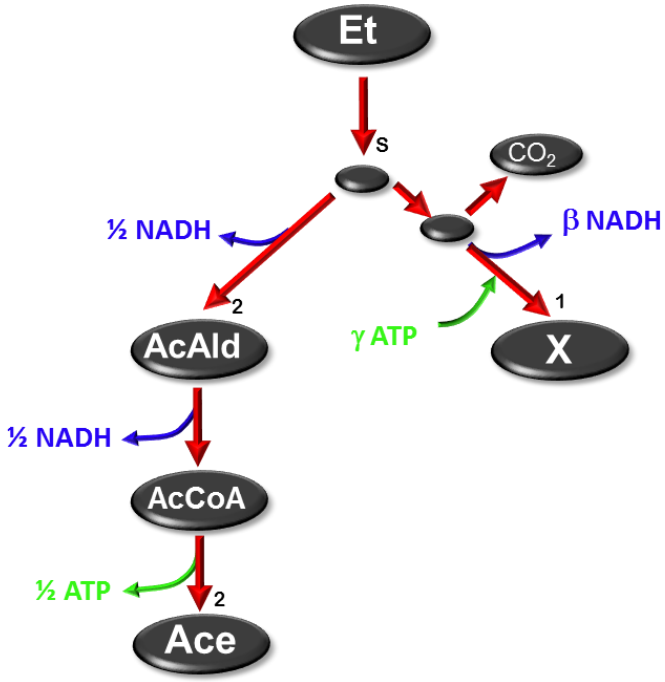
You will note the reverse of the TCA cycle is used up to succinic acid (SA) and that oxaloacetate (OXA) is formed by carboxylation of phosphoenolpyruvate (PEP) and not from pyruvate like in eukaryotes. The PEP carboxylation step is also associated with the formation of ATP via the specialized enzyme 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.

a) Set up the cmol pathway map and include the formation of biomass. You can assume the standard biomass composition, an α value of 0.1 mol CO_2/(cmol X)and a γ value of 1.8 mol ATP/(cmol X).
b) Set up the flux model and show that there is a single degree of freedom.
c) Assume zero pyruvate dehydrogenase action (your single specification) and determine all the yield coefficient on a cmol basis. [Y_{SX}=0.245, Y_{SSA}=0.536, Y_{SAc}=0.219, Y_{SF}=0.109].
d) Determine the CO_2 production/consumption in (c). [Y_{SC}=-0.109]
e) Any idea how to test the answer in (d)?
f) Assume zero formate formation (only pyruvate dehydrogenase) and determine the yield coefficients. [Y_{SX}=0.241, Y_{SSA}=0.686, Y_{SAc}=0.147, Y_{SC}=-0.737]
g) Why is the SA yield in (f) higher than in (c)?
h) The organism does not grow under high acid conditions, and rather use its generated ATP for cell maintenance processes. Under these conditions the SA yield is even higher since carbon is not wasted to make biomass. Determine the maximum possible mass based yield of SA under non-growth conditions. You will have to discard the ATP balance and replace it with a zero biomass specification. You will also have to specify the preferred pyruvate dehydrogenase route. [Y_{SSA}=0.874 g/g]

i) In order to further increase the SA yield one can delete certain genes on the *A.succinogenes* genome. It was decided to block acetate excretion by deleting the gene that codes for acetate kinase (the enzyme that converts acetyl-CoA to acetate). Note that acetyl-CoA cannot be excreted and that the whole C_3 flux from PEP is effectively blocked. Consider the case where zero biomass is formed under high acid conditions in the extra cellular space. Will the genetic modification work? Give clear reasons.

Tutorial 8

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:

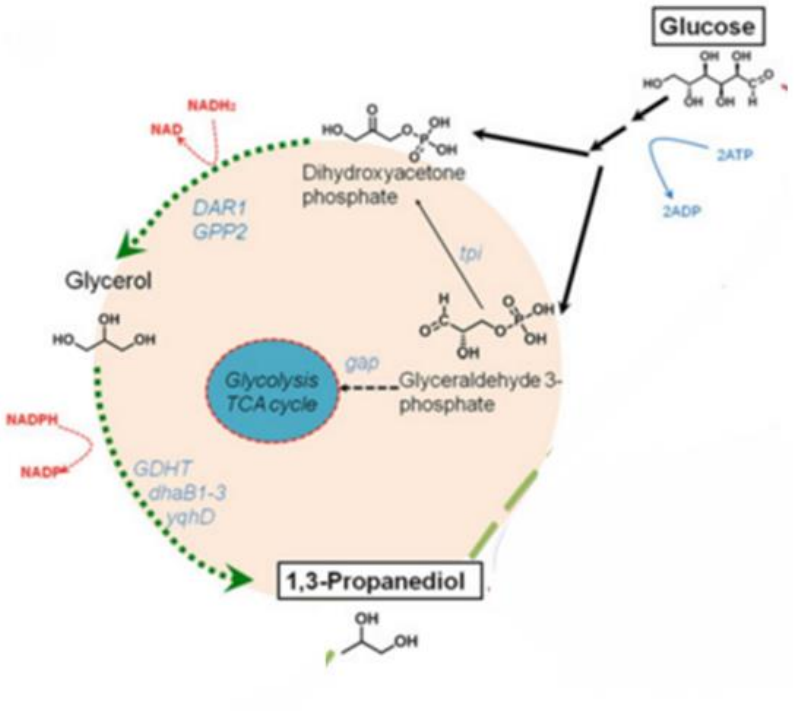


Biomass (X) can be represented by the standard elemental formula ($CH_{1.8}O_{0.5}N_{0.2}$). The value of γ is known to be 2.4 mol ATP/(cmol X). The generation of biomass is associated with the formation of 1.26 mol NADH/(cmol X). Oxygen is used to convert the excess of NADH to ATP with an (P/O) value of 1.6.

a) Determine the mass based yield of biomass on ethanol. [0.821 g/g]
b) What will the mass based Y_{SX} become if γ is 3.5 mol ATP/(cmol X)? Explain your answer. [Y_{SX}=0.586]

Tutorial 9

Determine the true γ value of the PDO calculations performed in tutorial 3 by setting up a proper flux model.



Use a biomass split point from the incoming glucose stream. Model glycolysis up to DHAP where a split point between the PDO branch and the rest of glycolysis occur. Assume NADH and NADPH equivalence. Ignore the L-aspartate route to PDO (given in tut 3). The glycolytic flux below DHAP is solely used for respiration and all carbon will end up as CO_2. You can work with an α value of 0.1 mol CO_2/(cmol X). Use the specified fluxes in your calculation.

a) What is the true value of γ for this example? [tip use sympy and work through the Python tricks in Chapter 5 Part 3]
b) If the oxygen flux is decreased from the specification in tutorial 3, how will Y_{SX} vary and why?
c) The theoretical maximum yield of a targeted product is typically calculated by ignoring the ATP balance and by specifying Y_{SX}=0. Use a zero oxygen flux and determine the maximum PDO yield on glucose on a mass basis. [0.633 g/g]
d) What is the maximum yield of PDO on glucose if the ATP balance is obeyed? For this calculation zero biomass formation is still assumed while the oxygen flux is solved for. Is this yield physically feasible? Why is oxygen required in this calculation? [0.619 g/g].

Info from tut 3

Biomass =$CH_{1.91}O_{0.48}N_{0.22}$

Given yields: Y_{SX} = 0.0822 g/g , Y_{SO} = -0.00267 mol O_2/g

Oxidative phosphorylation: $(P/O)_{NADH}$ and $(P/O)_{FADH}$ of 1.5.

Tutorial 10

We'll continue with the MSG example from chapter 4. You will have to construct your own flux model and cmol map for this question. Note that glutamate is formed from α-ketoglutarate, check the internet to see the redox changes in this enzymatic step. Given the TCA intermediate of α-ketoglutarate you will have to model the complete TCA cycle in this tutorial (see example in chapter 5_2). Given the 'drainage' of C_5 from the TCA cycle, you will have to include a formation step for oxaloacetate (OXA) other than its formation through the TCA cycle itself. Use the conventional carboxylation (CO_2 addition) step from pyruvate to OXA whereby a mole of ATP is required per mole of pyruvate that reacts. You can use an α value of 0.1 mol CO_2/(cmol X)and a γ value of 2.5 mol ATP/(cmol X). Include lactic acid in your matrix construction, but specify a zero flux unless instructed otherwise. You can use a (P/O) value of 1.5 and assume $FADH_2$ and NADH equivalence.

a) Define an oxygen flux of 0.25 mol O_2/(cmol glucose) and determine the biomass and glutamate yield. [Y_{SX}=0.362 cmol/cmol, Y_{SG}=0.411 cmol/cmol].
b) Assume zero biomass formation (ignore the energy balance). Determine the maximum glutamate yield. This will be achieved when the flux from α-ketoglutarate to oxaloacetate is zero. Remember you can use sympy to solve for this condition. [Y_{SG}=0.833 cmol/cmol, Y_{SO}=0.25].
c) Confirm the result with a black box model where you specify Y_{SO}.
d) Include biomass formation and calculate the oxygen yield that will result in the maximum yield of glutamate on glucose. [Y_{SG}=0.583 cmol/cmol].
e) Model the system as an anaerobic system by allowing for a lactic acid flux. Is it possible to produce glutamate? Give reasons.

Tutorial 11

Two micro-organisms (A and B) are inoculated into a 500 liter batch fermenter. Both consume the same substrates (glucose, ammonia, vitamins and minerals). You can assume that no substrate limitation is reached within the considered timespan. The idea is to investigate the concentration of the two types of biomass as a function of time.

a) By using the mole balance definition in chapter 6 (part 2) and the definition of biomass based reaction rates (part1), prove the following to yourself:

$$\frac{dC_{X_1}}{dt} = \mu_1 C_{X_1} \quad and \quad \frac{dC_{X_2}}{dt} = \mu_2 C_{X_2}$$

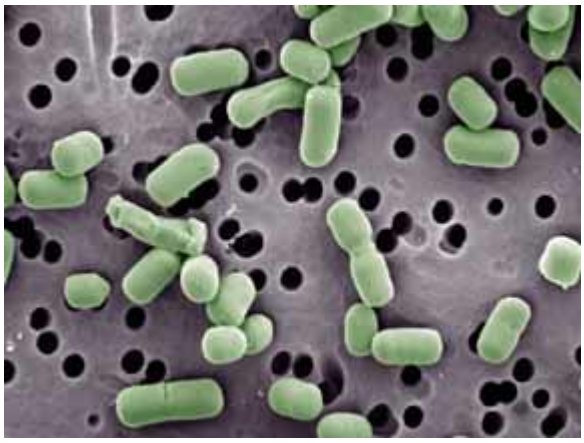
b) After inoculation, the concentration of both A and B is 0.0001 cmol/L. After 24 hours of growth microbe A is at a concentration of 0.01 cmol/L, while the concertation of microbe B is at 1 cmol/L. Determine the specific growth rate for both organisms. [0.19 and 0.38 h^{-1}]

c) How will the θ-values influence the outcome in (b). You can assume that the respective θ-values are different.

d) Assume that the θ-values are negligible for the rest of this question. Organism 1 produces catabolic product A and organism 2 catabolic product B. Given the same growth yield of product per biomass (Y_{XP}^{G}) for both organims what will be the ratio of product B to product A after 24 hours?

e) How will the ratio defined in (d) vary with time?

Tutorial 12



Lactobacillus brevis produces lactic acid, ethanol and formic acid from the anaerobic catabolic breakdown of glucose (no pyruvate dehydrogenase activity). The following physiological properties of the organism are known:

X (elemental)	$CH_{1.85}O_{0.55}N_{0.18}$	
α	0.12	cmol CO_2/(cmol X)
γ	1.85	mol ATP/(cmol X)
μ_{max}	0.55	1/h
θ_{max}	0.2	mol ATP/(cmol X.h)

It is also known that lactic acid in the extra cellular space inhibits the growth rate (μ) of the organism. The mathematical function describing the inhibition is given by:

$$\mu = \mu_{max}\left[1 + \frac{C_{LA}}{K_p}\right]^{-1}$$

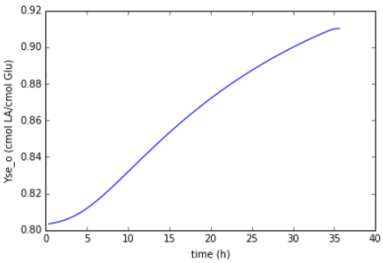
where C_{LA} is the lactic acid concentration in g/L and K_p an inhibition parameter in g/L.

a) Plot the given inhibition function with μ on the y-axis and C_{LA} on the x-axis. Use a K_p value of 4 g/L.
b) Determine the growth stoichiometric equation (i.e. $CH_2O \rightarrow Y_{SX}X$ . . .). You can assume that the complex organic nitrogen source is in excess. [Y_{SATP}=0.277 cmol/cmol]
c) Determine the maintenance stoichiometric equation.
d) Determine the observed lactic and ethanol yield on glucose as a function of the extracellular lactic acid concentration. [@C_{LA}=0 Y_{SLA}=0.803 &Y_{SE}=0.035, @C_{LA}=40 g/L Y_{SLA}=0.918 &Y_{SE}=0.014]
e) Assume that the fermentation is performed in a batch fermenter. Draw a qualitative plot of the lactic acid yield as a function of time.
f) Increase the θ-value to 0.4 mol ATP/(cmol X.h) and plot the lactic acid yield against lactic acid concentration (include the plot of lactic acid in (d)). Explain the observation. [@C_{LA}=0 Y_{SLA}=0.827, @C_{LA}=40 g/L Y_{SLA}=0.95]

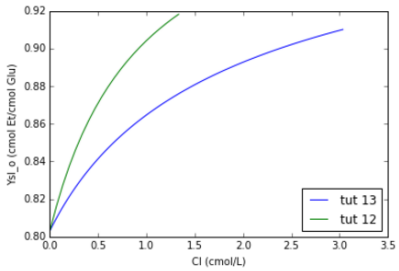
Tutorial 13

The lactic acid production of tutorial 12 will now be performed in a batch fermenter. The fermenter has an initial volume of 500L, while the initial concentration (time zero) of biomass in the fermenter is 0.04g/L. Use Monod kinetics to incorporate for substrate depletion in growth and maintenance. The Monod constant is known to be 0.008 cmol/L. Use a feed glucose concentration of 100 g/L.

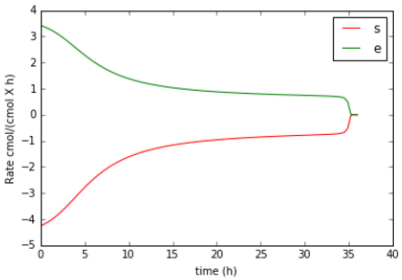
a) What is the lactic acid concentration in the fermenter after 24h of operation? [49.2 g/L]
b) Roughly how long does it take to convert all the glucose? [35h]
c) Plot the accumulated lactic acid yield as a function of time.



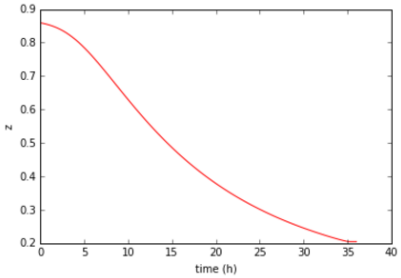
d) Plot the accumulated lactic acid yield against lactic acid concentration (x-axis). Plot the data of tutorial 12(d) on the same plot. Why is there is difference?



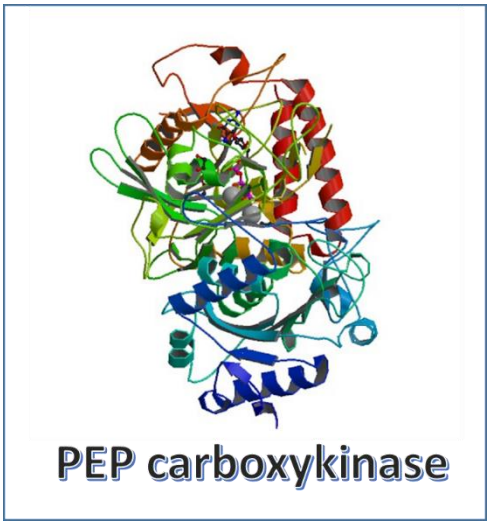
e) Plot the lactic acid production rate and the glucose consumption rate as a function of time.



f) Given that yield is defined as the ratio of rates, plot Y_{SL}^{o} from the data as a function of lactic acid concentration on the plot in (d). Clarify to yourself the meaning of instantaneous and accumulated yield.
g) Plot the z-value as a function of time and explain.



Tutorial 14



This tutorial is very close to the example in 5.7 (part 3). The only difference is that the 'magic' gene from *Actinobacillus succinogenes* (see tut 7) that codes for PEP carboxykinase is now inserted into the already modified *E. Coli*. This implies that the reductive TCA stream can start form PEP and **obtain an ATP in making OXA** unlike the PEP to OXA route in the example that is ATP neutral (the gene coding for this route was knocked out in the organism).

The following physiological parameters are given

X (elemental)	$CH_{1.8}O_{0.5}N_{0.2}$	
α	0.1	cmol CO_2/(cmol X)
γ	1.8	mol ATP/(cmol X)
μ_{max}	0.25	1/h
θ_{max}	0.32	mol ATP/(cmol X.h)

In this example a 2000 L batch fermenter will be used. The initial concentration (time zero) of biomass in the fermenter is 0.02 g/L. You can use Monod kinetics to incorporate for substrate depletion in growth and maintenance. The Monod constant is known to be 0.006 cmol/L. Use a feed glucose concentration of 100 g/L.

The growth inhibition characteristics is given by:

$$\mu = \mu_{max}\left[1 - \frac{C_{SA}}{C_{SA}^*}\right]$$

Where the terminal succinic acid concentration (C_{SA}^*) is 35 g/L. The maintenance production is also inhibited by via a different function given as:

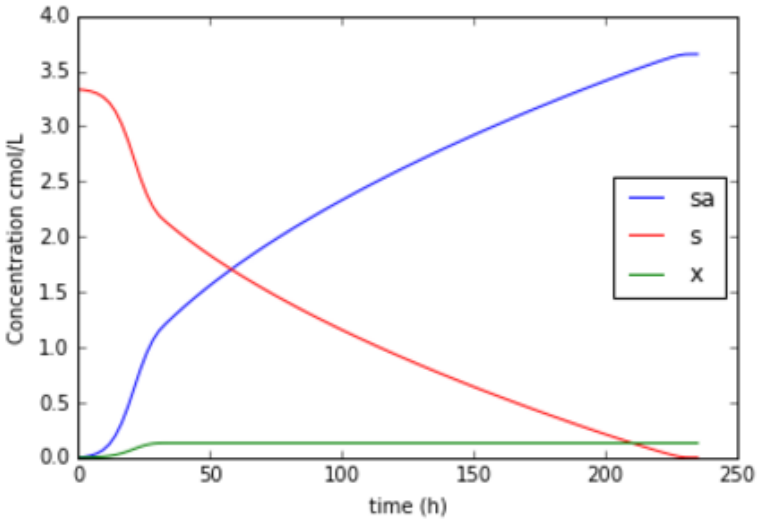
$$\theta = \theta_{max}\left[1 + \frac{C_{SA}}{K_p}\right]^{-1}$$

The K_p value is known to be 8 g/L. The fermentation is performed under anaerobic conditions.

a) Determine the growth and maintenance stoichiometry's:

[Gluc+0.109CO2 -> 0.938SA + 0.1706X + 0.307ATP, Gluc + (1/7)CO_2 → (8/7)SA + 0.381ATP]

b) Obtain the time dependant concentration profiles in the fermenter.



c) Explain the shape of the curves

d) Why does it take so long to convert all the substrate? Any ideas on improving the productivity?

e) What is the average succinic acid productivity of the run in g/(L.h). [0.46]