# Environmental Bioprocess Engineering
# Assignment #2 (A.2.1 & A.2.2)

Marc Deront EPFL-LBE

Don't remain alone and stuck if you don't' understand a step
and if you have difficulties with the problem.

**SHARE TOGETHER and again, use intensively the Moodle forum!**

**This assignment is the study of the biodegradation of organic pollution by heterotrophic biomass under aerobic conditions. The following aspects will be studied**

- **Microbial growth kinetics and yields with kinetics parameters (A.2.1)**
- **Analytical determination of the bioprocess (chemostat) behavior (Steady state study) (A.2.2)**
- **Dynamic simulation of the bioprocess, modeling (Simplified ASM approach) and simulation (Aquasim) (A.2.3)**

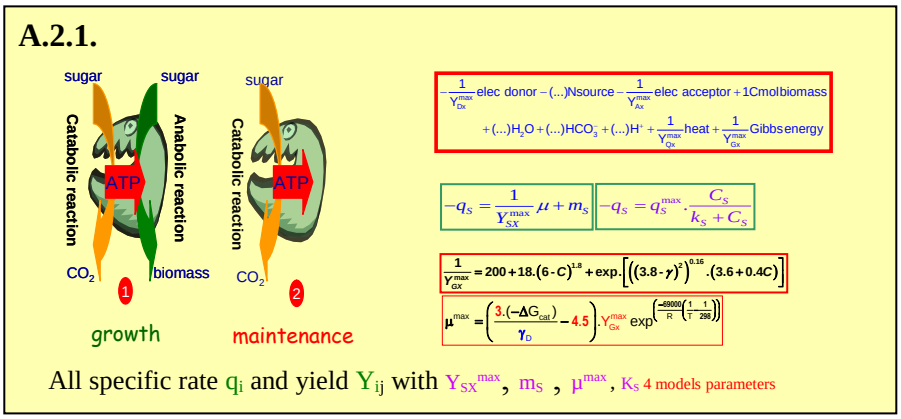
For A.2.1 and A.2.2., you will provide an Excel file and its PDF version.

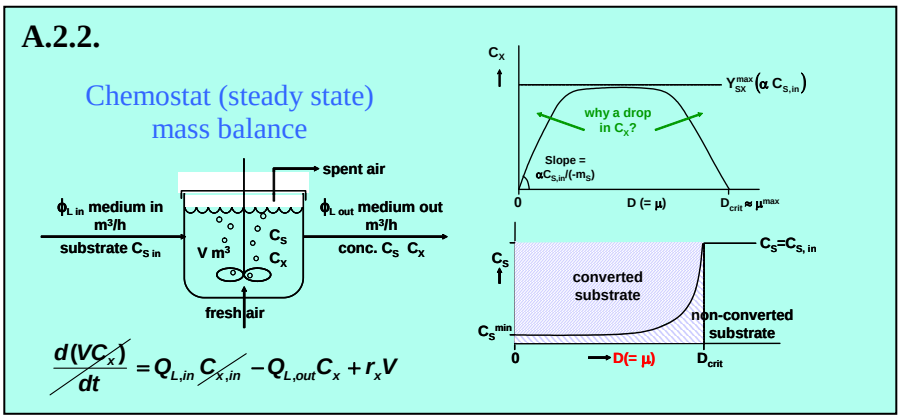
**A.2.1. Stoichiometry and kinetics of microbial growth study (in 1 Excel sheet)**

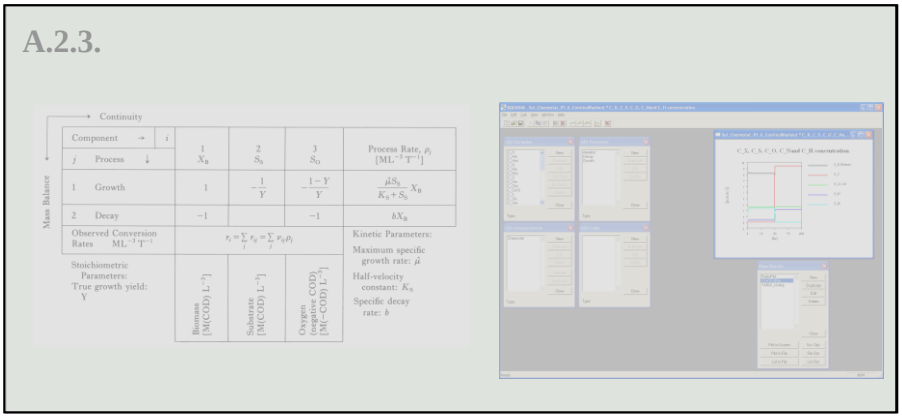
One considers the study of the microbial aerobic heterotrophic growth of biomass on acetate with $NH_4^+$ as N-Source at atmospheric pressure and 25°C.

Available acetate stock is in the form sodium acetate $CH_3COONa$ with an $\Delta^f_G{}^{01}$ of -369.41 $kJ.mol^{-1}$ (≡ Acetate)

a. (4) Using the required Gibbs Energy of heterotrophic growth $Y_{GX}^{max}$ correlation, compute and provide the global growth reaction. Then provide all $Y_{IX}^{max}$ stoichiometric coefficients of global growth reaction…
   You will see that the growth will produce protons and bicarbonate ions ($H^+$ and $HCO_3^-$). According the stoichiometry of the growth reaction, is the bioprocess alkalinizing or acidifying?
b. (2) Establish the catabolic reaction, which gives the $\Delta G_{cat}$ of the catabolic reaction.
c. (2) Establish the anabolic reaction.
d. (3) Check that the global reaction is the sum of the anabolic and the catabolic respiration of the growth (using $1/Y_{OX}$ stoichiometric yield) of global growth reaction)
e. (3) Using the Gibbs Energy correlations:
   - compute the maximal specific growth rate $\mu^{max}$
   - and from Gibbs Energy $m_G$ correlation, provide the rates $m_i$ required for the maintenance
f. (4) Express all specific rates $q_I$ (biomass, Acetate, $O_2$, $NH4^+$, $H^+$…) and yields $Y_{IX}$ as function of growth rate $\mu$
   Provide a double Y scatter plot of the $q_I$ rates and the yields $Y_{IX}$ vs $\mu$ [0 – 1.2 $\mu^{max}$]




**A.2.2. Reactor study (Steady state) (in 1 Excel Sheet)**

According this previous A.2.1, one wants to do an experiment to study this growth biosystem to determine its biological kinetic parameters. For this purpose, a 1.5 L of CSTR chemostat bioreactor will be used. Assume an inlet organic loading concentration of $C_{Sin}$ 1500 mg COD/L. Suppose $K_S$ the affinity constant of the biomass for this substrate is 20 mg COD/L

a. (1) As A.2.1. Stoichiometry and kinetics of microbial growth study is developed and expressed in C-mole of biomass and mole of sodium acetate, convert units of $C_{Sin}$ and $k_S$.

b. (5) Provide the expression of putative concentrations of Biomass $C_X$, Substrate $C_S$ as function of dilution rate D (use Chemostat lecture notes)

- Build mass balance for Biomass $C_X$, Substrate $C_S$ state variables, provide volumetric rates

- From two known expressions of $q_S$ (Herbert-Pirt and hyperbolic link $q_S$ to $\mu$ and $C_S$ provide.

  - expression for $C_S = f(D)$ which doesn't depend on $C_{Sin}$ inlet concentration
  - expression for $C_X = f(C_S)$
  - expression $\mu = f(C_S)$ using minimal substrate concentration (for maintenance) $C_{Smin}$ which occurs when $\mu=0$, but when residual $C_S$ allows biomass maintenance (see lecture on Chemostat).

c. (2) From $\mu = f(C_S)$ expression, give and compute the maximum critical dilution rate and determine DCrit, which occurs when $C_S=C_{Sin}$...

   Compute $C_{Smin}$ of minimal residual substrate concentration for maintenance

d. (4) Then, from Cs mass balance $r_S=q_S.C_X$, using observed Ysx yield ($q_X/q_S$ rate ratio ) or using one of the $q_S$ expression, give 3 expression of
   - $C_X = f(C_S, Y_{SX}, D)$
   - $C_X = f(D, C_S)$ Herbert Pirt $q_S$
   - $C_X = f(D, C_S)$ Hyperbolic $q_S$

   Then with expressions of $C_X = f(\mu) = f(D)$ and $C_S = f(\mu) = f(D)$ from [0 to DCrit] in 30 steps compute $C_S$ and $C_X$ (also all qi for all global growth components)
   Check same calculated for $C_S$ by the 3 expressions

   Plot them on one graph, the two 2 state variables $C_X$ & $C_S$ as function of D (DCrit)

e. (6) From mass balances, express all other state variables as function of D ($NH_4^+$, dissolved $O_2$, dissolved $CO_2$ and $H^+$) using:
   - C_Nin = YSN * C_Sin * 120%, YSN obtained from $Y_{SX}$ and $Y_{NX}$ of global growth equation
   - C_Oin=0 mole.$m^3$, Kla = 90 $hr^{-1}$, C_02sat 25°C =0.258 mole.$m^{-3}$. We consider that there is no limitation, and there no $O_2$ switch function. [same recommendations that will be used in **A.2.3** (below)]

   Once all $C_i$ states variables can be expressed from their respective mass balance, compute and plot on one graph, all these $C_i$ component concentrations of global growth equation using same dilution rates of d.)

   Do the same for all $r_i$ volumetric rates.

f. (2) Check $K_S$ affinity constant effect on chemostat behavior, by modifying ks.
   Note: $C_X$, $C_S$ and DCritical depend on to $\mu^{max}$ or $q_S^{max}$ and $K_S$. Compute $C_X$ and $C_S$ as function $f(K_S,\mu) = f(K_S,D)$, for Half Ks, Ks and 2 times Ks. Then plot Cs and Cs vs dilution rate D. Comment!

g. (4) The aim of this chemostat study will be to use an experimental chemostat for the estimation of the 4 biological kinetic parameters ($\mu^{max}$, $Y_{SX}^{max}$, $k_S$, and $m_S$) of heterotrophic biomass growing aerobically on sodium acetate.
   According estimation tools (See Chemostat lecture: Lineweaver-Burk and Hanes-Woolf linearization), the quality of the estimation depends on the covered range of Cs substrate residual concentration, which is fixed by the dilution rate D chosen, for each chemostat Steady State.

Using the $C_S$=f(D) expression (provide 10 dilution rate values, providing 10 $C_S$ values which cover the range from 0.1x$C_{Sin}$ to $C_{Sin}$ , in an equal distributed manner…

h. (3) During Practical Labs Chemostat experiment, even if $C_{sin}$ and D are supposed to be chosen and fixed, pumping reality of inlet medium, real outlet flow, bioreactor volume as well as inlet substrate concentration make observed/measured $C_{Sin}$ and D quite different from what has been supposed to be chosen. As residual substrate and biomass concentration depends mainly on these 2 applied operating parameters $C_{Sin}$ and D, provide an easy calculation of $C_S$ and $C_X$ using applied observed $C_{Sin}$ and D dilution rate. Use $C_S$ and $C_X$ expressions above.