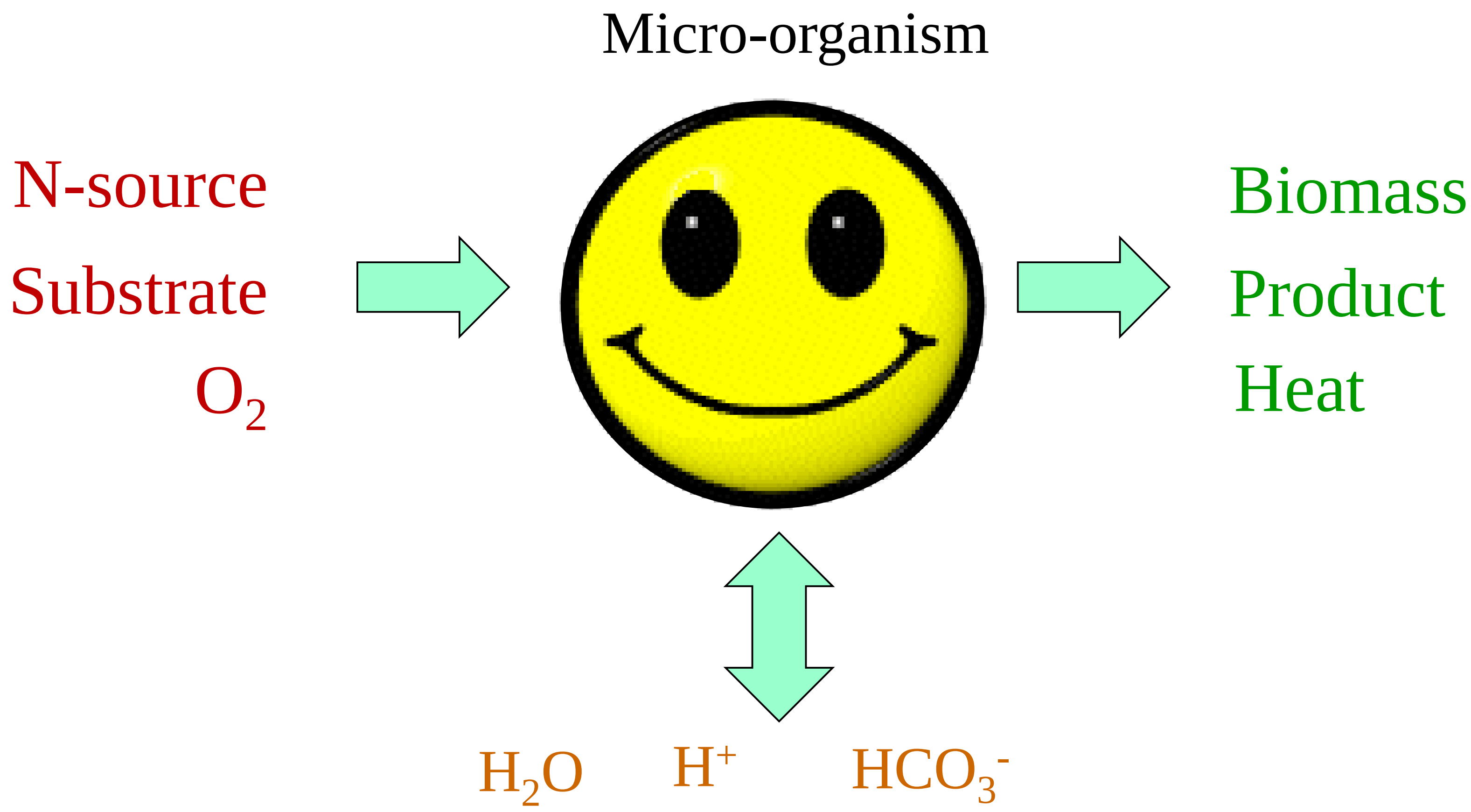
Microbial Growth Stoichiometry



Stoichiometry calculations for undefined biochemical systems

Previous methods apply to biosystems where organic compounds are known (C, H, O, N, P…).

However often, for undefined chemical systems this information is not available:

Typically the case of:

- Biological wastewater treatment
- Soluble and non-soluble compounds treatment
- Bio-fermentation of complex media

Stoichiometry calculations in undefined biochemical systems

Where undefined chemical systems are concerned, lumped measurements give global estimations about key parameters such as C and N content or degree of reduction of organic matter or effluent wastewater …

TOC	Total Organic Carbon can be used in Carbon-balance
COD	Chemical Oxygen Demand, to be used in COD-balance (γ balance)
Kj-N	Kjeldahl-nitrogen for all reduced nitrogen (organically bounded and NH_4^+), can be used in N-balance
Non-suspended, colloidal organic fractions	Total Solids can be used in TS-balance

COD-measurement

In Chemical Oxygen Demand COD analysis, all organic compounds are chemically oxidized. (e.g. using chromic acid or other oxidizing agent)

ThOD: Theoretical Oxygen Demand ?
If the oxidation is complete: COD = ThOD , not complete COD < ThOD

Can be estimated using full oxidation reaction (with Ox/Red couples)
→ Products are : $\mathbf{HCO_3^-}$, $\mathbf{H_2O}$, $\mathbf{H^+}$, NH_3, SO_4^{2-}, Fe^{3+}
for which COD = 0
and for which **γ = 0 (reference chemical compounds)**

→ **There is a direct link between γ and COD**

Note: Chemical Oxygen Demand : **COD** (US) DCO (FR)
Dissolved Organic Carbon : DOC (US) **COD** (FR)
Unit [$gO_2.m^{-3}$] mass concentration, [$gO_2.kg^{-1}$] mass fraction

COD or ThOD-calculation and γ

Complete RedOx chemical reaction of glucose:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6HCO_3^- + 6H^+$ (or 6 CO_2+6 H_2O)

→ $COD_{glucose}$ = 6∗32 gO_2/$mole_{glucose}$ = 192 g COD/mole
= 192 / 180 = 1.0667 g COD/g glucose

Each compound, each atom has : - a defined degree of reduction γ
- and thus a defined amount of COD

Indeed, the consumption of 1 mole O_2 allows the acceptance of 4 moles of electron (γO_2 = - 4) and 1 mole O_2 = 32g O_2 = – 32 g COD

γ Degree of reduction and COD content are linked :

1 γ unit ≡ e-mole ≡ 8 gCOD = ¼ mole O_2

Therefore, this allows **Calculation of COD values using γ values** (and reversely).

COD calculation - Biomass

$1\ \gamma$ unit $\equiv$ e-mole $\equiv$ 8 g COD = ¼ mole O_2

→ Calculation of COD values using γ values

Biomass $C_1H_{1.8}O_{0.5}N_{0.2}$

$Mw_{biomass}$ = 24.6 g /C_mole

$\gamma_{biomass}$ = 4.2 e-mole /C_mole

$$\text{COD Biomass} = \frac{4.2\ \text{e-mole}}{\text{C\_mole X}} \times \frac{8\ \text{g COD}}{\text{e-mole}} = \mathbf{33.6\ g\ COD/C\_mole\ X}$$

COD Biomass = 33.6 / 24.6 = 1.366 g COD/ g X = **1.366 g COD/g VSS**

Comments:

1. **Biomass can be expressed in g COD**…! As used in ASM Matrix formulation
2. In practice values of 1.3-1.5 gCOD/g organic matter are found (wastes vs biomass).
3. Reversely, this agreement shows the validity of assumed biomass composition

Relation between γ and COD/TOC ratio for organic compounds values

The wastewater composition or organic compounds (waste or wastewater) can easily be characterized by measured COD and TOC values. These values are directly related to the degree of reduction. It determines de degree of reduction per C-mole:

$\gamma \equiv$ Electron content of 1 C-mole of <u>organic compound</u> in [e-mole / C-mole]

1 γ unit ≡ e-mol ≡ 8 gCOD = ¼ mol O_2

As there is 8 g COD per e-mole → 1 g COD is equivalent 1/8 e-mole
As there is 12 g C per C-mole → 1 g TOC is equivalent 1/12 C-mole

Degree of reduction γ of organic matter is linked to COD/TOC ratio…

Thus :

COD_{unit} [g COD / L] = 1 / 8 e-mole / L
TOC_{unit} [g C / L] = 1 / 12 C-mole / L

$$\gamma = 1.5 \cdot \frac{COD_{g\,COD}}{TOC_{g\,C}} \left[\frac{e\_mole}{C\_mole}\right]$$

Thus, for undefined organic waste or wastewater, from COD and TOC, one can build a γ Balance… ;-)

$$\left(\frac{COD}{TOC}\right); \quad \frac{COD_{unit}}{TOC_{unit}} = \frac{\left[{}^{g\,COD}/_{L}\right]}{\left[{}^{g\,C}/_{L}\right]} = \frac{g\,COD}{g\,C} = \frac{{}^{1}/_{8}\,e\_mol}{{}^{1}/_{12}\,C\_mol} = 1.5\frac{e\_mol}{C\_mol} = 1.5 \cdot \gamma_{unit}$$

$$\gamma Biomass = 1.5 \cdot \frac{33.6}{12} = 4.2$$

COD balance / γ balance

As γ related to COD
COD balance ≡ γ balance

1 γ unit ≡ e-mole ≡ 8 g COD = ¼ mole O_2

Thus assuming that COD = ThOD, from global growth reaction…

– (...) substrate – (...) acceptor + (...) NH_4^+ + (...) biomass + (...) H_2O + (...) H^+ + (...) CO_2

$-(\ldots)\,\gamma_{substrate} - (\ldots)\,\gamma_{acceptor} + (\ldots)\,\gamma_{biomass}$

$-(\ldots)\,\gamma_{substrate}.8g[COD] - (\ldots)\,\gamma_{acceptor.}(-1).8g[COD] + (\ldots)\,\gamma_{biomass}.8g[COD]$

Consumed COD in substrate + COD accepted by acceptor + COD fixed in biomass

COD-balance applications (i.e. γ Balance) allows to

1. Calculate **Biomass production yield $Y_{COD} \equiv Y_{SX}$**
2. Calculate **O_2-consumption = BOD** (Biological Oxygen Demand)

→ Assignment #1 BOD_5

COD-based stoichiometry → $Y_{COD} \equiv Y_{SX}$

From $-(\ldots)\,\gamma_{substrate}.8g[COD] - (\ldots)\,\gamma_{acceptor}.(-1).8g[COD] + (\ldots)\,\gamma_{biomass}.8g[COD]$

→ $-\mathbf{1}\ g_{COD}.\text{Substrate} - \mathbf{Y_O}.g_{COD}O_2 + \mathbf{Y_{COD}}.g_{COD}\text{Biomass}$

↖ $\text{sign}(\ g_{COD}.O_2) = (-)$

COD Balance: $-\mathbf{1}.S_{[gCOD]} - \mathbf{Y_O}.O_{2\ [g=(-)gCOD]} + \mathbf{Y_{COD}}.X_{[gCOD]}$

$$Y_{COD} = \frac{g_{COD}\ \text{biomass produced}}{g_{COD}\ \text{substrate consumed}} < 1$$

2nd law thermodynamics Conservative Principle & Energy requirement of growth !

COD based yield :

→ Mole based yield Y_{SX}

→ Mass based yield Y_{SX}

As for a compound C_i : $x[\text{mole}C_i] = x.\gamma i.8\,[g_{COD}C_i]$

$$1[g_{COD}C_i] = \frac{1}{\gamma i.8}[\text{mole}C_i] = \frac{MW_i}{\gamma i.8}[gC_i]$$

$$Y_{COD}\left[\frac{g_{COD}X}{g_{COD}S}\right] = Y_{COD}\left(\frac{1/\gamma_X.8\ [\text{Cmole\_X}]}{1/\gamma_S.8\ [\text{mole\_S}]}\right)$$

$$= Y_{COD}.\frac{\gamma_S}{\gamma_X}\left[\frac{\text{C-mole\_X}}{\text{mole\_S}}\right] = Y_{SX}\left[\frac{\text{C-mole\_X}}{\text{mole\_S}}\right]$$

$$Y_{COD}\left[\frac{g_{COD}X}{g_{COD}S}\right].\frac{\gamma_S}{\gamma_X}.\frac{24.6}{\text{MW\_S}} \equiv Y_{SX}\left[\frac{\text{gVSS\_X}}{\text{g\_S}}\right]$$

COD-based stoichiometry → BOD

COD Balance: $-\mathbf{1}.S_{[gCOD]} - \mathbf{Y_O}.O_{2\,[g=(-)gCOD]} + \mathbf{Y_{COD}}.X_{[gCOD]}$

$$-1 + Y_O + Y_{COD} = 0$$

$$Y_O = \frac{g_{COD}\,O_2 \text{ consumed}}{g_{COD} \text{ substrate consumed}} < 1$$

1. If S = organic pollutant content of a wastewater sample?
   → Y_O is the **BOD content** of
   1 g_{COD} of the sample
2. **Y_O** can be seen as the **Aeration requirement** of the bioprocess !

Definition: Biochemical Oxygen Demand or BOD_5, is the amount of dissolved oxygen needed for aerobic biological degradation of an organic pollutant present in a given water sample, given 25^oC during 5 days.

1^{st} Example COD-balance (1/2)

The measured aerobic growth yield on oxalic acid, is 0.052 g biomass per gram oxalic acid → $\mathbf{Y_{SX[g/g]}}$= 0.052 [gX/gOxal.ac.]

→ for COD balance Y_{COD} : [g_{COD}X/g_{COD}Oxal.ac.] is required.

COD Equivalence for each compound :

1 g oxalic acid	= (1 /90) * 2 * 8 = 0.178 g COD	MW_{C2O4H2} = 90 g/mole; γ_{C2O4H2} = 2
1 g O_2	= (1 / 32) * (-4) * 8 = – 1 g COD	MW_{O2} = 32 g/mole; γ_{O2} = -4
1 g biomass	= (1 / 24.6) * 4.2 * 8 = 1.36 g COD	$MW_{biomass}$ = 24.6 g/mole; γ_{C2O4H2} = 4.2

→ $\mathbf{Y_{COD}}$ = 0.052*(1.36 / 0.178) = **0.4** [g_{COD}X /g_{COD}Oxal.ac]

$$Y_{COD}\left[\frac{g_{COD}X}{g_{COD}S}\right]\cdot\frac{\gamma_S}{\gamma_X}\cdot\frac{24.6}{MW\_S} = Y_{SX}\left[\frac{gVSS\_X}{g\_S}\right] \quad\Rightarrow\quad Y_{COD}\left[\frac{g_{COD}X}{g_{COD}S}\right] = \frac{\gamma_X}{\gamma_S}\cdot\frac{MW\_S}{24.6}\cdot Y_{SX}\left[\frac{gVSS\_X}{g\_S}\right]$$

→ $\mathbf{Y_{COD}}$ = 0.052 *(4.2/2)*(90/24.6) = **0.4** [g_{COD}X /g_{COD}Oxal.ac]

1^{st} Example COD-balance (2/2)

The measured aerobic growth yield on oxalic acid, is 0.052 g biomass per gram oxalic acid → $\mathbf{Y_{SX[g/g]}}$= 0.052 [gX/gOxal.ac.]

MW_{C2O4H2}	= 90 g/mole;	γ_{C2O4H2}	= 2
MW_{O2}	= 32 g/mole;	γ_{O2}	= -4
$MW_{biomass}$	= 24.6 g/mole;	γ_{C2O4H2}	= 4.2

$$Y_{COD}\left[\frac{g_{COD}X}{g_{COD}S}\right] = \frac{\gamma_X}{\gamma_S} \cdot \frac{MW\_S}{24.6} \cdot Y_{SX}\left[\frac{gVSS\_X}{g\_S}\right]$$

$$\mathbf{Y_{COD}} = 0.052 *(4.2/2)*(90/24.6) = \mathbf{0.4} \quad [g_{COD}X / g_{COD}Oxal.ac]$$

COD Balance : – 1 g_{COD} Oxalic acid **+ $\mathbf{Y_O}$** g_{COD} O_2 **+ 0.4** g_{COD} Biomass

→ $\mathbf{Y_O}$ = 1 – 0.4 = 0.6 =

O_2 Cons. = 0.6 [$g_{COD}O_2$ / g_{COD}Oxal. ac.

= 0.6 * (1/(-1))/(1/0.178)

= - 0.106 [gO_2/gOxal.ac.]

1 g oxalic acid = (1 /90) * 2 * 8 = 0.178 g COD

1 g O_2 = (1 / 32) * (-4) * 8 = – 1 g COD

→ Aeration requirement!

2cd Example COD-balance

In the anaerobic growth on glucose with ethanol production, where measured biomass yield on glucose is 0.2 g dry biomass (15% ash) per gram glucose. $\mathbf{Y_{SX[g/g]}}$=0.2 * 0.85 [g X /g Glucose]

→ for COD balance Y_{COD} [g_{COD}X/g_{COD}Glc] is required

COD equivalence for each compound :

1 g glucose = (1 / 180) * 24 * 8 = 1.067 g COD	MW:180 g/mole;	γ:24
1 g ethanol = (1 / 46) * 12 * 8 = 2.087 g COD	MW:46 g/mole;	γ:12
1 g biomass = (1 / 24.6) * 4.2 * 8 = 1.366 g COD	MW:24.6 g/mole;	γ:4.2

→ $\mathbf{Y_{COD}}$ = Y_{SX} *(1.366 / 1.067) = **0.22** [g_{COD}X/g_{COD}glucose]

COD Balance : – 1 g_{COD} glucose **+** $\mathbf{Y_O}$ g_{COD} ethanol **+ 0.22** g_{COD} biomass

→ $\mathbf{Y_O}$ =1 – 0.22 = 0.78

Ethanol Production = 0.78 [g_{COD}Eth/g_{COD}Glc]

Estimation = 0.78 * (1/2.087)/(1/1.067)

= 0.39 [gEth/gGlc]

COD Balance / BOD-measurement

A volume of wastewater sample containing a given organic pollution COD = [g O_2.m^{-3}] = [g COD/m^{-3}]) is inoculated with aerobic heterotrophic micro-organisms from activated sludge, for a batch growth at 25 °C during 5 days where O_2-consumption is measured

$$BOD = \frac{\text{amount } O_2 \text{ consumed}}{\text{volume of sample}} \left[\frac{gO_2}{m^3}\right]$$

BOD [g O_2.m^{-3}] and COD **[g O_2.m^{-3}]** … Correlation?

Common opinion :

If organic matter is fully biodegradable then BOD $\overset{=}{\ldots}$ COD

If organic matter is partially degradable then BOD $\overset{<}{\ldots}$ COD

COD and BOD correlation

Assumption: organic matter is perfectly/fully biodegradable

From: COD Balance: $\mathbf{-1}.S_{[gCOD]} - \mathbf{Y_O}.O_{2\,[g=(-)gCOD]} + \mathbf{Y_{COD}}.X_{[gCOD]}$

→ $-\ m._{S[gCOD]} - Y_O.\ m._{S[gCOD]}\ O_2 + Y_{COD.}\ m._{S[gCOD]}$

Using the BOD of the sample: The O_2 consumption respiration [gCOD] ≡ electron acceptor consumption [(-)gCOD]

→ $-\ m._{S[gCOD]} + BOD_{[gO2]} + Y_{COD.}\ m._{S[gCOD]}$

Thus: $\mathbf{-\ COD_S + BOD + Y_{COD}*COD_S = 0}$

Consumed substrate in sample	BOD content in sample	COD fixed in produced biomass in BOD measurement

$$- COD_S + BOD + Y_{COD}*COD_S = 0$$

$$\frac{BOD}{COD} = 1 - Y_{COD}$$

Note: BOD < COD Even for fully biodegradable organic matter !!!

COD and BOD correlation

From: COD Balance : $- 1.S_{[gCOD]} - Y_O.O_{2\,[g=(-)gCOD]} + Y_{COD}.X_{[gCOD]}$

→ $- COD_S + BOD + Y_{COD} * COD_S = 0$

$$\frac{BOD}{COD} = 1 - Y_{COD} < 1$$

$$\text{for} \quad Y_{COD} = \begin{matrix} 0.40 \\ 0.60 \\ 0.70 \end{matrix} \left[\frac{g\,COD\,X}{g\,COD\,S}\right]; \quad \frac{BOD}{COD} = \begin{matrix} 0.6 \\ 0.4 \\ 0.3 \end{matrix}$$

1) For <u>fully</u> biodegradable organic matter a high BOD/COD ratio only indicates a poor quality substrate with a poor Y_{COD} biomass yield

2) For <u>partially</u> biodegradable organic matter, BOD content of the biodegradability fraction of the total COD could be accessed with

$$\frac{BOD}{COD_{Total}} = \frac{(1 - Y_{COD}).COD_S}{COD_{Total}} = \frac{(1 - Y_{COD}).f_{biodegradable}.COD_{Total}}{COD_{Total}}$$

$$\text{for} \quad \frac{COD_{Biodegradable}}{COD_{Total}} = 60\% = \frac{COD_S}{COD_{Total}}$$

$$Y_{COD} = \begin{matrix} 0.40 \\ 0.60 \\ 0.70 \end{matrix} \left[\frac{g\,COD\,X}{g\,COD\,S}\right]; \quad \frac{BOD}{COD_{Total}} = \begin{matrix} 0.36 \\ 0.24 \\ 0.18 \end{matrix}$$

Population Equivalent (PE)

PE = amount of biodegradable organic matter produced by one person per day including (feces, urine, washing, etc…) [g O_{2}.d^{-1}].

Biodegradable organic matter consists for 1 PE:

- Organics → COD value = 90 $g.d^{-1}$
- Reduced nitrogen → Kj-N = 10 g $N.d^{-1}$

→ 1 PE = 90 + 4.57 ∗ 10 = 136 g $O_2.d^{-1}$

$$1PE\ g_{O2}.d^{-1} = g_{COD}.d^{-1} + 4.57 * g_{Kj\text{-}N}.d^{-1}$$

BOD content of NH_4^+ oxidized to NO_3^-

$\gamma NH_4^+ = 0;\ \gamma NO_3^- = -8;\ \gamma O_2 = -4;$

$1\,gN_{Kj} = \frac{1}{14}$ mole N_{Kj} which will requires transfer of 8 emole to NO_3^-

→ there will be accepted by 2 mole $O_2 = 32\,g\,O_2$ (2 x 4 emole)

$1\,gN_{Kj} => \frac{1}{14}\ .\ 2\ .\ 32 = 4.57\,g\,O_2$