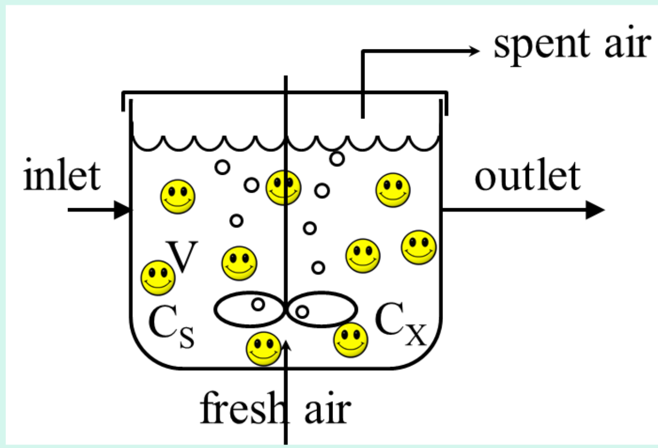
Bioprocesses in Bioreactors
→ Transports

The job to be done: Design and operate Bioprocesses towards best efficiency and productivity, at minimal cost!

Biomass, which plays the key role inside bioreactors, if formed of microorganisms requiring an **optimal microenvironment**!

Consumption
(..) substrate
+ (..) O_2
+ (..) N-source
+ …



Production
Biomass
+ (..) CO_2
+ (..) H^+
+ (..) heat
+ …

→ Requirement of efficient transport processes in bioreactors

Fundamentals of Transport processes

WHAT, is Transported/Transferred into or from bioreactors:

- Gas : O_2, CO_2 …
- Fluids: Organic pollution, medium and substrates (or products)
- Solids: Organic matter (which must dissolved), minerals
- Heat

1. **Mass transfer** (liquid and gas): by means of Convection due to Diffusion and Advection (mass displacement, f(density), f(T))
2. **Heat transfer** : Radiation (space), Conduction (solid), Convection (fluid, density), Advection

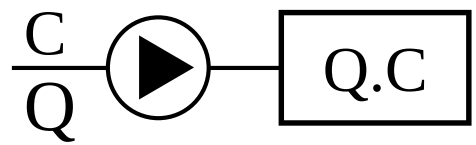
Transport process, is an **interdependent chain/network** of 3 types of transports mechanisms.

Rate limitation of one transport mechanism governs the overall transport process.

3 types of transport in bioprocess

1. Transport: FAST in ONE phase

Feeding, liquid pump, Aeration compressor
Mixing of stirred vessels or bubble columns



2. Transfer: SLOW between TWO phases

O_2 and CO_2 gas-liquid transfer in bioreactor
heat transfer in bioreactor

$$K_L A(C^* - C)$$

3. Transfer (Fick Diffusion) very SLOW in ONE phase

in biofilms
immobilized enzymes/organisms
in stagnant films at surfaces

$$-AD \frac{dC}{dX}$$

In WWTP bioprocess, the most important nutriment, after substrate, is O_2
→ O_2 transfer is the most important transport process to provide!

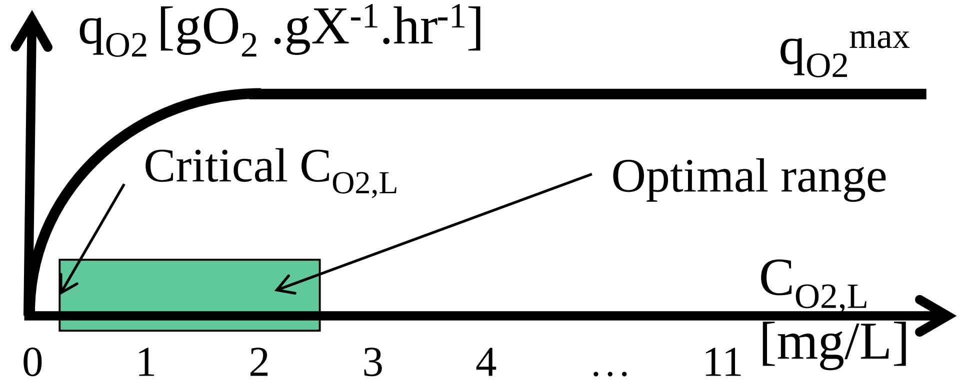
O2 transfer for biomass respiration

Many environmental bioprocesses are aerobic bioprocesses which REQUIRE **oxygen transfer for biomass respiration** (electron acceptor requirement, see COD balance). At steady state:

Biomass respiration **=** **Oxygen transfer rate**

$r_{O2} = q_{O2} \cdot C_X$ $[gO_2 \cdot m^{-3} \cdot hr^{-1}]$ $OTR = k_La\,(C_{O2,L}^{*} - C_{O2,L})$

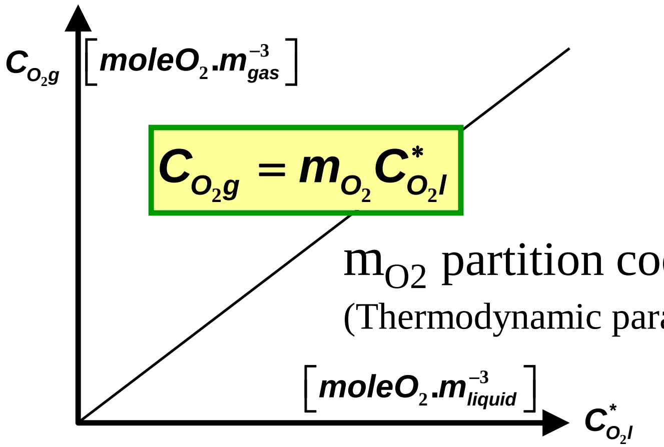
Optimal dissolved O_2 concentration
→ Optimization



- Oxygen transfer is expensive… about 20 to 60% WWTP operating energy
- Maximal dissolved oxygen is low, and in the range of mg O_2/L. (About 8.6, 9,1 or 11 mg O_2/L for 25, 20 or 10°C which be compared to 10^2 to 10^5 mg COD/L of electron donor (substrate or organic pollutant)

Henry's law

O_2 Gas-liquid partition

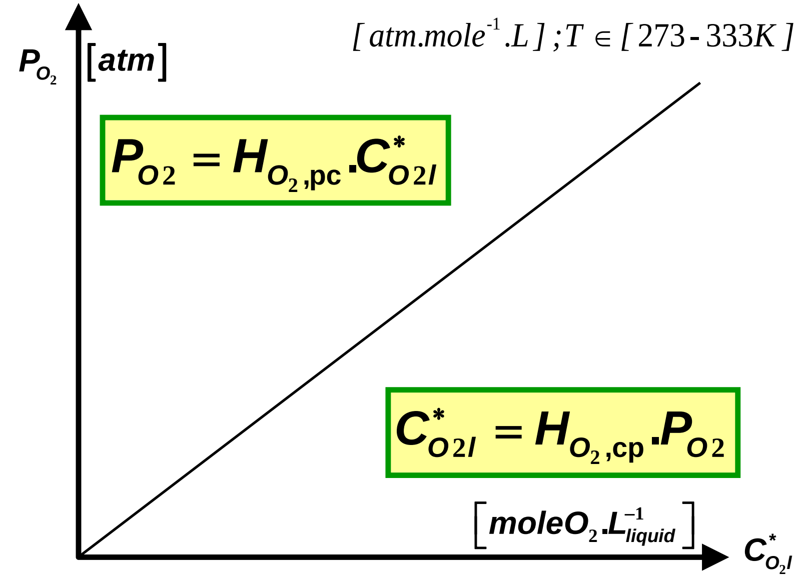


m_{O2} partition coefficient = 32 at 298K
(Thermodynamic parameter)

Henry's law H_{O2}

$$H_{O_2,pc} = exp(-8391.24/T - 23.24323 ln(T) + 167.2367)$$

$[atm.mole^{-1}.L]$; $T \in [273-333K]$



"cp" vs. "pc"
c: O_2 Concentration in liquid
p: O_2 partial Pressure in gas

In air, X_{O2g} molar fraction of O_2 in gas = 0.21
$\mathbf{P_{O2} = X_{O2g}. P}$ = 0.21*1= 0.21 [atm]

From Ideal Gas Law: $\mathbf{P_{O2}.V = n_{O2}.R.T}$
As $\mathbf{C_{O2g}}$ = n_{O2}/V = $\mathbf{P_{O2}/(R.T) = m_{O2}.C^*_{O2L}}$
As $\mathbf{C^*_{O2L} = P_{O2}/H_{O2,pc}}$ → $\mathbf{m_{O2}}$ and $\mathbf{H_{O2,pc}}$ are linked:

$$H_{O_2,pc} = m_{O_2} RT$$

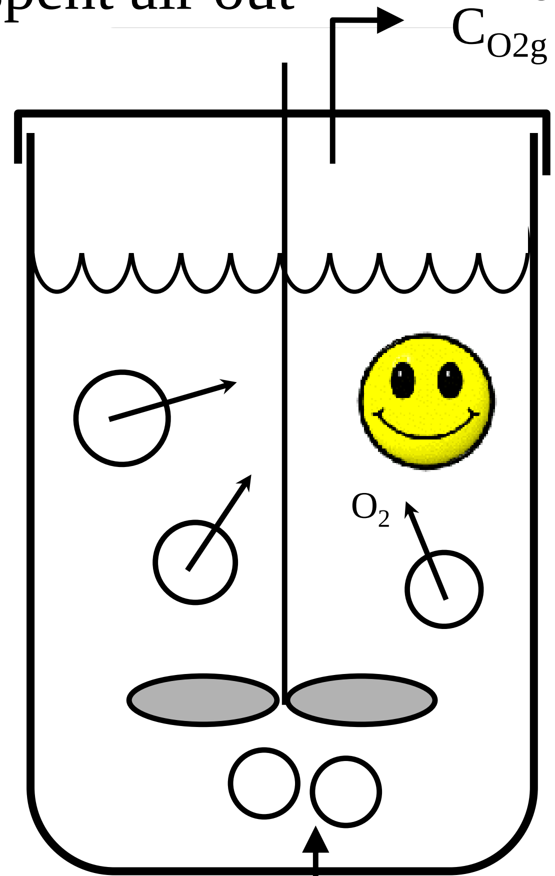
Calculate C^*_{O2L}, at T=298°K ; $H_{O2,pc}$ =780 [atm.mole^{-1}.L]
$H_{O2,pc}$ Henry'a law : C^*_{O2L}=(0.21/780)*32*1000=**8.6 [mg.L^{-1}]**
Ideal gas law : C_{O2g}=0.21/(0.08205*298)=0.0086 [mole.L^{-1}]
m_{O2} Partion C^*_{O2L}=(0.0086/32)*32*1000=**8.6 [mg.L^{-1}]**

R Values	Unités
8,314472	J·mole^{-1}·K^{-1}
0,0820578437	L·atm·K^{-1}·mole^{-1}

O_2 transfer for biomass respiration

spent air out

X_{O2g} outlet (reactor) molar fraction of O_2
C_{O2g} outlet O_2 gas concentration [moleO_2.m^{-3}_{gas}]



fresh air in

[$mole_{gas}$.h^{-1}]

X_{O2g}^{in} inlet molar fraction of O_2
C_{O2g}^{in} inlet O_2 gas concentration [moleO_2.m^{-3}_{gas}]

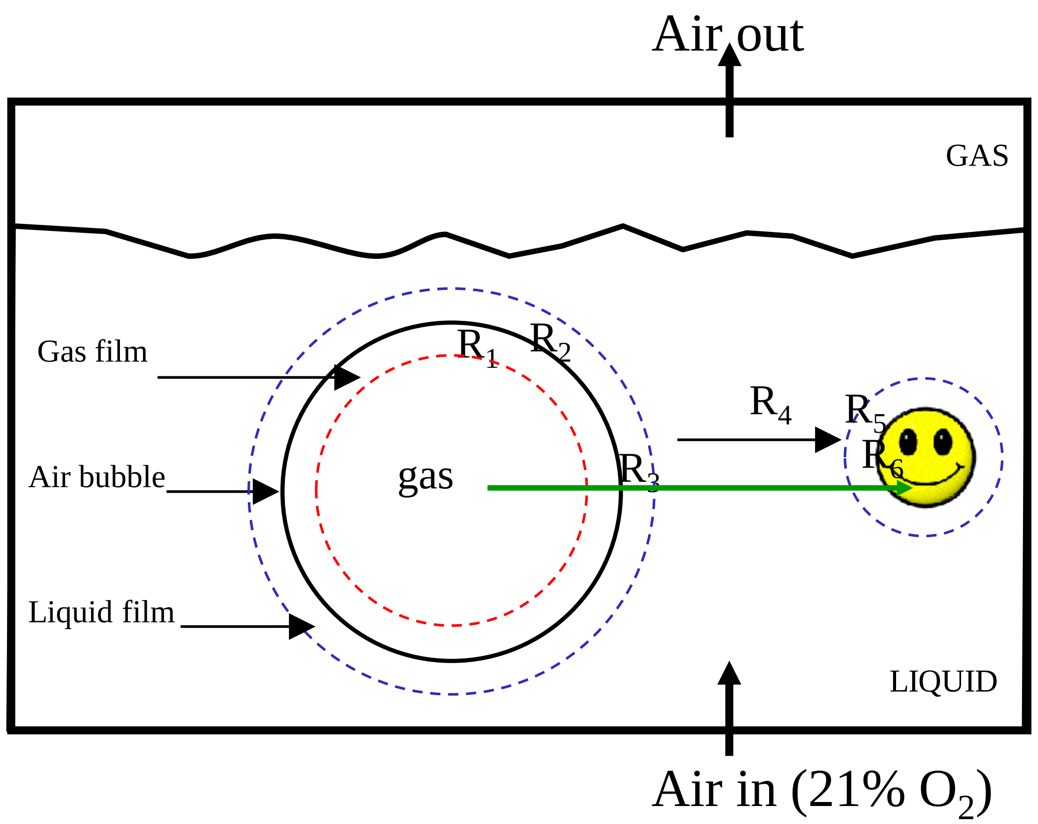
In WATER: Max. dissolved O_2 (Saturation in water) below **9 [mg.L^{-1}] ≈ 0.3 [mole.m^{-3}]**

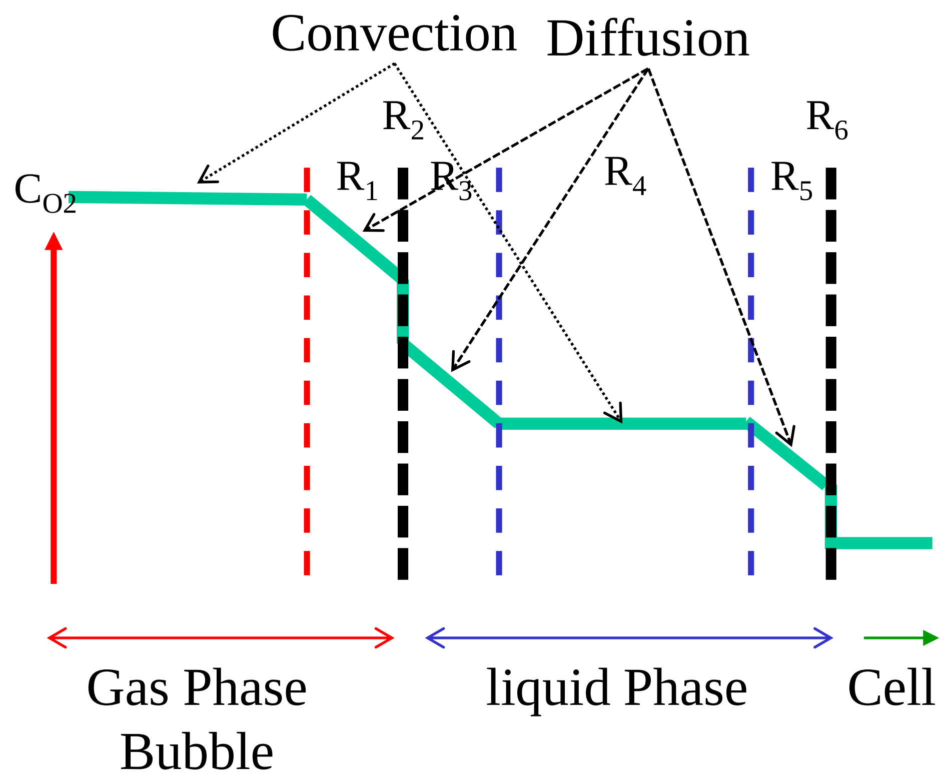
In GAS: O_2 concentration (Air) : ≈ **0.3 [g.L^{-1}] = 9.37 [mole.m^{-3}]**

<u>Biomass consumes ONLY dissolved oxygen !</u>

→ $\mathbf{X_{O2g}^{in} > X_{O2g}}$ and $C_{O2g}^{in} > C_{O2g}$
Often in bioreactor $C_{O2g} \approx C_{O2g}^{in}$

O_2 transfer. A chain transport mechanisms





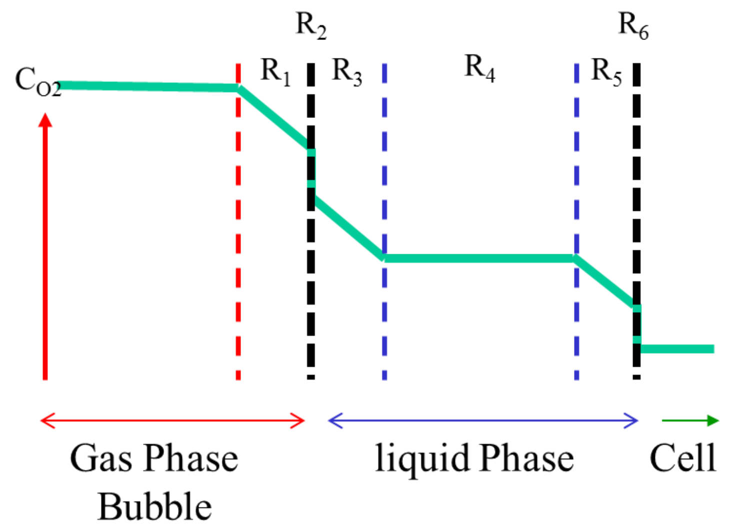
Transport of oxygen, so crucial for biomass of aerobic bioprocesses, from gas bubble to microorganisms through medium, is hindered by several transfer resistances !

O_2 transfer. A chained transport mechanisms

Bubbles side:
- R_1, Within the gas film itself
- R_2, At the gas-liquid interface
- R_3, Within the liquid film itself

In the medium: R_4, Liquid bulk resistance

Microorganisms side
- R_5, Within the liquid film surrounding the microorganism
- R_6, At the liquid-microbe interface



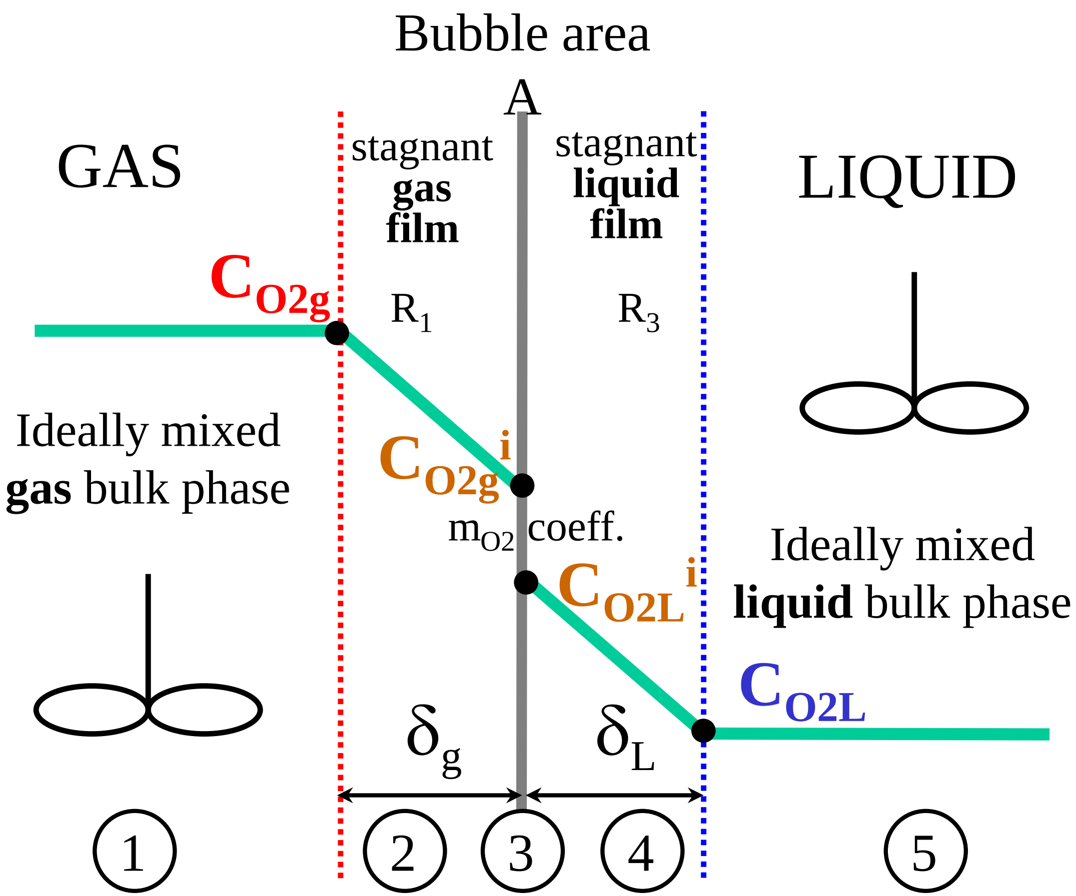
Which is the greatest resistance limiting overall transfer rate?

Negligible Quantities:
- Gas phase diffusivity >> Liquid phase diffusivity, hence, $R_1 << R_3$
  → R1 usually negligible
- Assuming Gas/Liquid interface is in partition equilibrium $Cg_i = m_i.Cl_i$
  →R2 Interfacial resistance is small to be neglected
- In well mixed , inviscid systems, there is no resistance through the liquid phase →R4 bulk resistance negligible (specially under good mixing)
- The liquid-microbe interfacial resistance is small (particularly face to liquid-bubble interface) → R_6 can be neglected
- For small sized cells (e.g yeast and bacteria), Cell. diam. (~1-10µm) << Bubble diam. (~1-5 mm) → it results in a larger cellular interfacial area, hence, $R_5 << R_3$ → R5 negligible

→ Diffusion inside <u>liquid film of gas bubble</u> R3 is the rate controlling for overall transfer resistance.

Note: For large microbial pellets [(4-5 mm) relative to the size of a bubble (4-5mm)] e.g. microbial pellets or fungi, the liquid film surrounding the pellet can be the rate limiting resistance…

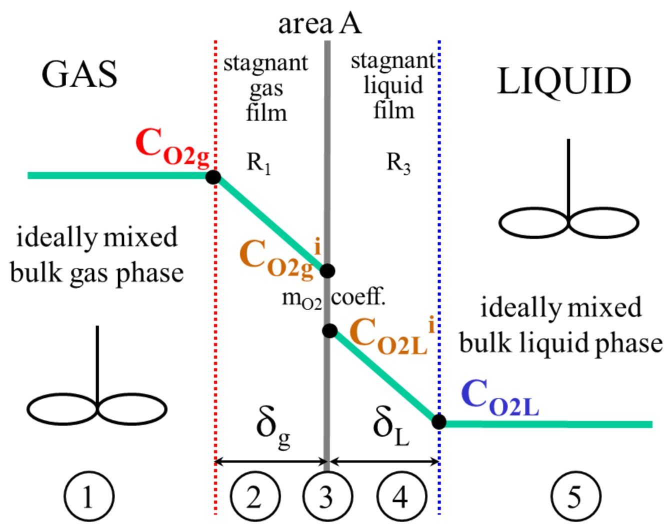
Gas-Liquid Mass Transfer (1)



1. Convective transport to gas film
2. Diffusion in gas film over distance $\boldsymbol{\delta_g}$
3. Gas/liquid O_2 partition equilibrium at bubble interface of **area A**
4. Diffusion in liquid film over distance $\boldsymbol{\delta_L}$
5. Convective transport from liquid film

At interfaces, there are always more or less stagnant layers, of thickness δ where only diffusion is possible, hindering gas transfer by R1 and R3 resistances.
The interfacial equilibrium constant $\mathbf{m_{O2}}$, is determined by the solubility of the gas in the liquid phase (gas-liquid partition or Henry's law).

Gas-Liquid Mass Transfer (2)



Rate of transfer through a stagnant gas film (Fick)

$$rate_{gas} = -\frac{AD_g}{\delta_g}\left(C^i_{O_2g} - C_{O_2g}\right) = Ak_g\left(C_{O_2g} - C^i_{O_2g}\right) \text{ where } k_g \overset{def}{=} \frac{D_g}{\delta_g}$$

Rate of transfer through a stagnant liquid film (Fick)

$$rate_{liq.} = -\frac{AD_l}{\delta_l}\left(C_{O_2L} - C^i_{O_2L}\right) = Ak_l\left(C^i_{O_2L} - C_{O_2L}\right) \text{ where } k_l \overset{def}{=} \frac{D_l}{\delta_l}$$

Equilibrium at interface (Henry's law)

$$C^i_{o_2g} = m_{O2}.C^i_{o_2L} \ ; \ mO_2 = \ 32 \ at \ 298K$$

Assuming **steady state** along transport chain: **rate$_{gas}$= rate$_{liq}$**

3 unknowns (C_{O2L}^{i}, C_{O2g}^{i}, rate) 3 equations… solving for C_{O2L}^{i}.

$$Ak_g\left(C_{O_2g} - C^i_{O_2g}\right) = Ak_l\left(C^i_{O_2L} - C_{O_2L}\right)$$

$$C^i_{o_2g} = m_{O2}.C^i_{o_2L} \qquad \Rightarrow C^i_{O_2L} = \frac{k_g m_{O2}\left(\frac{C_{O_2g}}{m_{O2}}\right) + k_l C_{O_2L}}{k_g m_{O2} + k_l}$$

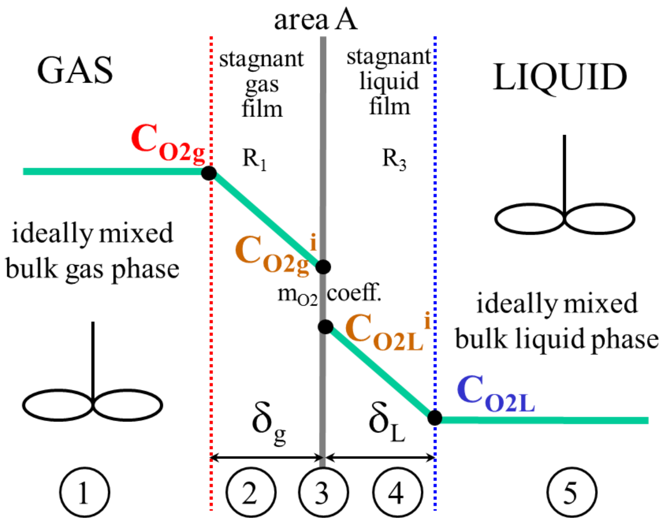
If **gas** diffusion ↑ or stagnant layer ↓

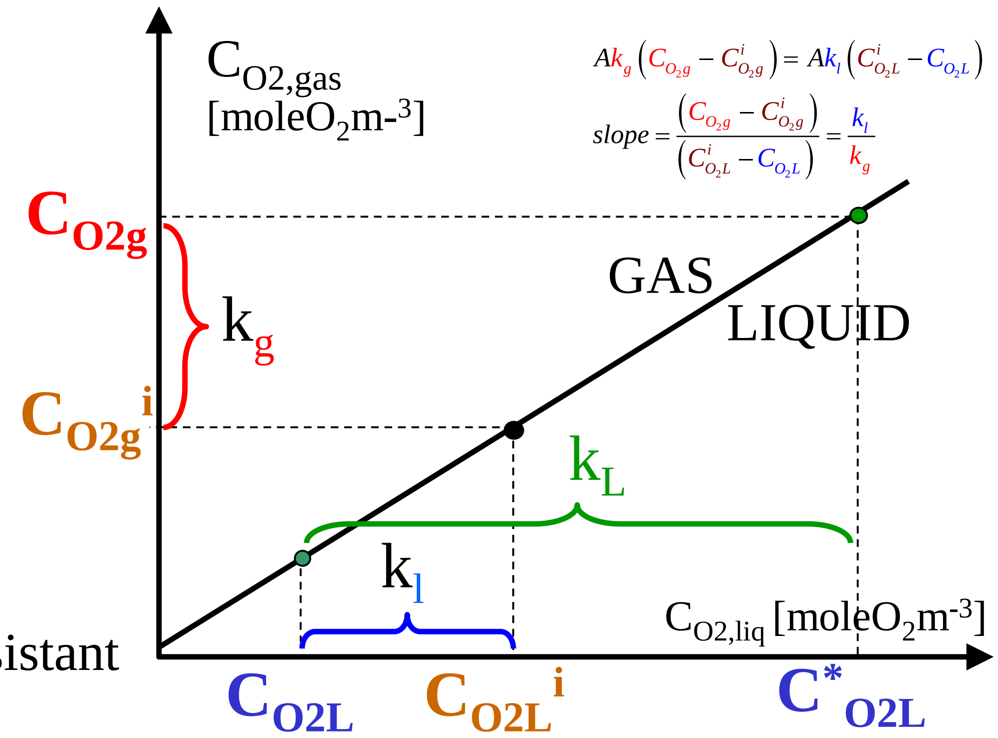
$$k_g = \frac{D_g}{\delta_g} \rightarrow \infty; \quad C^i_{O_2L} \approx \frac{C_{O_2g}}{m_{O2}} = C^*_{O_2L}$$

If **liquid** diffusion ↑ or stagnant layer ↓

$$k_l = \frac{D_l}{\delta_l} \rightarrow \infty; \quad C^i_{O_2L} \approx C_{O_2L}$$

K_L overall mass transfer parameter (1)





$$Ak_g\left(C_{O_2g}-C_{O_2g}^{i}\right)=Ak_l\left(C_{O_2L}^{i}-C_{O_2L}\right)$$

$$slope=\frac{\left(C_{O_2g}-C_{O_2g}^{i}\right)}{\left(C_{O_2L}^{i}-C_{O_2L}\right)}=\frac{k_l}{k_g}$$

? What at S.S.?

Considering overall transport, from bubble gas phase to medium liquid phase, each resistant step rates should be equal, and equal to an **overall gas transfer rate** from gas bubble to liquid medium…

$$\text{rate}=Ak_g\left(C_{O_2g}-C_{O_2g}^{i}\right)=Ak_l\left(C_{O_2L}^{i}-C_{O_2L}\right)=K_LA\left(C_{O_2L}^{*}-C_{O_2L}\right)\quad with\ C_{O_2L}^{*}=m_{O2}.C_{O_2g}$$

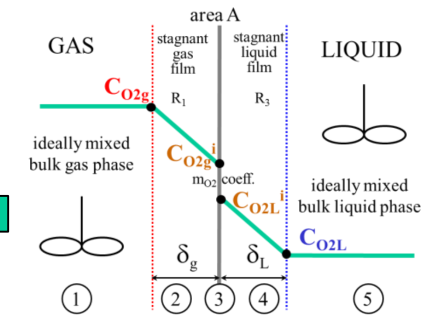
→
$$\left(C_{O_2L}^{*}-C_{O_2L}\right)=\left(C_{O_2L}^{*}-C_{O_2L}^{i}\right)+\left(C_{O_2L}^{i}-C_{O_2L}\right)$$
$$=\left(\frac{C_{O_2g}}{m_{O2}}-\frac{C_{O_2g}^{i}}{m_{O2}}\right)+\left(C_{O_2L}^{i}-C_{O_2L}\right)$$
$$\frac{rate}{K_LA}=\frac{rate}{m_{O2}Ak_g}+\frac{rate}{Ak_l}$$

Thus overall K_L depends on gas-liquid partition coefficient, and resistances of gas and liquid stagnant layers :

$$\frac{1}{K_L}=\frac{1}{k_l}+\frac{1}{m_{O2}.k_g}$$

K_L overall mass transfer parameter (2)

As K_L overall transfer coefficient: $\frac{1}{K_L} = \frac{1}{k_l} + \frac{1}{m_{O2}.k_g}$

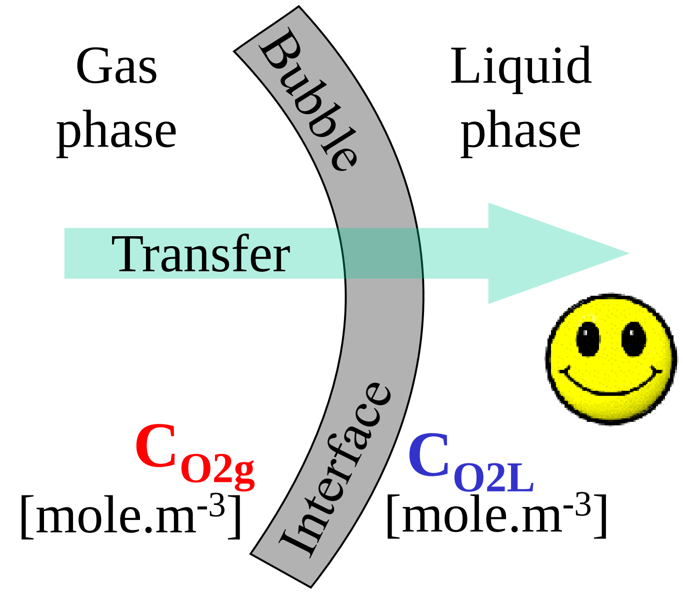


In bioprocesses, as $D_g >> D_l$, then $m.k_g >> k_l$

As: O_2 diffusion coefficient in water $D_l = 10^{-9}[m^2.s^{-1}]$

Stagnant layer thickness : $\delta_l = 10^{-5}[m]$

$$\rightarrow K_L \approx k_L \overset{def}{=} \frac{D_l}{\delta_l} = \text{ in the order of } 10^{-4}\left[\text{m.s}^{-1}\right]$$



Interfacial Area A [m^2]

Thus : $Rate = K_L \cdot A \cdot \left(C^*_{O_2L} - C_{O_2L}\right)$

[$mole.s^{-1}$] with : K_L [$m.s^{-1}$]; A [m^2];

$\mathbf{C^*_{O2L} = C_{O2g}/m}$ [$mole.m^{-3}$] ($H_{O2,pc} = m.RT$)

Gas-Liquid Mass Transfer

Oxygen Mass Transfer Rate [mole.m^{-3}.s^{-1}]:

$$Rate = K_L \cdot A \cdot \left(C^*_{O_2L} - C_{O_2L}\right) \rightarrow \frac{Rate}{V} = K_L.a\left(C^*_{O_2L} - C_{O_2L}\right)$$

with Specific gas/liquid surface area $a = \frac{A}{V}\ [m^{-1}]$

$$OTR = K_L.a\left(C^*_{O_2L} - C_{O_2L}\right)$$

- **No transfer**, if $\mathbf{C^*_{O2L} = C_{O2L}}$, no driving force.
- **Maximal transfer** rate occurs when $\mathbf{C_{O2L}}$= 0. **MaxOTR =$\mathbf{K_L a.C^*_{O2L}}$** (which is thermodynamically determined by solubility, and bioreactor)
- In bioreactor, after inoculation, **biomass respiration increases demand**, decreasing $\mathbf{C_{O2L}}$, ($\mathbf{C^*_{O2L} - C_{O2L}}$) driving force and O_2 transfer rate increase.

The rate of oxygen mass transfer in fermentation broths is highly influenced by several physical and chemical factors that change either :

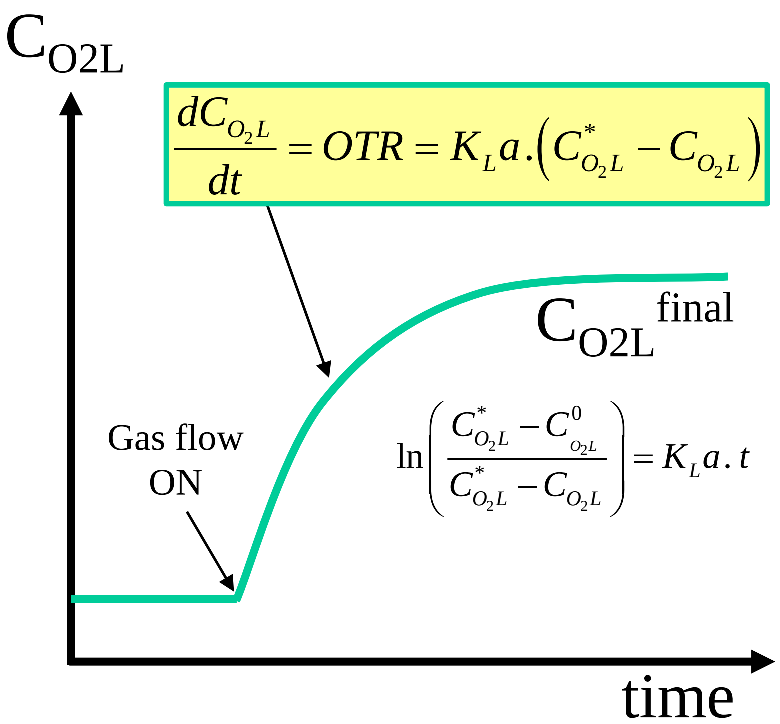
- the value of $\mathbf{K_L}$ or the value of **interfacial area *a***
- the **driving force** for mass transfer, ($C^*_{O2L} - C_{O2L}$)

Even if it can estimated, the precise value of gas transfer coefficient **$\mathbf{K_L}$a** for a given bioprocess often requires **Experimental $\mathbf{K_L}$a determination**!!!

Experimental Determination of $K_L a$

1. **WITHOUT Biomass**
Common absorption method
(pO_2 probe)

$$\frac{dC_{O_2L}}{dt} = OTR = K_L a.\left(C^*_{O_2L} - C_{O_2L}\right)$$



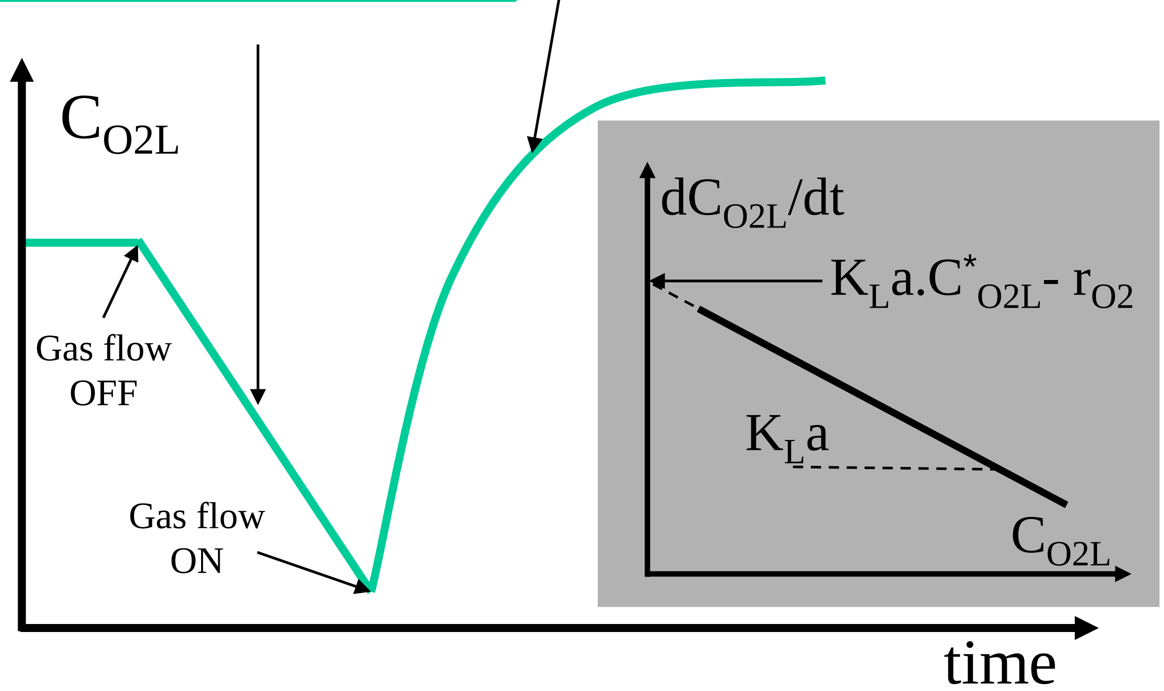
$$\ln\left(\frac{C^*_{O_2L} - C^0_{O_2L}}{C^*_{O_2L} - C_{O_2L}}\right) = K_L a.t$$

2. **WITH Biomass** "Dynamic" method

$$\frac{dC_{O_2L}}{dt} = -rO_2 = -q_{O_2}.C_x = O.U.R.$$

O.U.R.: Oxygen Uptake Rate

$$\frac{dC_{O_2L}}{dt} = K_L a.\left(C^*_{O_2L} - C_{O_2L}\right) - rO_2$$



Volumetric gas mass transfer coefficient K_La

The volumetric gas mass transfer in bioreactors is determined by agitation (liquid mixing) and/or the aeration rate : $K_l a \div (\text{agitation}, \text{aeration})$

1. In CSTR bioreactor (Continuously Stirred Tank Reactor)
   P_g stands for Power [$W.m^{-3}$] (**Agitation**)
   V_{sg} stands for superficial gas velocity [$m.s^{-1}$] (**Aeration**)
   c1, α and β are constants for given combination of the fluid and bioreactor geometry

$$K_L a = c_1 \left(\frac{P_g}{V} \right)^{\alpha} V_{sg}^{\ \beta}$$

2. In Bubble column of Airlift reactor
   Agitation term becomes negligible

$$K_L a = c_1 V_{sg}^{\ \beta}$$

for commonly gas flow rate
$5 < V_{sg} < 30$ [$cm.s^{-1}$]
one can use for calculations

$$K_L a = 0.32 . V_{sg}^{\ 0.7} \ [s^{-1}] \ \ ; V_{sg} [m/s]$$

Volumetric gas mass transfer coefficient $K_l a$ (2)

Practically, as a general rule of thumb: in bioreactor, **K_L coefficient** liquid phase depends on bubble diameter:

- For bubbles diameter > 2-3 mm, $K_L \approx 3\text{-}4 \times 10^{-4}$ m/s and K_L is relatively constant and insensitive to conditions.
- For smaller bubble diameter → $K_L \approx 1 \times 10^{-4}$ m/s depending on bubble rigidity

→ To substantially improve mass transfer rates, it is usually more productive to focus on **the interfacial area *a* increase** .

$$K_L \approx 3-4 \times 10^{-4} \left[m.s^{-1} \right] \; then \text{ if } a \uparrow \;\; \Rightarrow \;\; K_L a \;\; \uparrow$$

How ?

In bubble column, specific gas/liquid surface area ***a*** is a function of:

- Bubble diameter $\mathbf{d_b}$ (average 6 mm)
- Gas holdup **ε** (reactor volume expansion – Aeration)

$$a = 6 \frac{\varepsilon}{d_b}$$

So in bubble column or airlift bioreactor, it's easy to measure **ε** gas holdup which depends on gas superficial velocity $\mathbf{V_{sg}}$.