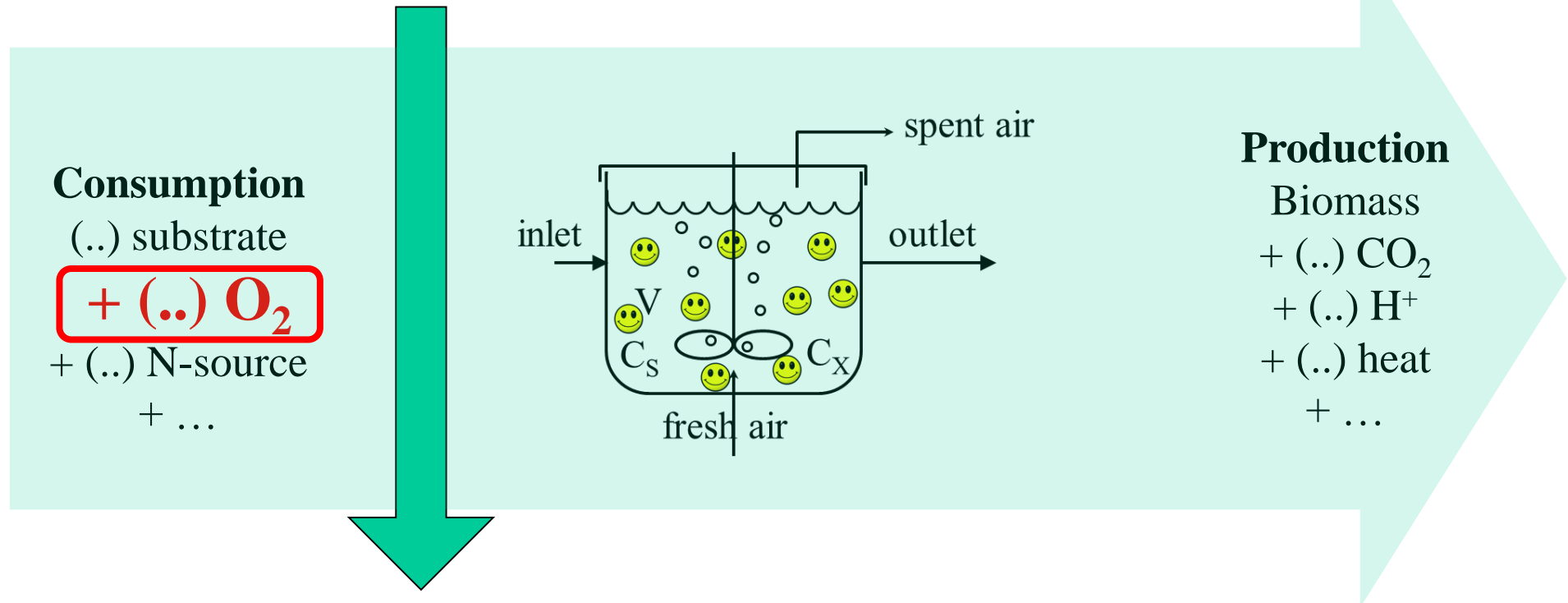


Coupled transport / conversions in Bioreactors

Transport limitation



→ Leads to **Growth limitation !!!** $r_X = Y_{OX} \cdot (-r_{O_2}) = Y_{OX} \cdot OTR$

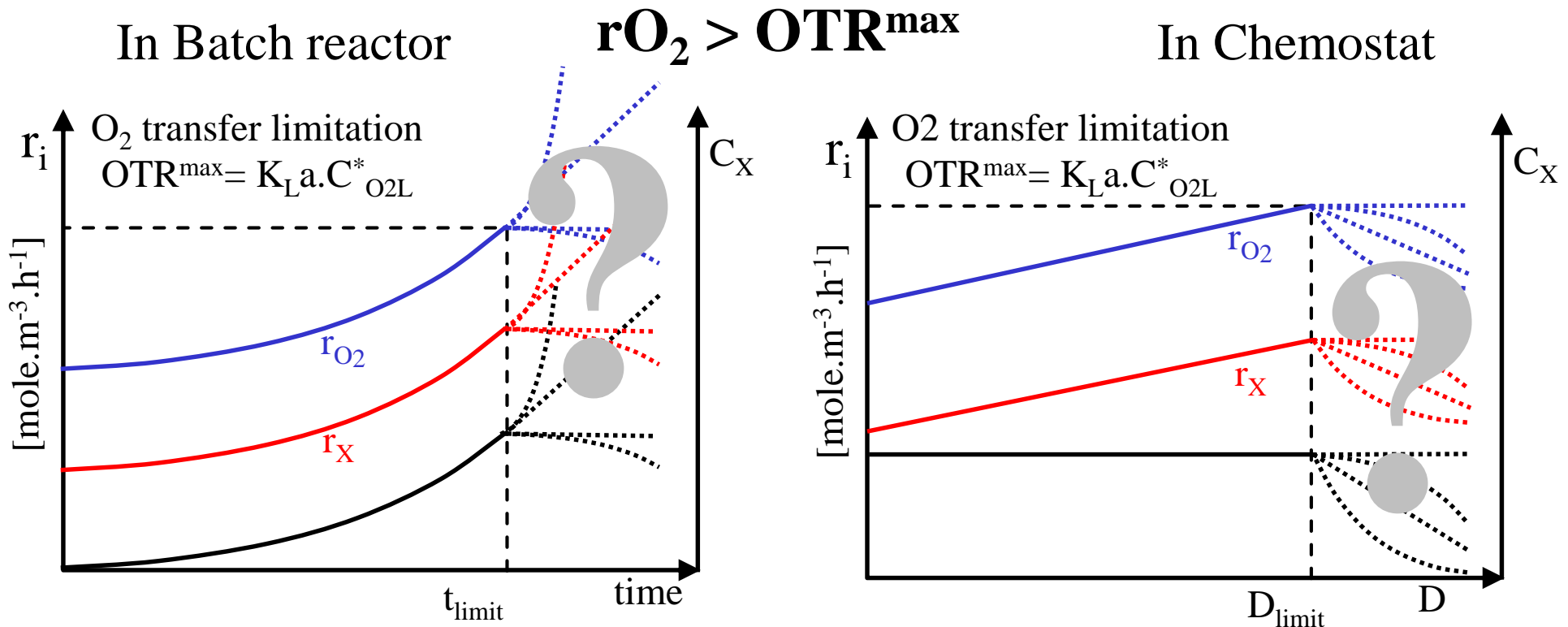
Effect of gas transport limitation in bioreactors:

- O₂ transfer limitation in batch reactors
- O₂ transfer limitation in chemostat

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

Growth limitation when O₂ transfer limitation

Growth limitation is an effect of **gas transport limitation** in bioreactors and occurs when r_{O_2} exceeds maximal O₂ transfer capacity OTR^{max}



$$(-r_{O_2}) = \frac{1}{Y_{OX}} r_X = \frac{1}{Y_{OX}} \mu \cdot C_X \approx \frac{1}{Y_{OX}} \mu^{max} \cdot C_X$$

$$(-r_{O_2}) = \frac{1}{Y_{OX}} r_X \approx \frac{1}{Y_{OX}} D \cdot C_X$$

O₂ transfer limitation in Batch reactor (1)

1. Maximal O₂ transfer in batch reactor

Neglecting maintenance, with:

Air flowrate = 6500 [m³.h⁻¹]; Volume = 100 [m³]

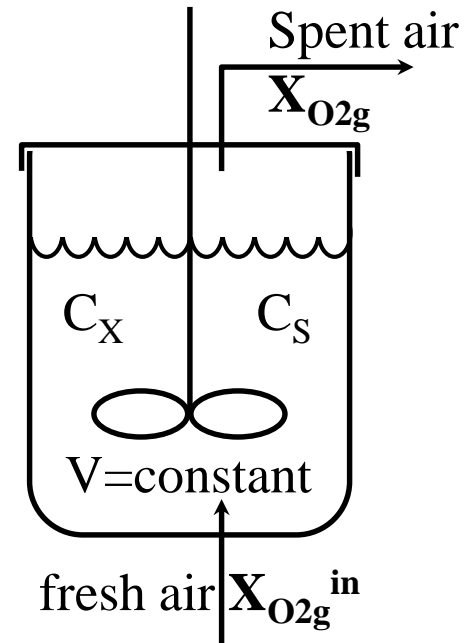
→ 1.08 VVM [$V_{\text{gas}} \cdot V_{\text{liq}}^{-1} \cdot \text{mn}^{-1}$]

Specific superficial area a 200 [m².m⁻³]

T = 300 [K]; P = 1.5 10⁵ [N.m⁻²]; X_{O_{2g}}ⁱⁿ = 0.21

Maximal O₂ transfer occurs when dissolved O₂ = 0 !

$$OTR^{\max} = K_L \cdot a C_{O_2L}^* = K_L \cdot a \frac{C_{O_2g}}{m_{O_2}}$$



Assuming X_{O_{2g}} = X_{O_{2g}}ⁱⁿ = 0.21 (No O₂ depletion in gas bubbles)

With m_{O₂} = 32;

K_L = 1 [m.h⁻¹]

$$C_{O_2g} = \frac{n}{V} = \frac{P_{O_2}}{RT} = \frac{X_{O_2g} P}{RT} = \frac{0.21 \times 1.5 \times 10^5}{8.314 \times 300} = 12.63 \text{ [moleO}_2 \cdot \text{m}^{-3}]$$

→ Maximal volumetric O₂ transfer rate OTR^{max} = 78.94 [moleO₂.m⁻³.h⁻¹]

Thus, the maximal O₂ mass rate transfer in the reactor is:

$$\begin{aligned} &= OTR^{\max} \cdot V \\ &= \mathbf{7894 \text{ [moleO}_2 \cdot \text{h}^{-1}]} \end{aligned}$$

O₂ transfer limitation in Batch reactor (2)

2. Assumption check $X_{O_{2g}} \approx X_{O_{2g}}^{in} = 0.21$

Amount fresh AIR supplied to the reactor [moleAir.h⁻¹] through aeration:

With: Aeration flowrate = 6500 [m³.h⁻¹] and using Ideal Gas Law. $PV=nRT$
 6500 [m³.h⁻¹] $\rightarrow (1.5 \cdot 10^5 \times 6500) / (8.314 \times 300) = \mathbf{390906}$ [moleAir.h⁻¹]

With O₂ molar fraction $X_{O_{2g}}^{in} = 0.21$

\rightarrow **Amount of O₂ supplied to the reactor = 82090 [moleO₂.h⁻¹]**

As $OTR^{max} = 78.94$ [mole_{O₂}.m⁻³.h⁻¹] and reactor volume = 100 [m³]

O₂ content of spent air = 82090 – (7894) = 74196 [moleO₂.h⁻¹]

Thus, reactor O₂ molar fraction $X_{O_{2g}} = [74196 / (390906 - 7894)] = \mathbf{0.1937}$

$\rightarrow X_{O_{2g}}$ **changes only of about 7.7% (from $X_{O_{2g}}^{in} = 0.21$)**

Often with good aeration (VVM of 1 or 1.5), O₂ molar fraction in reactor gas phase and O₂ dissolved concentration are constant over the bioreactor:

$\rightarrow X_{O_{2g}} \approx X_{O_{2g}}^{in} = 0.21 \quad \rightarrow X_{O_{2g}} \approx cst / C_{O_{2g}} \approx cst$

O₂ transfer limitation in Batch reactor (3)

3. Dissolved oxygen C_{O_{2L}} evolution $\frac{dV C_{O_2L}}{dt} = -r_{O_2}V + K_L a (C_{O_2L}^* - C_{O_2L}) \cdot V$
 From C_{O_{2L}} mass balance →

In batch growth: $Y_{OX} \approx Y_{OX}^{\max}$; $\mu \approx \mu^{\max}$; $m_s \approx 0 \Rightarrow r_{O_2} = -\frac{1}{Y_{OX}} r_X = -\frac{\mu}{Y_{OX}} C_X \approx -\frac{\mu^{\max}}{Y_{OX}^{\max}} C_X$

C_X follows from mass balance: $\frac{d(C_X V)}{dt} = r_X V = \mu C_X V \approx \mu^{\max} C_X V \Rightarrow C_X = C_{X0} \exp(\mu^{\max} t)$

As $C_{O_2L} < C_{O_2L}^* \approx 0.3$ [moleO₂.m⁻³], C_{O_{2L}} changes slowly $d(C_{O_2L} \cdot V)/dt \approx 0!$

Assuming **pseudo steady state** for C_{O_{2L}}:

$$\frac{dV_L C_{O_2L}}{dt} = -\left(\frac{\mu^{\max} C_{X0}}{Y_{OX}^{\max}}\right) \exp(\mu^{\max} t) V_L + K_L a (C_{O_2L}^* - C_{O_2L}) V_L \approx 0$$

Solving → $C_{O_2L}^* - C_{O_2L} = \frac{\mu^{\max} C_{X0}}{Y_{OX}^{\max} K_L a} \exp(\mu^{\max} t)$

O₂ transfer limitation in Batch reactor (4)

3. C_{O2L} dynamics

A) Before O₂ limitation

(C_{O2L}ⁱⁿ - C_{O2L}) increases exponentially as
 $\mu = \mu^{\max}$ with low change of d(C_{O2L}·V)/dt

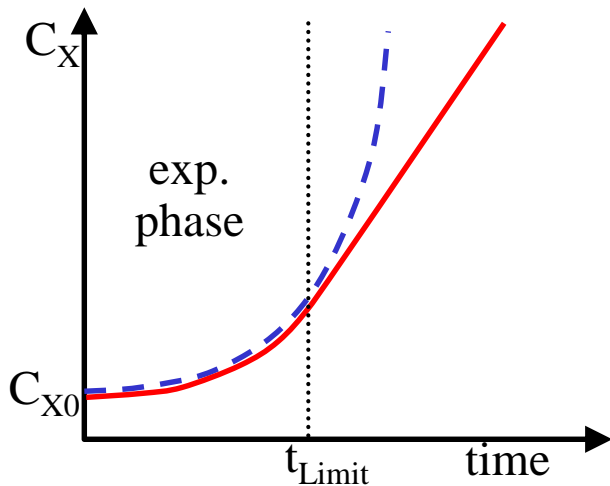
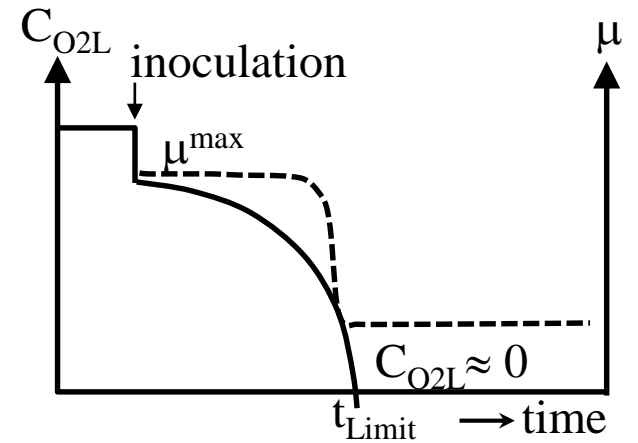
t_{Limit} can be calculated when C_{O2L} = 0

B) After O₂ limitation

As C_{O2L} = 0, C_{O2L}^{*} = cst ≈ 0.3 [moleO₂·m⁻³] then OTR^{max} = cst = K_La C_{O2L}^{*}

Consumed O₂ = transferred O₂; r_{O2} = OTR^{max}, (neglecting m_s) r_{O2}, r_X, r_S = cst

$$C_{O2L}^* - C_{O2L} = \frac{\mu^{\max} C_{X0}}{Y_{OX}^{\max} K_L a} \exp(\mu^{\max} t)$$

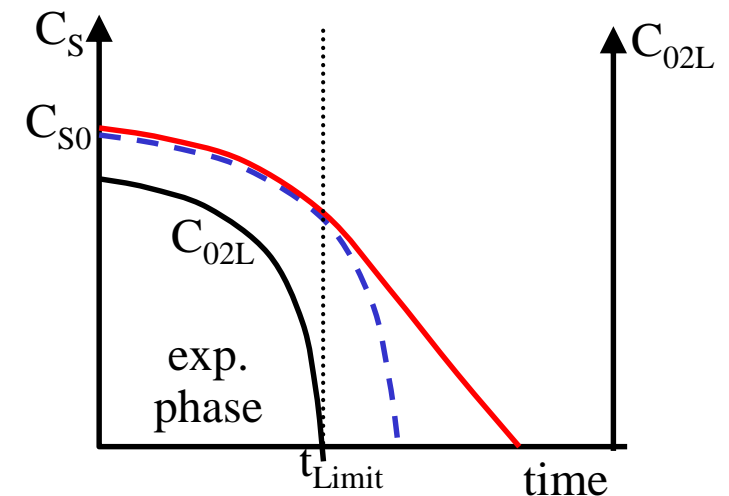


After O₂ limitation
 → linear phase

$$-r_{O2} = K_L a \cdot C_{O2L}^* = Cst$$

$$r_X = Y_{OX}^{\max} \cdot K_L a \cdot C_{O2L}^* = Cst$$

$$\frac{dC_X}{dt} = r_X = Cst$$



O₂ transfer limitation in Chemostat (1)

Assumptions:

$C_{S,in} \gg C_S$ residual concentration

Neglecting m_S , $Y_{OX} \approx Y_{OX}^{\max}$

Then $-r_{O_2} \approx \frac{1}{Y_{OX}^{\max}} r_X$ because $C_{S,in} \gg C_S$;

$$-r_S = D.(C_{S,in} - C_S) \approx D.C_{S,in} \Rightarrow r_X = Y_{SX}^{\max} . r_S \approx Y_{SX}^{\max} . D . C_{S,in}; \quad -r_{O_2} \approx \frac{Y_{SX}^{\max}}{Y_{OX}^{\max}} D C_{S,in}$$

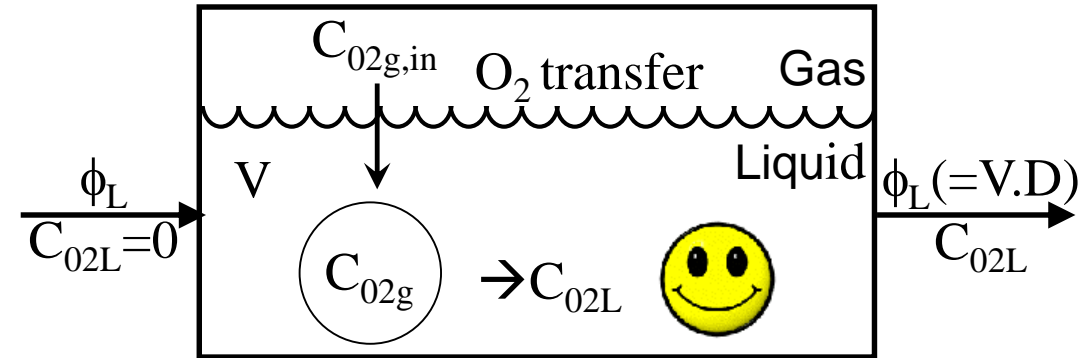
At steady state: $\frac{d(V.C_{O_{2L}})}{dt} = -r_{O_2} V + K_L a (C_{O_{2L}}^* - C_{O_{2L}}) V \approx 0$

Assuming $X_{O_{2g}} \approx X_{O_{2g}}^{in} \rightarrow C_{O_{2g}} \approx \text{cst}$,
then $C_{O_{2L}}^* \approx \text{cst}$

$$C_{O_{2L}}^* - C_{O_{2L}} = \frac{Y_{SX}^{\max} . C_{S,in} . D}{Y_{OX}^{\max} . K_L a}$$

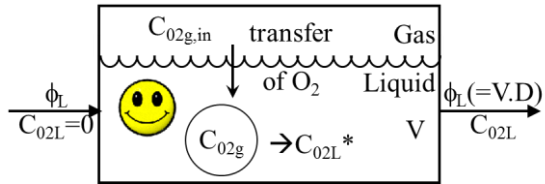
With $C_{O_{2L}} = 0$, one can estimate D_{Limit} value of dilution rate, when O₂ limitation occurs. As **OTR is maximal**, then r_{O_2} , r_X , $r_S = \text{cst}$!

Then $C_X = r_X/D = \text{cst}/D$ and $(C_{S,in} - C_S) = \text{cst}/D$

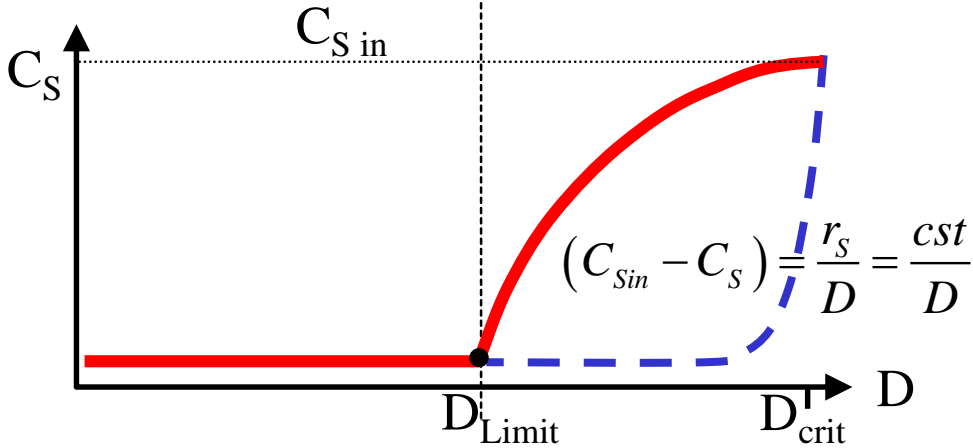
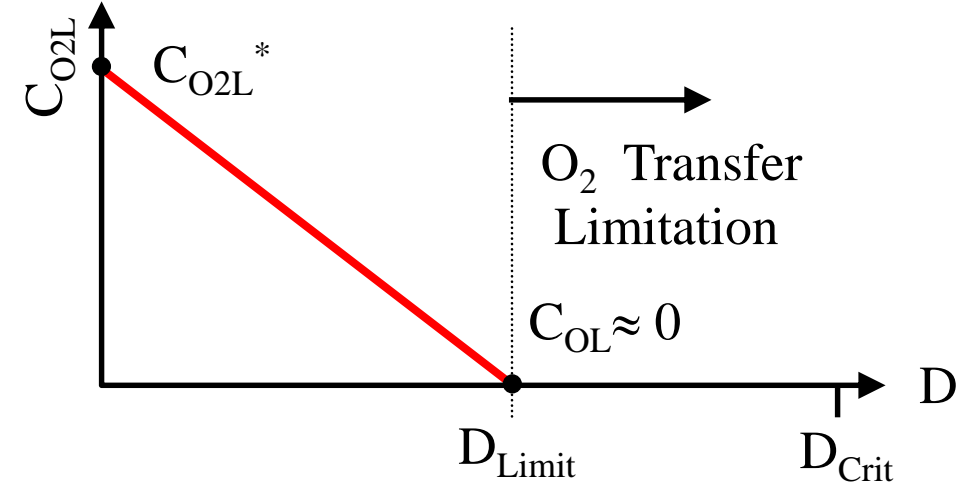
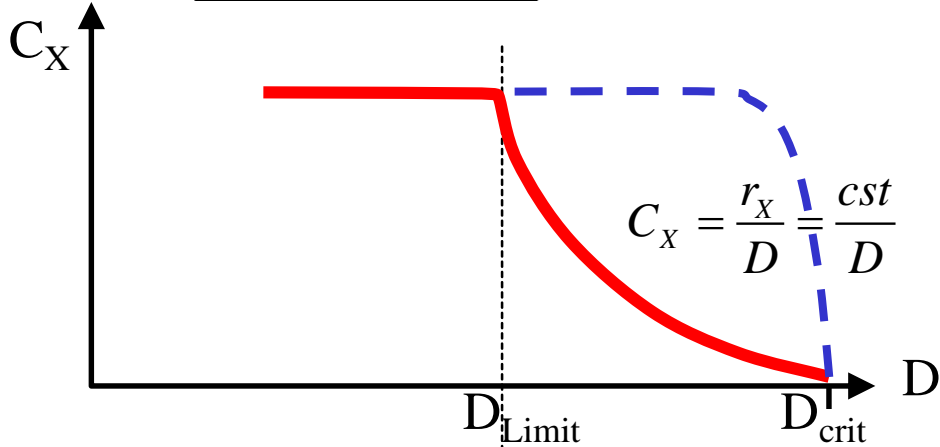


O₂ transfer limitation in Chemostat (2)

In chemostat bioreactor, before O₂ transfer limitation dissolved oxygen depends linearly on D



$$C_{O2L}^* - C_{O2L} = \frac{Y_{SX}^{max} \cdot C_{S,in} \cdot D}{Y_{OX}^{max} \cdot K_L a}$$



After O₂ transfer limitation, C_{O2L} = 0 and C_X decreases with D⁻¹ from D_{Limit} to critical dilution rate D_{crit}. Similarly, (C_{Sin}-C_S) decreases with D⁻¹.

O₂ transfer limitation with gas depletion (1)

Gas depletion ? Considering Chemostat bioreactor as an ideal CSTR and perfect mixed gas/liquid phase.

O₂ mass balance (in liquid phase)

Assuming that O₂ pseudo steady state in liquid

$$0 = r_{O_2} V_L + K_L a (C_{O_2L}^* - C_{O_2L}) V_L \rightarrow -r_{O_2} = K_L a (C_{O_2L}^* - C_{O_2L})$$

→ How to calculate r_{O_2} when $X_{O_2g} < X_{O_2g}^{in}$?
($C_{O_2L}^*$ or X_{O_2g} is required !)

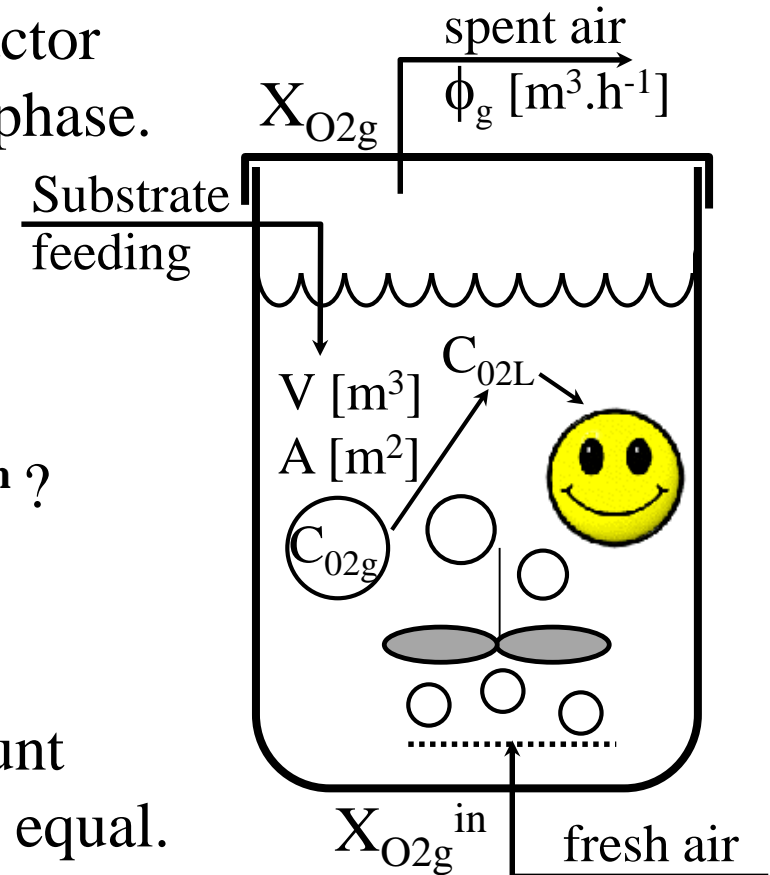
O₂ mass balance (in gas phase)

With pseudo steady state in gas phase, the amount of O₂ supplied (gas) and transferred (liquid) are equal.

$$0 = \phi_g (X_{O_2g}^{in} - X_{O_2g}) \frac{P}{RT} - K_L a (C_{O_2L}^* - C_{O_2L}) V_L$$

$$\text{With } C_{O_2L}^* = \frac{C_{O_2g}}{m_{O_2}} = \frac{X_{O_2g} P}{RT m_{O_2}}$$

$$\rightarrow X_{O_2g} = \frac{X_{O_2g}^{in} + \frac{K_L a V_L}{\phi_g} \frac{RT}{P} C_{O_2L}}{1 + \frac{K_L a V_L}{\phi_g} \frac{1}{m_{O_2}}}$$



O₂ transfer limitation with gas depletion (2)

O₂ molar fraction in bubble gas in bioreactor depends:

- On **K_La** gas/liquid transfer coefficient
- Inlet gas flux **Φ_g** into bioreactor

$$X_{O_2g} = \frac{X_{O_2g}^{in} + \frac{K_L a V_L}{\phi_g} \frac{RT}{P} C_{O_2L}}{1 + \frac{K_L a V_L}{\phi_g} \frac{1}{m_{O_2}}}$$

1. If **K_La/Φ_g** small, when gas/liquid transfer is small in front of inflow mass rate

→ **negligible depletion** $X_{O_2g} \approx \frac{X_{O_2g}^{in} + 0}{1 + 0} \approx X_{O_2g}^{in}$

2. If **K_La/Φ_g** large, when gas/liquid transfer is large in front of inflow mass rate

→ **equilibrium** $C_{O_2L} \approx X_{O_2g} \frac{P}{RT} \cdot \frac{1}{m_{O_2}} = \frac{C_{O_2g}}{m_{O_2}} = C_{O_2L}^*$

Substituting X_{O_2g} in r_{O_2} expression

→ $r_{O_2} \uparrow$ with a , P , $X_{O_2g}^{in}$, Φ_g **and** \downarrow if $C_{O_2L} \uparrow$

$$-r_{O_2} = K_L a \left(C_{O_2L}^* - C_{O_2L} \right) = \frac{K_L a \left(\frac{P}{RT} \frac{X_{O_2g}^{in}}{m_{O_2}} - C_{O_2L} \right)}{\left(1 + \frac{K_L a V_L}{\phi_g} \cdot \frac{1}{m_{O_2}} \right)}$$

For given $K_L a$, P , ϕ_g gas flow rate, and $X_{O_2g}^{in}$ composition, **r_{O_2} will be determined by O₂ gas/liquid mass transfer capacity !**

→ According stoichiometry or yields, as r_{O_2} is fixed by O₂ transfer, one can calculate r_X , r_S , r_i rates, can **set operation and substrate feed strategy...**

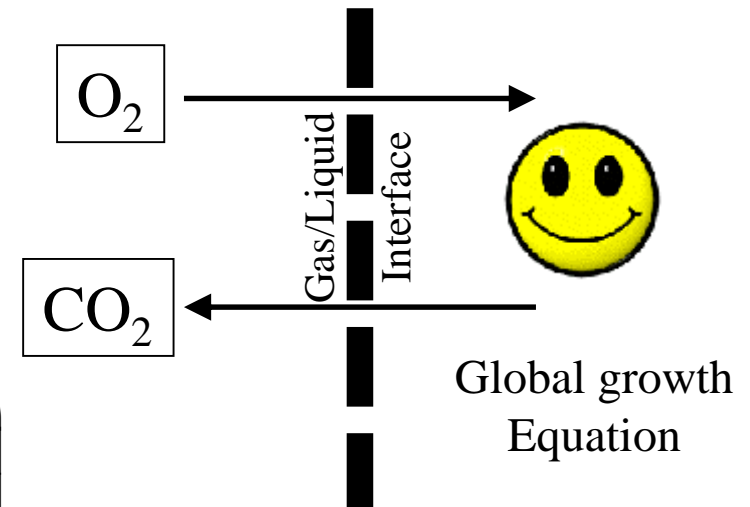
O₂ and CO₂ coupled transport in bioreactors (1)

In bioreactors, biomass consumes O₂ and (usually) produces CO₂.
 Gas/Liquid interface is same for O₂ and CO₂, with same transfer limitation.
 → Same **K_LA** volumetric transfer coefficient...!

Thus similarly to O₂, production and transport of produced CO₂ are coupled.
 From CO₂ mass balance:
 (but with no CO₂ in inlet gas)

$$\rightarrow X_{CO_2g} = \frac{\frac{K_L a V_L}{\phi_g} \frac{RT}{P} C_{CO_2L}}{1 + \frac{K_L a V_L}{\phi_g} \frac{1}{m_{CO_2}}} \quad r_{CO_2} = K_L a \left(\frac{C_{CO_2L}}{1 + \frac{K_L a V_L}{\phi_g} \frac{1}{m_{CO_2}}} \right)$$

Thus, if biomass produces CO₂ according microbial growth equation, at biomass growth rate μ , biomass growth will determine r_{CO_2} and thus, the dissolved CO₂ concentration C_{CO_2L} in bulk, and outlet molar fraction X_{CO_2} .



O₂ and CO₂ coupled transport in bioreactors (2)

Usually r_{O_2} and r_{CO_2} are stoichiometrically coupled by global growth equation. → Thus there is a coupling between C_{CO_2L} and C_{O_2L} .

Often $-r_{O_2} = 1 \cdot r_{CO_2}$:

$$\rightarrow C_{CO_2L} = \frac{\left(1 + \frac{K_L a V_L}{\phi_g} \cdot \frac{1}{m_{CO_2}}\right)}{\left(1 + \frac{K_L a V_L}{\phi_g} \cdot \frac{1}{m_{O_2}}\right)} \left(\frac{P}{RT} \frac{X_{O_2g}^{in}}{m_{O_2}} - C_{O_2L} \cdot \left(1 + \frac{K_L a V_L}{\phi_g} \cdot \frac{1}{m_{O_2}}\right) \right)$$

$$-r_{O_2} = \frac{K_L a \left(\frac{P}{RT} \frac{X_{O_2g}^{in}}{m_{O_2}} - C_{O_2L} \right)}{\left(1 + \frac{K_L a V_L}{\phi_g} \cdot \frac{1}{m_{O_2}}\right)}$$

$$r_{CO_2} = K_L a \left(\frac{C_{CO_2L}}{1 + \frac{K_L a V_L}{\phi_g} \cdot \frac{1}{m_{CO_2}}} \right)$$

→ For constant microenvironment, gas/liquid transfer and microbial growth stoichiometry determine C_{CO_2L} and C_{O_2L}

Increasing in $K_L a$, P , ϕ_g and $X_{O_2g}^{in}$ will increase r_{CO_2} (and r_{O_2}), and thus C_{CO_2L} will also increase.

Thus, there is very little freedom for changes in $K_L a$, P , ϕ_g and $X_{O_2g}^{in}$ to fix C_{O_2L} independently of C_{CO_2L} .