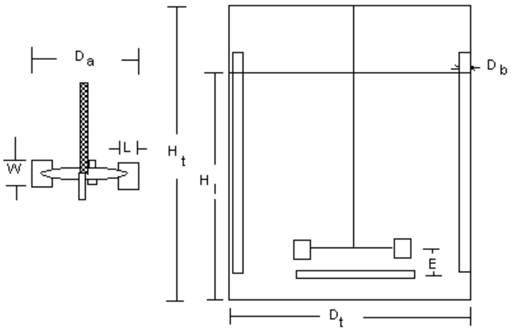
Contacts: weijin.wang@epfl.ch;
manfred.zinn@epfl.ch

Biochemical Engineering
Exercise Session 2 - Solutions

1) Design aspects of a bioreactor



A stirred tank bioreactor is approximately cylindrical in shape. It has a total volume (V_t) of 100,000 liters. The geometry of the reactor is defined by the following ratios: D_t:H_t=0,5 ; D_a:D_t=0,33 ; D_b:D_t = 0,1

a) Is it an aerated system? Yes the bioreactor is aerated. The sparger positioned beneath the agitator provides the air.
b) Is this type of bioreactor advantageous for mammalian cells? The aspect ratio of this bioreactor is 2. Bioreactors for mammalian cells have aspect ratios of 1, while microbial cells cultures in a STR have a aspect ratio of 3. In this limiting case it is still possible to culture mammalian cells, even though the aspect ratio is above 1.
c) Calculate: D_t, H_t, D_a, D_b

$$D_t = \left(\frac{2V_t}{\pi}\right)^{1/3} = 4m \quad with \qquad 2D_t = H_t \qquad and \qquad V_t = 100m^3$$

It is important to convert the volume in SI units.

$$H_t = 8m$$
$$D_a = 0{,}33 \cdot 4m = 1{,}33$$
$$D_h = 0{,}4m$$

2) Cell concentration in aerobic culture

A strain of *Azotobacter vinelandii* is cultured in a 15m^3 stirred Fermenter for alginate production. Under current operating conditions k_La is 0.17 s^{-1}. Oxygen solubility in the broth is approx. 8 x 10^{-3} kg m^{-3}.

a) The specific rate of oxygen uptake is 12.5 mmol g^{-1} h^{-1}. What is the maximum possible cell concentration?

<u>*Unit Transformation*</u>:

$$k_L a = 0.17\ s^{-1} \cdot 3600\ s/h = 612\ h^{-1}$$

$$C^*_{O_2} = 8 \times 10^{-3}\ kg \cdot m^{-3} \cdot 1000\ g/kg \cdot 0.001\ m^3/L = 8 \times 10^{-3}\ g \cdot L^{-1}$$

$$\begin{cases} OUR = 12.5\ mmol \cdot g^{-1} \cdot h^{-1} \cdot 0.032\ g/mmol = 0.4\ g \cdot g^{-1} \cdot h^{-1} \\ MW_{O_2} = 32\ g/mol \cdot 0.001\ mol/mmol = 0.032\ g/mmol \end{cases}$$

$$\begin{cases} OTR = OUR \cdot X \\ OTR = k_L a \left(C^*_{O_2} - C_{O_2} \right) \end{cases}$$

The maximum cell concentration will be for maximum OUR, which is for
$OTR_{\max} = k_L a \times C^*_{O_2}$

Therefore, $X_{\max} = \dfrac{OTR_{\max}}{OUR} = 12.24\ g/L$

b) The bacteria suffer growth inhibition after copper sulphate is accidently added to the fermentation broth. This causes a reduction in oxygen uptake rate to 3 mmol g^{-1} h^{-1}. What maximum cell concentration can now be supported by the fermenter?

New $OUR = 3\ mmol \times g^{-1} \times h^{-1} \times 0.032\ g/mmol = 0.096\ g \times g^{-1} \times h^{-1}$

Therefore $X_{\max} = 51\ g/L$

3) Specific oxygen uptake in *E. coli* culture

It is assumed, that the specific oxygen uptake rate (q_{O2}) of *E. coli* is 5.0 mmol g^{-1} h^{-1}.

a) Which cell concentration X can be reached in a laboratory reactor with a k_La of 25 h^{-1} when C_L = 10 % C* and for the medium at 37 °C is C* = 0.17 mmol L^{-1}.

Working according to the exercise (2) we have

$$OUR = 0.16\ g \cdot g^{-1} \cdot h^{-1}$$
$$C_L^* = 5.44 \times 10^{-3}\ g/L$$
$$C_L = 0.1 \cdot C_L^* = 5.44 \times 10^{-4}\ g/L$$
$$OTR = k_L a \cdot \left(C_L^* - C_L\right) = 0.1224\ g \cdot L^{-1} \cdot h^{-1}$$
$$X = \frac{OTR}{OUR} = 0.765\ g/L$$

4) Oxygen consumption

Estimate how fast the dissolved oxygen concentration is consumed in a bioreactor with k_La 1000 h^{-1}, containing a 10g/l culture growing with μ = 0.5h^{-1} if the aeration is interrupted.

a) Calculate the quasi-steady state oxygen concentration. Assume $Y_{X/O}$=1g/g and the oxygen solubility in the medium equilibrium with C^*=7mg/L.

The consumption of dissolved oxygen while we provide oxygen to the bioreactor will be given by the equation:

$$\frac{dC_L}{dt} = k_L a\left(C_L^* - C_L\right) - \frac{1}{Y_{X/O}}\mu X$$

from where by assuming quasi steady state $\left(\frac{dC_L}{dt} = 0\right)$

$$C_L = 0.002\ g/L$$

b) In what time will the culture become completely anaerobic?

When we cut the oxygen we can approximately set

$$\frac{dC_L}{dt} \approx \frac{\Delta C_L}{\Delta t} = -\frac{1}{Y_{X/O}}\mu X \Rightarrow \Delta t = \frac{C_L^{final} - C_L^{initial}}{-\frac{1}{Y_{X/O}}\mu X} = \frac{0 - 0.002}{-0.5 \cdot \frac{1}{3600} \cdot 10}\sec = 1.44\sec$$

5) Oxygen storage capacity of a fermentation broth

With a OTR (=OUR) of growing bacteria of 1 g O_2/(l·h) per 1 g/l cell dry mass and approximately 100 g/l cell dry mass at the end of the growth phase, an oxygen uptake rate of 100 g/(l·h) will develop. The oxygen solubility in the fermentation broth at 28°C is calculated at 7.76 mg/l.

a) How long will the oxygen supply last?

Note: OUR per 1 g cell dry weight = q_{O2}

O_2 consumption per h with 100 g biomass = 100 g_{O2} L^{-1} h^{-1}

$$t_{oxygen\ supply} = \frac{7{,}76 \cdot 10^{-3} \frac{g}{l\cdot} \cdot 3600}{100 \frac{g}{l \cdot h}} = 0{,}279s$$

6) Calculating saturation concentration

Calculation of the oxygen saturation concentration at different temperatures and partial oxygen pressures using the correlation of Tresdale:

T [°C]	28	37	60	28	37	60
P_{O2} [bar]	0.2121	0.2121	0.2121	1.0133	1.0133	1.0133
$C^*_{O_2}$ **[mg/L]**	7.76	6.86	4.32	37.06	32.80	20.63

Equation is found on p.60 of the slides from the corresponding theory and the calculations are made by using the above values in °C and bar. The oxygen saturation concentration will be given in mg/L.

7) Oxygen transfer in a sparged stirred tank bioreactor

Which of the following would have the highest oxygen transfer rate characteristics?

a) A sparged stirred tank bioreactor being stirred at 200 rpm
b) A non-sparged stirred tank bioreactor being stirred at 200 rpm
c) A shake flask being mixed at 200 rpm
d) All of the above would have equivalent oxygen transfer rate characteristics

A sparged stirred tank bioreactor being stirred at 200 rpm.

Short Resume

Dissolved oxygen is an important substrate in aerobic fermentations. Since oxygen is sparingly soluble in water, it may be the growth-limiting substrate in these fermentations. For bacteria and yeast cultures, the *critical oxygen concentration* is about 10% to 50% of the saturated DO (dissolved oxygen concentration).

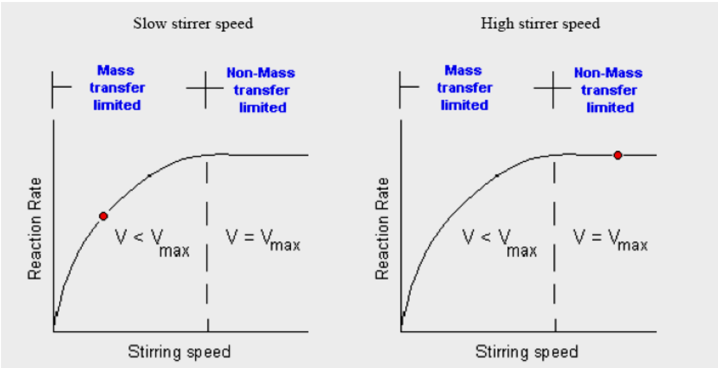


Figure 1: If the bulk liquid is well mixed, the convective transport of oxygen through the bulk liquid is much faster than compared to the diffusion in the liquid films.

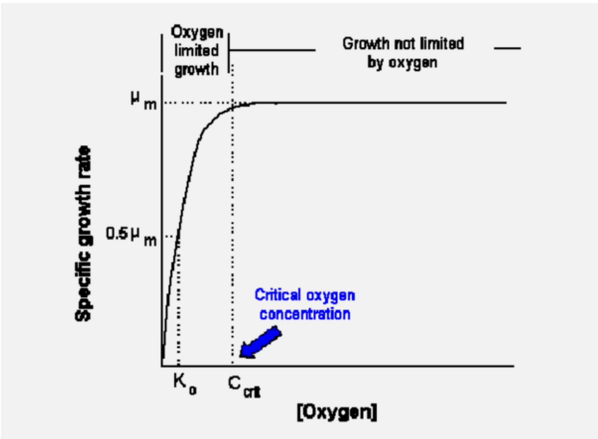


Figure 2: Oxygen as a substrate

Equation of transport

$$OTR = k_L a(c^* - c_L) = X \cdot q_{O_2}$$

k_l: is the oxygen transport coefficient [=] L/t

a: is the gas-liquid interfacial area per volume of reactor [=] L^{-1}

k_la: is the volumetric oxygen gas transfer coefficient [=] t^{-1}

c*: saturated dissolved oxygen concentration [=] m/L^3 (approximately 7 mg/l at 25°C and 1 atm)

c_L: actual dissolved oxygen concentration in the liquid [=] m/L^3

X: is the biomass concentration in the liquid [=] m/L^3

q_{o2}: specific oxygen uptake rate [=] mol/m/t

k_La: Is the product of the mass transfer coefficient k_L and a the interfacial area of mass transfer.

- Surface active agents, such as antifoaming agents decrease k_La
- An increase in the salt concentration reduces the gas bubble size, thus k_La increases
- An increase in viscosity leads to thicker liquid films, thus k_La decreases
- An increase in viscosity leads to bubble coalescence, thus k_La decreases
- An increase of mixing leads to an increase of the relative velocity between the gas bubbles and fluid phase, thus k_La increases