H. Oxygen

Oxygen is a unique substrate in aerobic microbial processes since (unlike carbon, nitrogen, phosphorus sources, etc.) it *must be continuously supplied.* The solubility of oxygen is about 7-8 mg/L or 0.2 mM, over 15,000 times less soluble than glucose on a molar basis.

1. Oxygen Uptake Rate

Oxygen uptake rate (OUR or Q_O) is the rate at which oxygen is consumed on a volumetric basis.

The rate of oxygen consumed is proportional to the rate of new cell formation (for the moment, assuming negligible maintenance). If oxygen were like typical soluble substrates (e.g., glucose), we would write:

$$-\frac{dO}{dt} \propto \frac{dX}{dt}$$

And, in a fashion analogous to the utilization of other substrates, we could define a proportionality constant:

$$-\frac{dO}{dt} = \frac{1}{Y_{X/O}}\frac{dX}{dt}$$

where $Y_{X/O}$ is the oxygen yield coefficient (g cells formed/g oxygen consumed).

However, oxygen is continuously supplied. So, its utilization is not related directly to a change in oxygen concentration in the vessel. Instead, an oxygen uptake rate (OUR) is defined:

$$OUR = \frac{1}{Y_{X/O}} \frac{dX}{dt}$$

Note:

$$OUR = \frac{X}{Y_{X/O}} \frac{1}{X} \frac{dX}{dt}$$

$$Q_O = OUR = \frac{\mu X}{Y_{X/O}}$$ (mass O_2/L·h)

Specific Oxygen Uptake Rate = $q_O = \frac{OUR}{X}$

$$q_O = \frac{\mu}{Y_{X/O}}$$ (mass O_2/mass cells·h)

2. Cutting Off the Oxygen Supply

To illustrate the importance of oxygen, consider what would happen if the oxygen supply were shut off…

Conditions: Organism with:

$\mu = 0.5\ h^{-1}$

$X = 4.0\ g/L$

$Y_{X/O} = 0.75\ g\ cells/g\ O_2$

Calculation:

$$OUR = \frac{\mu X}{Y_{X/O}} = \frac{(0.5\ h^{-1})(4.0\ g\ cells/L)}{(0.75\ g\ cells/g\ O_2)}$$

$$OUR = \frac{\mu X}{Y_{X/O}} = \frac{(0.5\ h^{-1})(4.0\ g\ cells/L)}{(0.75\ g\ cells/g\ O_2)}$$

$$= 2.67\ g\ O_2/L \cdot h \quad = 0.74\ mg\ O_2/L \cdot s$$

If the vessel is saturated with oxygen in equilibrium with air then $c^{l}_{O2} \sim 7.0$ mg/L

Time to use up all the oxygen in the vessel $= \frac{7.0\ mg\ O_2/L}{0.74\ mg\ O_2/L \cdot s} =$ 9.5 s

Note: Fermentation media contains salts and proteins which decrease the solubility of oxygen.

3. Other Oxygen Sinks

Oxygen, like other substrates, is also consumed for cell maintenance and for product formation. That is,

$$-\left.\frac{dO}{dt}\right|_{Total} = -\left.\frac{dO}{dt}\right|_{Maint} - \left.\frac{dO}{dt}\right|_{Cells} - \left.\frac{dO}{dt}\right|_{Products}$$

$$OUR = m_O X + \frac{\mu X}{Y_{X/O}} + \frac{Q_P}{Y_{P/O}}$$

Usually, $m_O X$ is small

m_O typically 0.02 – 0.10 g/g·h

Using numbers from §2:

$$m_O X = (0.02 – 0.10 \text{ g/g·h})(4.0 \text{ g/L})$$

$$m_O X = 0.08 – 0.40 \text{ g/L·h}$$

Compared to $\dfrac{\mu X}{Y_{X/O}} = 2.67 \text{ g } O_2\text{/L·h}$

In this case, maintenance accounts for 3-13% of OUR.

Maintenance is less important the faster the cells grow.

…and $\frac{Q_P}{Y_{P/O}}$ is very small

(rarely does oxygen directly generate a product)

so $OUR = \cancel{m_O X} + \frac{\mu X}{Y_{X/O}} + \cancel{\frac{Q_P}{Y_{P/O}}}$

4. Oxygen Transfer Rate

Oxygen Transfer Rate (OTR) is the rate at which oxygen is supplied on a volumetric basis. That is, it is the oxygen transferred from the oxygen-containing bubbles to the bulk liquid.

OTR is related to the flux of oxygen across the boundary separating the two phases (Φ_O). Recall that this flux is proportional to the driving force for mass transfer:

$$\Phi_O = K_L (c^*_{O2} - c^l_{O2})$$

where K_L is the overall mass transfer coefficient

Since oxygen is sparingly soluble in water, the mass transfer is liquid-phase controlled, and

$$K_L \approx k_L$$

So, flux is described by

$$\Phi_O = k_L \left(c^*_{O2} - c^l_{O2}\right)$$

The oxygen transfer rate is related to flux by

$$\text{OTR} = \frac{\text{Flux} \times \text{Surface Area of Bubbles}}{\text{Volume of Liquid}}$$

The surface area divided by the volume (A/V) is typically incorporated into the mass transfer coefficient as the parameter “a”, so that the oxygen transfer rate becomes:

$$OTR = k_L a(c^*_{O2} - c^l_{O2})$$

$k_L a$ can have several different units…

h^{-1}

mmol/L·h·atm

$k_L a$ (mmol/L·h·atm) = 0.861 $k_L a$ (h^{-1}) at 25°C and 1 atm

Note: The maximum OTR occurs when the liquid phase oxygen concentration (c^{l}_{O2}) is zero. Such a situation would occur when all oxygen entering the bulk solution is rapidly consumed.

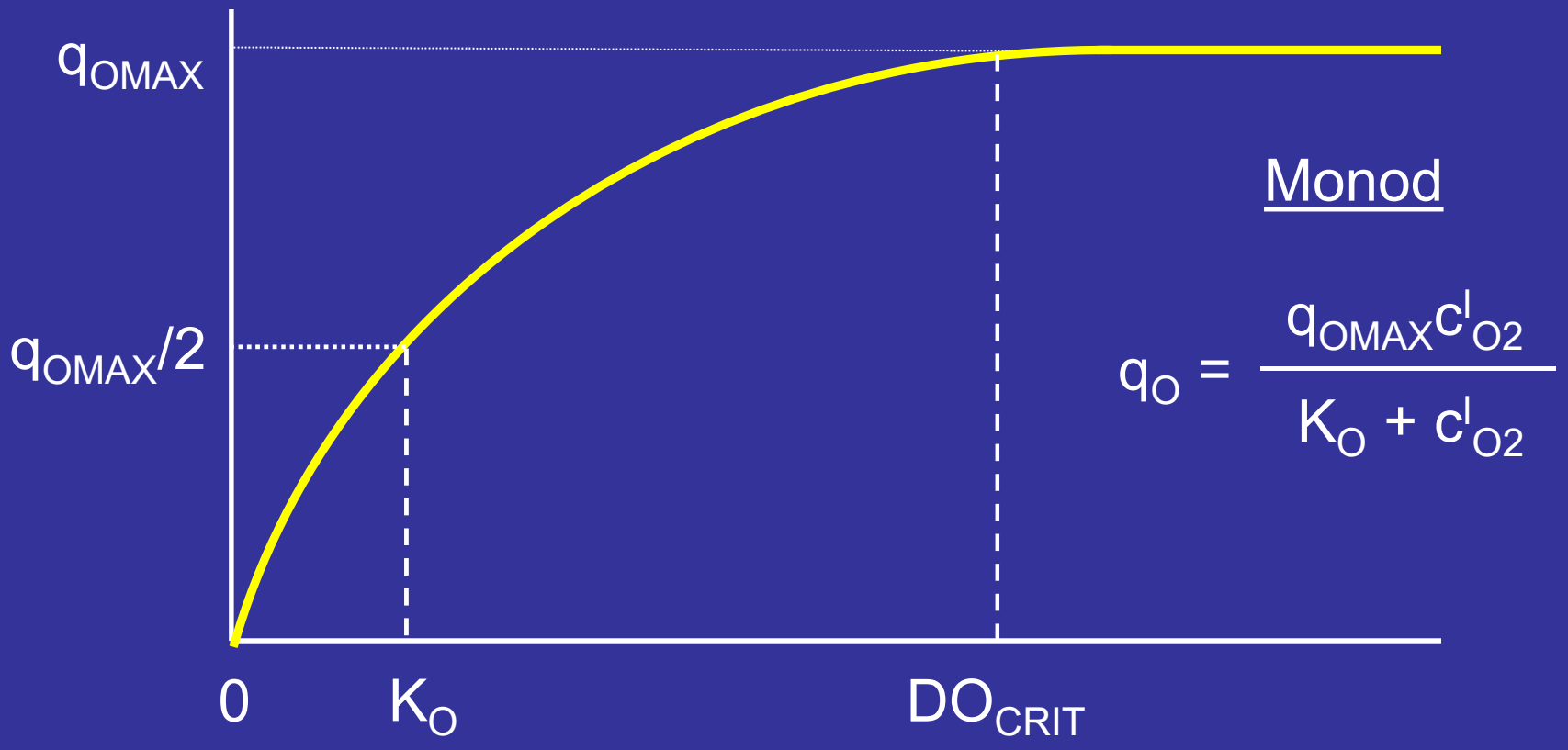
$$OTR_{MAX} = k_L a \, (c^{*}_{O2})$$

If OUR is much smaller than OTR_{MAX}, then $c^{l}_{O2} \approx c^{*}_{O2}$, and the rate of oxygen consumed by the cells is limited by microbial metabolism (or the number of cells).

If OUR is equal to OTR_{MAX}, then $c^{l}_{O2} \approx 0$, and the rate of oxygen consumed by the cells is limited by our ability to supply oxygen. (OUR cannot exceed OTR_{MAX}… there isn't enough oxygen being supplied!)

5. Kinetics of Specific Oxygen Consumption

The specific oxygen consumption rate (q_O) follows a Monod-type saturation kinetics.



Recall that dissolved oxygen concentration is often given in terms of the percentage of saturation (dissolved oxygen, DO):

$$DO = \frac{c^{l}_{O2}}{c^{*}_{O2}} \times 100\%$$

Maximum biomass production may be achieved by maintaining the DO above a "critical value" DO_{CRIT}.

However, often the goal is to produce a product rather than produce cell mass. Cell starvation may be advantageous to the formation of certain products. Alternatively, a DO far greater than DO_{CRIT} may be necessary to stimulate product formation.

Organism	DO_{CRIT} (mg/L)
Azotobacter vinelandii	0.58
Penicillium chrysogenum	0.70
Aspergillus niger	0.66
Serratia marcescens	0.48

6. Effect of Oxygen Uptake on Batch Kinetics

At steady-state, find the dissolved oxygen concentration. At steady-state:

$$OUR = OTR$$

$$Q_O = k_L a(c^*_{O2} - c^l_{O2})$$

Need an expression for Q_O…

$$\frac{\mu X}{Y_{X/O}} = k_L a(c^*_{O2} - c^l_{O2})$$

Need an expression for μ…

We will use the 2-substrate (S & O) Monod model:

$$\frac{\mu_{MAX}\, S}{K_S + S} \frac{c^l_{O2}}{K_O + c^l_{O2}} \frac{X}{Y_{X/O}} = k_L a(c^*_{O2} - c^l_{O2})$$

Note: Sorry about the inconsistency…

S = substrate concentration

c^l_{O2} = oxygen concentration

Here, we are interested in oxygen, so we will let μ_S be equal to that portion of the specific growth rate that is not a function of c^l_{O2}:

$$\mu_S = \frac{\mu_{MAX}\, S}{K_S + S}$$

So,

$$\frac{(\mu_S c^l_{O2} X/Y_{X/O})}{K_O + c^l_{O2}} = k_L a(c^*_{O2} - c^l_{O2})$$

Remember, we are trying to determine c^l_{O2}

We actually have already solved this equation!

$$Da = \frac{\text{Maximum oxygen uptake rate}}{\text{Maximum oxygen transfer rate}}$$

$$Da = \frac{(\mu_S X/Y_{X/O})}{k_L a(c^*_{O2})}$$

$$Da = \frac{\mu_S X}{Y_{X/O} k_L a (c^*_{O2})}$$

Typically, units of X [g/L] while units of c^*_{O2} [mg/L]

Also, define some dimensionless groups…

$$y = \frac{c^l_{O2}}{c^*_{O2}}$$

$$\alpha = \frac{K_O}{c^*_{O2}}$$

Solution is...

$$y = \frac{\beta}{2}\left[-1 \pm \sqrt{(1 + [4\alpha/\beta^2\,])}\right]$$

β = Da + α - 1

Select sign so that y>0

When β = 0 $\quad\quad$ $y = \sqrt{\alpha}$

What happens?

…depends on amount of substrate available

At onset of fermentation: X is low

S is high

$\frac{\mu_S X}{Y_{X/O}}$ & OUR are low

$OTR = k_L a(c^*_{O2} - c^l_{O2})$ is low

So, $c^l_{O2} \approx c^*_{O2}$

No oxygen limitation

No carbon (i.e., substrate) limitation

As fermentation proceeds: X is increasing

S is decreasing

$$OUR = \underbrace{\frac{\mu_{MAX}\, S}{K_S + S}}_{\mu_S} \frac{c^l_{O2}}{K_O + c^l_{O2}} \frac{X}{Y_{X/O}}$$

Whether OUR increases or decreases depends on the relative value of S and X. Either S will reach zero before oxygen becomes limiting, or X will increase to the point that oxygen becomes limiting.

If S reaches zero while X is still relatively low, then $\mu_S = 0$ and OUR = 0….so, cell growth terminates. The process was never oxygen limited.

$$OUR = \underbrace{\frac{\mu_{MAX}\, S}{K_S + S}}_{\mu_S} \frac{c^l_{O2}}{K_O + c^l_{O2}} \frac{X}{Y_{X/O}}$$

If there are plenty of soluble nutrients (C, N, S, P, etc.) in the system, then X will increase while μ_S remains unchanged. In this case,

$$\frac{\mu_S X}{Y_{X/O}}$$ & OUR increase

$OTR = k_L a(c^*_{O2} - c^l_{O2})$ increases

c^l_{O2} must decrease

Eventually, the second term in the Monod model decreases to the point that the growth rate decreases

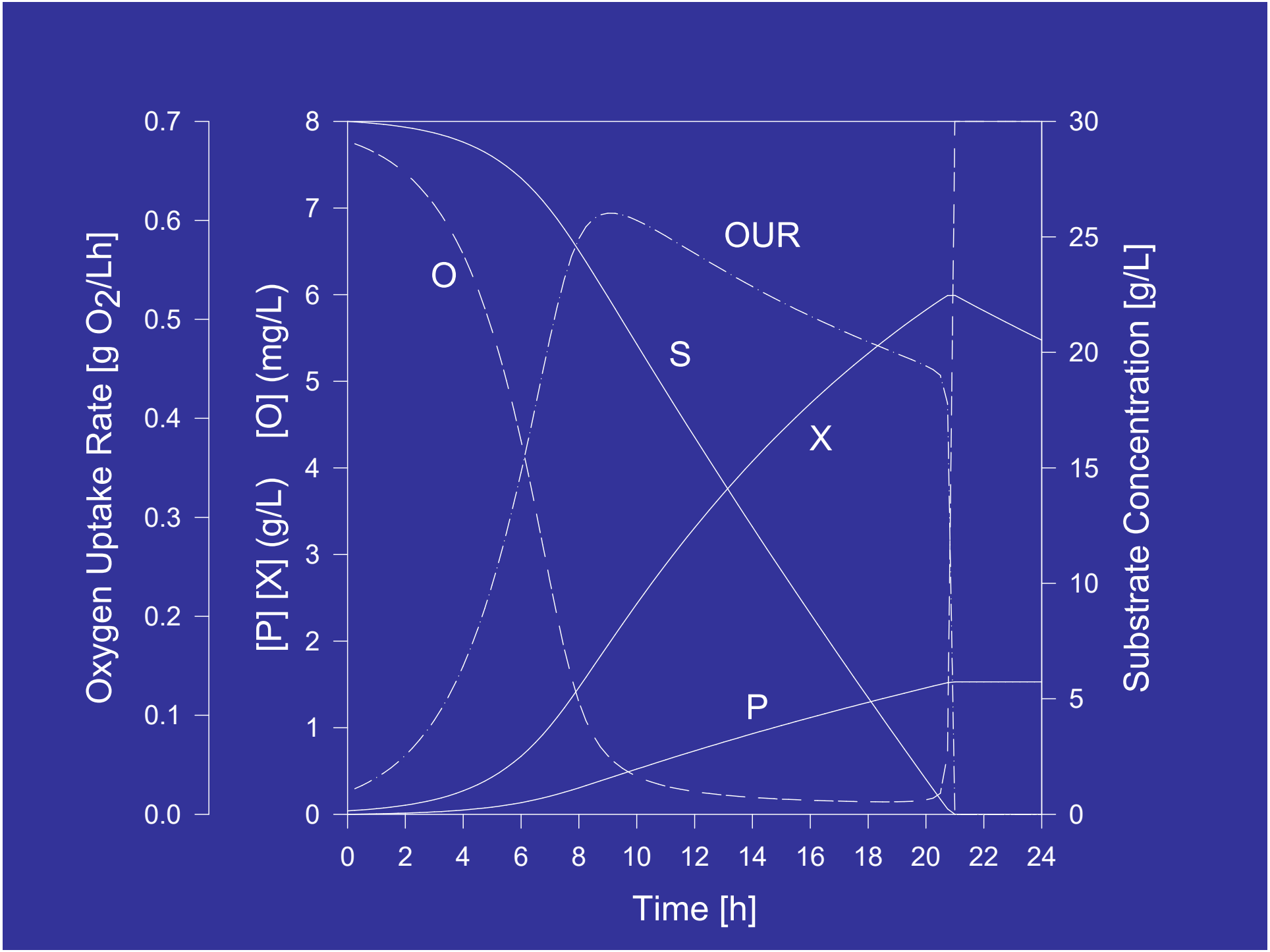
$$\mu = \frac{\mu_{MAX}\, S}{K_S + S} \cdot \frac{c^l_{O2}}{K_O + c^l_{O2}} \rightarrow 0$$

At that point, we will reach a maximum OUR.

c^l_{O2} will continue to decrease, but much more gradually.

We cannot supply oxygen fast enough to meet the maximum demand of the cells, but the cells do utilize what they are provided. The only way to increase OTR is by increasing $k_L a$ or c^*_{O2}.

We nearly achieve a steady-state dissolved oxygen concentration. Cells continue to be oxygen limited until substrate concentration decreases to the point that the substrate becomes limiting. At this point, the dissolved oxygen concentration will increase suddenly when the growth rate decreases to near zero, and the cells' oxygen demand decreases.


Oxygen Uptake Rate [g O2/Lh]
[P] [X] (g/L) [O] (mg/L)
Substrate Concentration [g/L]
Time [h]
O
OUR
S
X
P
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
0
2
4
6
8
10
12
14
16
18
20
22
24

7. Measurement of $k_L a$ and OUR

a. Sulfite Oxidation

(Cooper, Ind. Eng. Chem. 336, 504, 1944)

Relies on the rate of conversion of 0.5 M sodium sulfite to sodium sulfate in the presence of cobalt ion catalyst:

$$Na_2SO_3 + ½\ O_2 \rightarrow Na_2SO_4$$

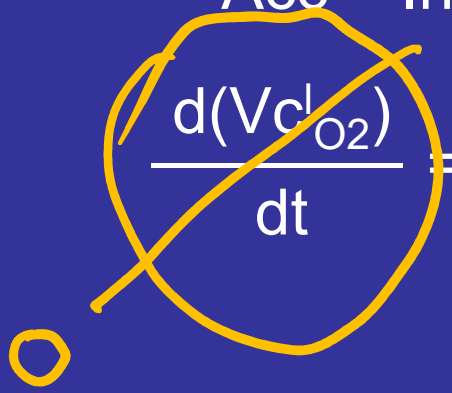
A sample is taken at a desired time…amount of unreacted sulfite is measured by titrating with iodine.

Advantages

- Does not rely on measurement of dissolved concentration ($c^{l}_{O2} = 0$). Don't need a probe.
- The rate of reaction is very fast. Thus, the O_2 never accumulates in the reactor, and the concentration of O_2 is zero throughout the experiment.

Mass Balance

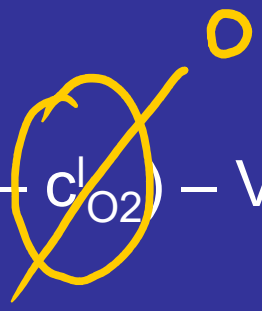
$$\text{Acc} = \text{In} - \text{Out} + \text{Gen}$$



$$\frac{d(Vc^{l}_{O2})}{dt} = k_L A(c^{*}_{O2} - c^{l}_{O2}) - VR_{O2}$$

R_{O2} = molar rate of reaction mol/Lh (measured)

Mass Balance…



$$0 = k_L A(c^*_{O2} - c^l_{O2}) - VR_{O2}$$

$$R_{O2} = k_L a(c^*_{O2})$$

Note that from stoichiometry…

$$R_{O2} = \tfrac{1}{2} R_{sulfite}$$

Solving for $k_L a$:

$$k_L a = \frac{\tfrac{1}{2} R_{sulfite}}{c^*_{O2}}$$

Disadvantages

- Expensive to fill large reactor with sulfite.
- The reactor must be consistent in physical and chemical parameters (pH, temperature, catalyst concentration).
- Cannot make measurement in presence of cells. Also, rheology of sodium sulfite is unlike cell culture!

b. Static Gassing Out
(Wise, J. Gen. Microbiol. 5, 167-177, 1951)

Scrub the solution free of oxygen by sparging with pure nitrogen. This deoxygenated liquid is then agitated and at the beginning of the experiment ("time equals zero"), oxygenation is commenced.

The dissolved oxygen concentration is measured with time.

Mass Balance

$$\text{Acc} = \text{In} - \text{Out} + \text{Gen}$$

$$\frac{d(Vc^{l}_{O2})}{dt} = \Phi_{O2}A = k_L A(c^{*}_{O2} - c^{l}_{O2})$$

$$\frac{dc^{l}_{O2}}{dt} = k_L a(c^{*}_{O2} - c^{l}_{O2})$$

$$\int_{0}^{c^{l}_{O2}} \frac{dc^{l}_{O2}}{c^{*}_{O2} - c^{l}_{O2}} = \int_{0}^{t} k_L a \, dt$$

$$- \ln(c^{*}_{O2} - c^{l}_{O2}) + \ln (c^{*}_{O2}) = k_L a \, t$$

$$\ln(c^*_{O2} - c^l_{O2}) = - k_L a\, t + \ln (c^*_{O2})$$

Plot $\ln (c^*_{O2} - c^l_{O2})$ versus time t

Slope = $-k_L a$

Advantages

- Does not rely on a chemical reaction. Thus, can alter properties of the fluid freely (viscosity, density, surface tension, etc.)

Disadvantages

- Still can't use with living, aerobic cells (need to purge fluid with nitrogen)

c. Dynamic Gassing Out

(Taguchi and Humphrey, J. Ferm. Technol. 44, 881, 1966)

Method depends on repeating a two-step cycle:

1. Turn off aeration – the decrease in dissolved oxygen concentration gives a measure of the oxygen uptake rate (OUR).

2. Turn on aeration – the increase in dissolved oxygen concentration with time gives a measure of $k_L a$.

Mass Balance

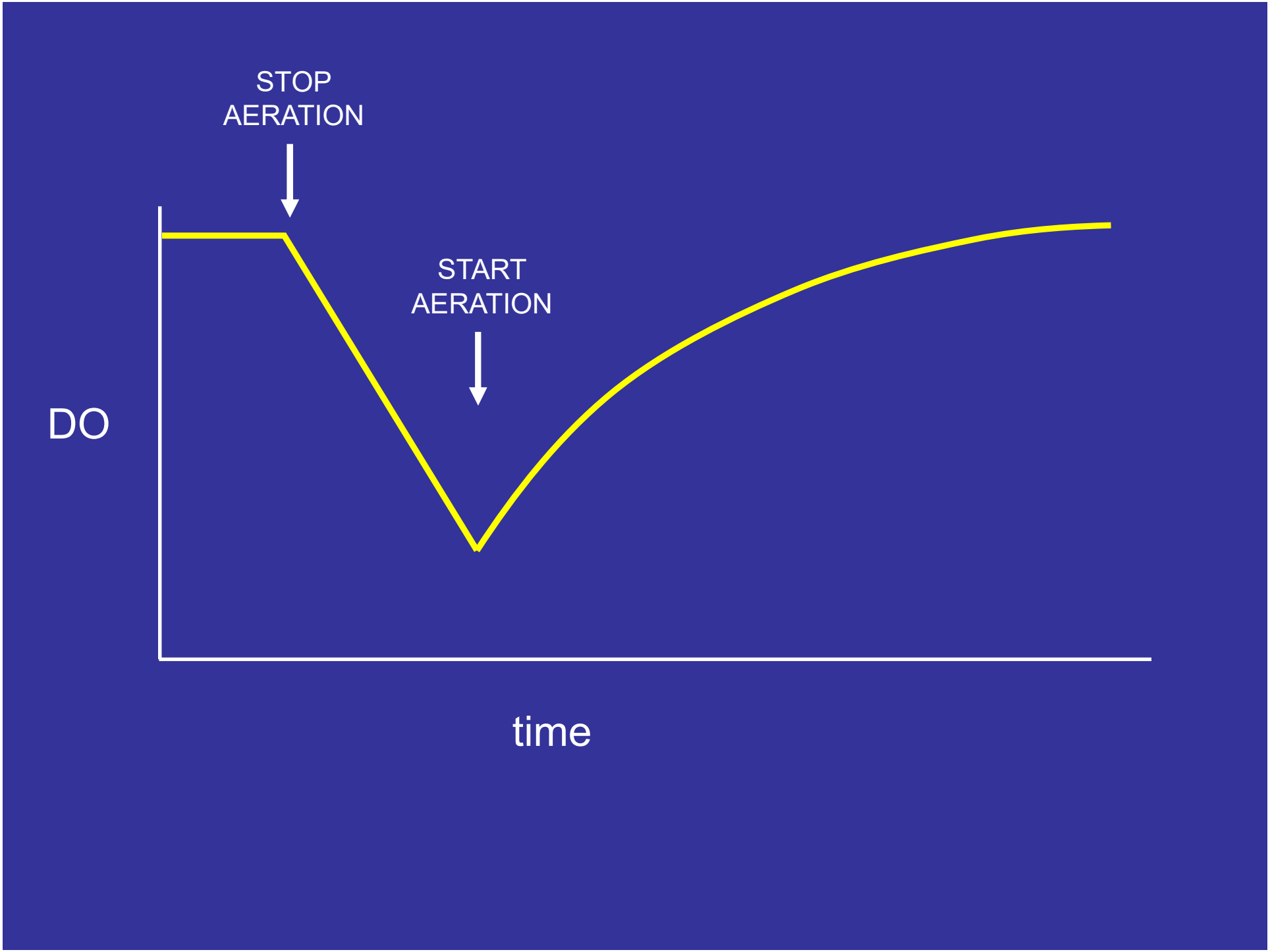
Acc = In – Out + Gen

$$\frac{d(Vc^{l}_{O2})}{dt} = k_L A(c^{*}_{O2} - c^{l}_{O2}) - q_O XV$$

$$\frac{dc^{l}_{O2}}{dt} = k_L a(c^{*}_{O2} - c^{l}_{O2}) - q_O X$$

Consumption of O_2 by cells

Think about the process going on in bioreactor in each part of the two-step cycle…


STOP
AERATION
START
AERATION
DO
time

1) During first phase (without aeration)

$$\frac{dc^{l}_{O2}}{dt} = k_L a(c^{*}_{O2} - c^{l}_{O2}) - q_O X$$

$$\frac{dc^{l}_{O2}}{dt} = - q_O X$$

$$q_O = - \frac{1}{X} \frac{dc^{l}_{O2}}{dt}$$ ..specific oxygen uptake rate

2) During second phase (with aeration)

$$\frac{dc^{l}_{O2}}{dt} = k_L a(c^{*}_{O2} - c^{l}_{O2}) - q_O X$$

Solve for c^{l}_{O2} (without solving differential equation)…

$$c^{l}_{O2} = -\frac{1}{k_L a}\left[\frac{dc^{l}_{O2}}{dt} + q_O X\right] + c^{*}_{O2}$$

Plot $\left[\frac{dc^{l}_{O2}}{dt} + q_O X\right]$ versus c^{l}_{O2}

(x) (y)

t	c^l_{O2}	$\frac{dc^l_{O2}}{dt}$	$\frac{dc^l_{O2}}{dt} + q_O X$
X	X	X	X
X	X	X	X
X	X	X	X
X	X	X	X
X	X	X	X
X	X		
	(y)		(x)

Advantages

- Fast
- Measures $k_L a$ during the actual fermentation
- Measures q_O during the actual fermentation

Disadvantages

- Potentially limited DO range since you should not pass below DO_{CRIT} during cycle.
- Can't use for viscous system in which bubbles have extended residence time.

d. Direct Oxygen Measurement

Measure the amount of oxygen transferred into the solution in a set interval of time. Basically measure the parameters in the ideal gas law for the inlet (i) and outlet (o).

$$OTR = \frac{7.31 \times 10^5}{V_L}\left(\frac{Q_i P_i y_i}{T_i} - \frac{Q_o P_o y_o}{T_o}\right)$$

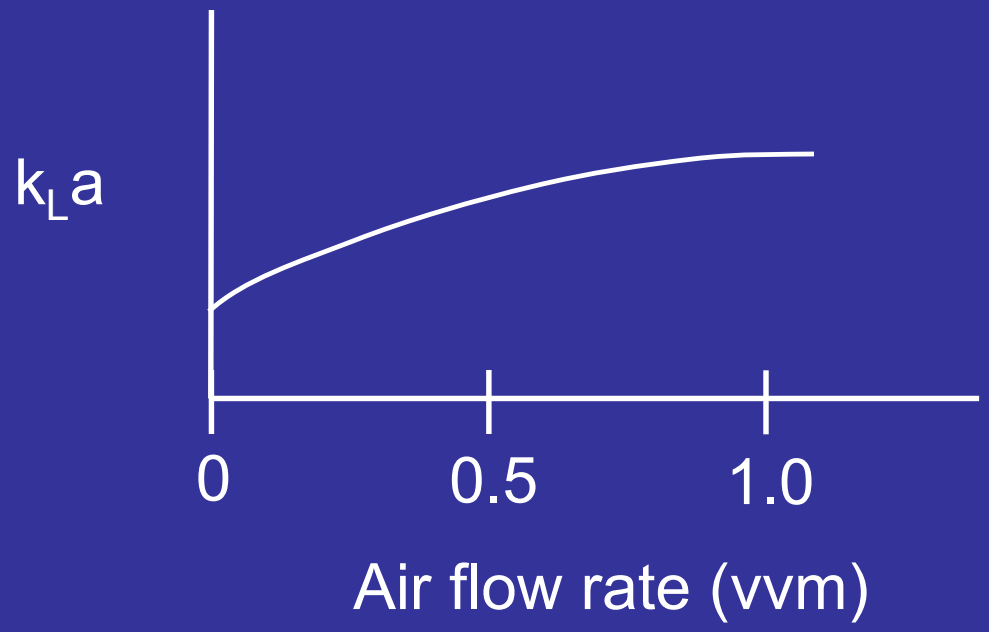
Q = volumetric air flow rate (L/min)
P = pressure (atm)
y = mole fraction
T = temperature (K)
V_L = liquid volume (L)
OTR (mmol/Lh)

7.31×10^5 → comes from ideal gas law…

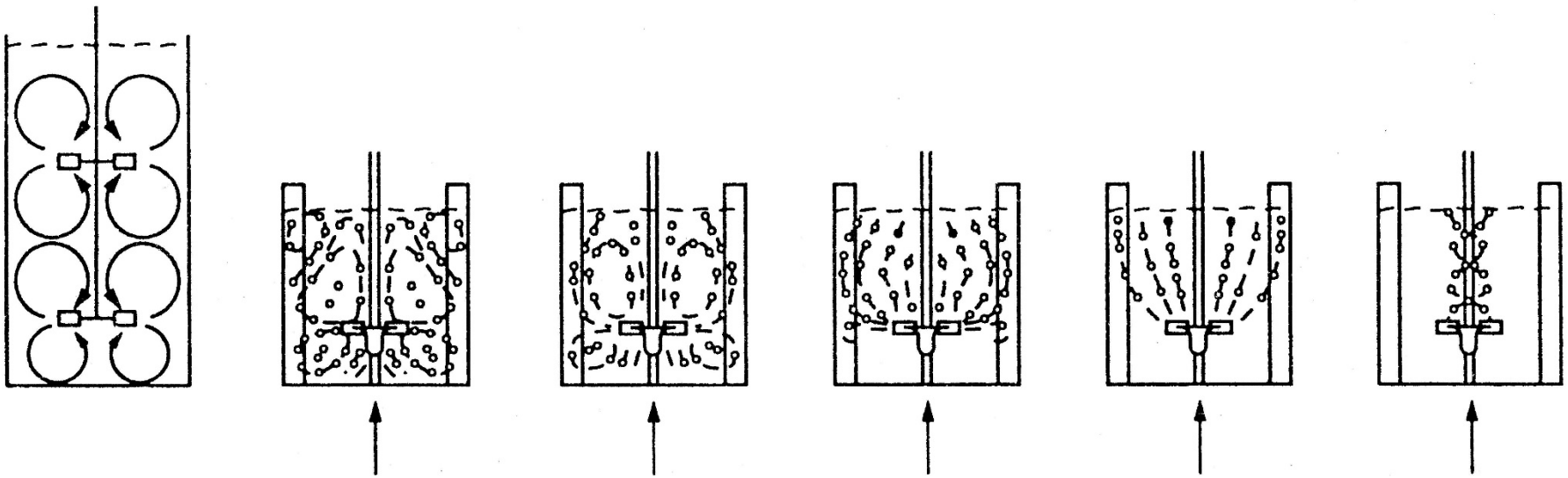
= 60x1000/0.0820575

8. Factors affecting OTR and k_La (see correlations in §J)

a. Air flow rate



If the impeller is unable to disperse the incoming air (because of its high velocity), flooding occurs. This condition means an inappropriate combination of air flow rate and agitation. See Figure….



Increasing Gas Flow Rate →

b. Agitation (more later…)

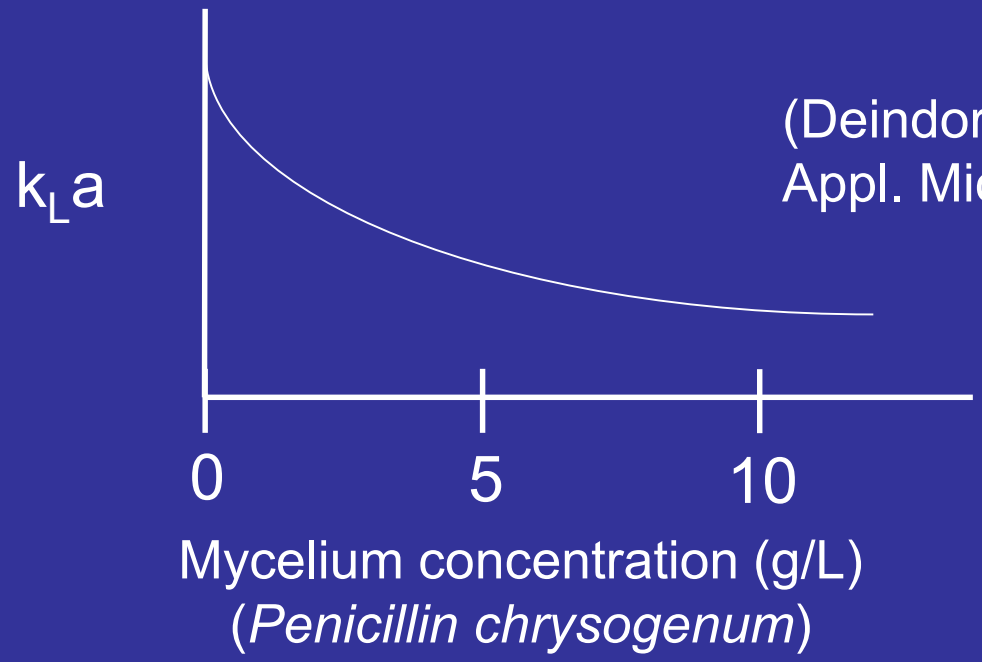
Agitation assists oxygen transfer in several ways:

- Agitation increases the surface area for transfer by dispersing air and forming smaller bubbles.
- Agitation delays the escape of air bubbles from the liquid.
- Agitation prevents coalescence of air bubbles.
- Agitation decreases the thickness of the liquid film layer at the gas-liquid interface.

c. Viscosity

$$k_L a \propto \frac{1}{\sqrt{\mu}}$$

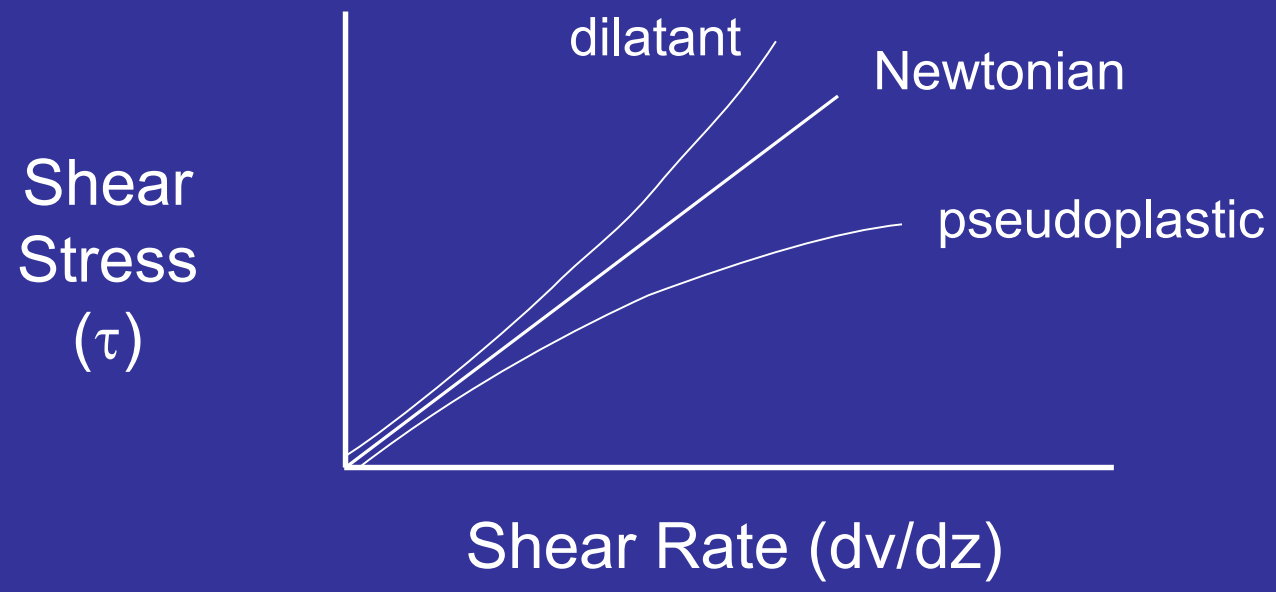
(Buckland et al., 1988)



(Deindorfer & Gaden, 1955,
Appl. Microbiol., 3, 253-257)

Most fermentations become more viscous as time progresses and cell density increases.

Many fermentations become pseudoplastic (non-Newtonian) as time progresses.



d. Air enrichment

$$OTR = k_L a(c^*_{O2} - c^l_{O2})$$

Increasing concentration of oxygen in air proportionally increases the equilibrium liquid phase oxygen concentration (c^*_{O2}).

Special membranes are available which have a higher N_2 permeability than O_2, and hence selectively remove N_2 from air. This technology has improved in the last 10-20 years to point that it may be economic to use enriched air (35-45% O_2).