

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:



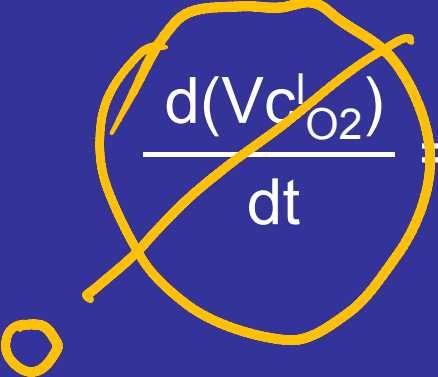
A sample is taken at a desired time...amount of unreacted sulfite is measured by titrating with iodine.

Advantages

- Does not rely on measurement of dissolved concentration ($c^l_{O_2} = 0$). 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

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


$$\frac{d(Vc^l_{O_2})}{dt} = k_L A (c^*_{O_2} - c^l_{O_2}) - VR_{O_2}$$

R_{O_2} = molar rate of reaction
mol/Lh (measured)

Mass Balance...

$$0 = k_L A (c_{O_2}^* - c_{O_2}) - VR_{O_2}$$

$$R_{O_2} = k_L a (c_{O_2}^*)$$

Note that from stoichiometry...

$$R_{O_2} = \frac{1}{2} R_{\text{sulfite}}$$

Solving for $k_L a$:

$$k_L a = \frac{\frac{1}{2} R_{\text{sulfite}}}{c_{O_2}^*}$$

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

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

$$\frac{d(Vc'_{O_2})}{dt} = \Phi_{O_2}A = k_L A (c^*_{O_2} - c'_{O_2})$$

$$\frac{dc'_{O_2}}{dt} = k_L a (c^*_{O_2} - c'_{O_2})$$

$$\int_0^{c'_{O_2}} \frac{dc'_{O_2}}{c^*_{O_2} - c'_{O_2}} = \int_0^t k_L a dt$$

$$-\ln(c^*_{O_2} - c'_{O_2}) + \ln(c^*_{O_2}) = k_L a t$$

$$\ln(c_{O_2}^* - c_{O_2}^l) = -k_L a t + \ln(c_{O_2}^*)$$

Plot $\ln(c_{O_2}^* - c_{O_2}^l)$ versus time t

$$\text{Slope} = -k_L a$$

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**

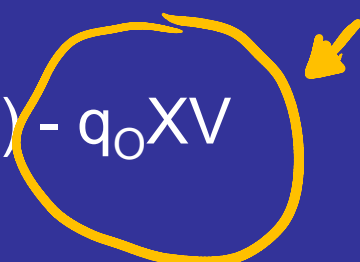
(Taguchi and Humphrey, J. Ferm. Technol.
44, 881, 1966)

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

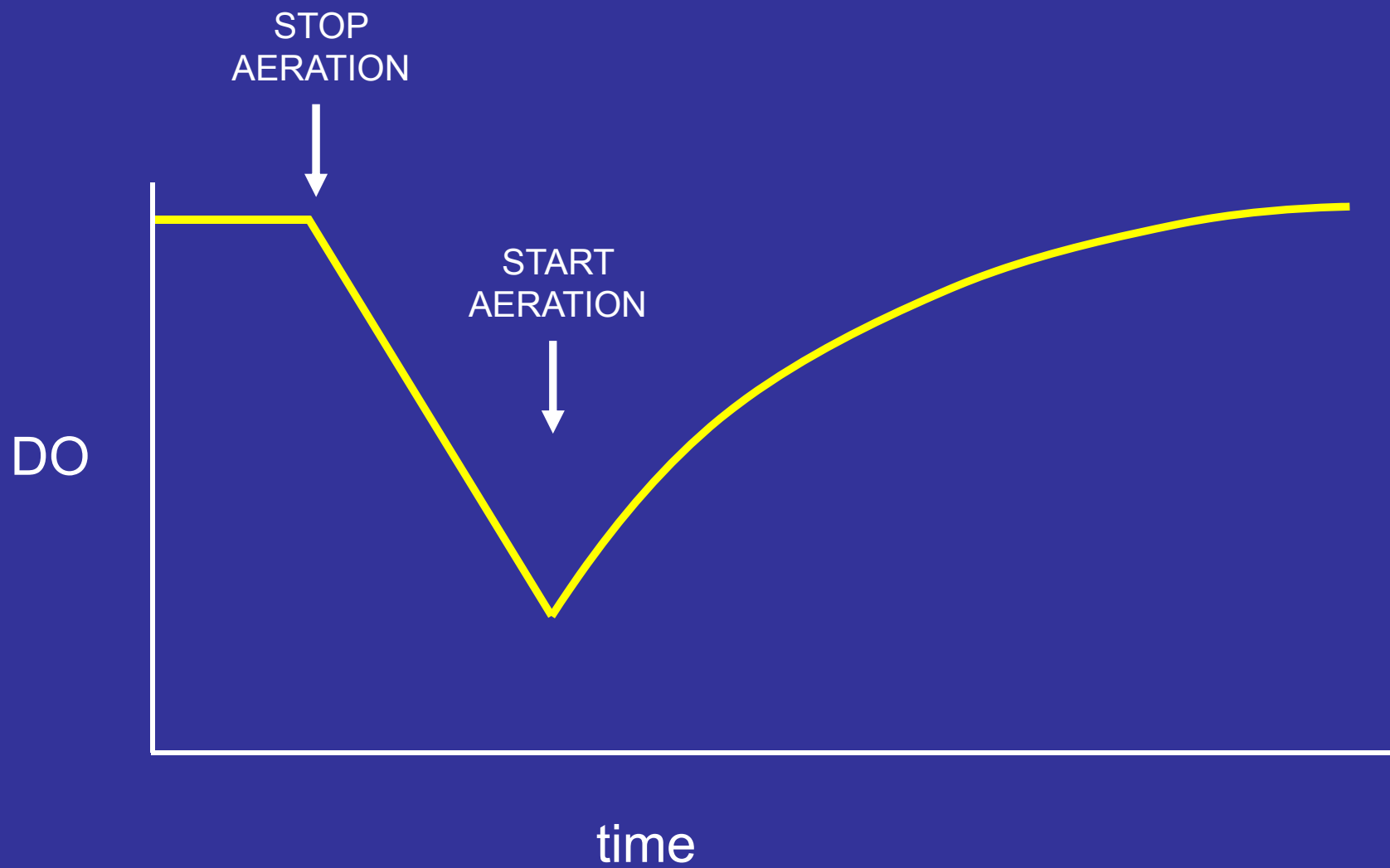
$$\text{Acc} = \text{In} - \text{Out} + \text{Gen}$$

$$\frac{d(Vc'_{O_2})}{dt} = k_L A (c^*_{O_2} - c'_{O_2}) - q_O XV$$


$$\frac{dc'_{O_2}}{dt} = k_L a (c^*_{O_2} - c'_{O_2}) - q_O X$$

Consumption
of O_2 by cells

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



1) During first phase (without aeration)

$$\frac{dc'_{O_2}}{dt} = k_L a (c^*_{O_2} - c'_{O_2}) - q_O X$$

$$\frac{dc'_{O_2}}{dt} = - q_O X$$

$$q_O = - \frac{1}{X} \frac{dc'_{O_2}}{dt} \quad \text{..specific oxygen uptake rate}$$

2) During second phase (with aeration)

$$\frac{dc^l_{O_2}}{dt} = k_L a (c^*_{O_2} - c^l_{O_2}) - q_O X$$

Solve for $c^l_{O_2}$ (without solving differential equation)...

$$c^l_{O_2} = - \frac{1}{k_L a} \left[\frac{dc^l_{O_2}}{dt} + q_O X \right] + c^*_{O_2}$$

Plot $\left[\frac{dc^l_{O_2}}{dt} + q_O X \right]$ versus $c^l_{O_2}$
(x) (y)

t	c'_{O_2}	$\frac{dc'_{O_2}}{dt}$	$\frac{dc'_{O_2}}{dt} + q_o X$
X	X	X	X
X	X	X	X
X	X	X	X
X	X	X	X
X	X	X	X
X	X	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).

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

Q = volumetric air flow rate (L/min)

P = pressure (atm)

y = mole fraction

T = temperature (K)

V_L = liquid volume (L)

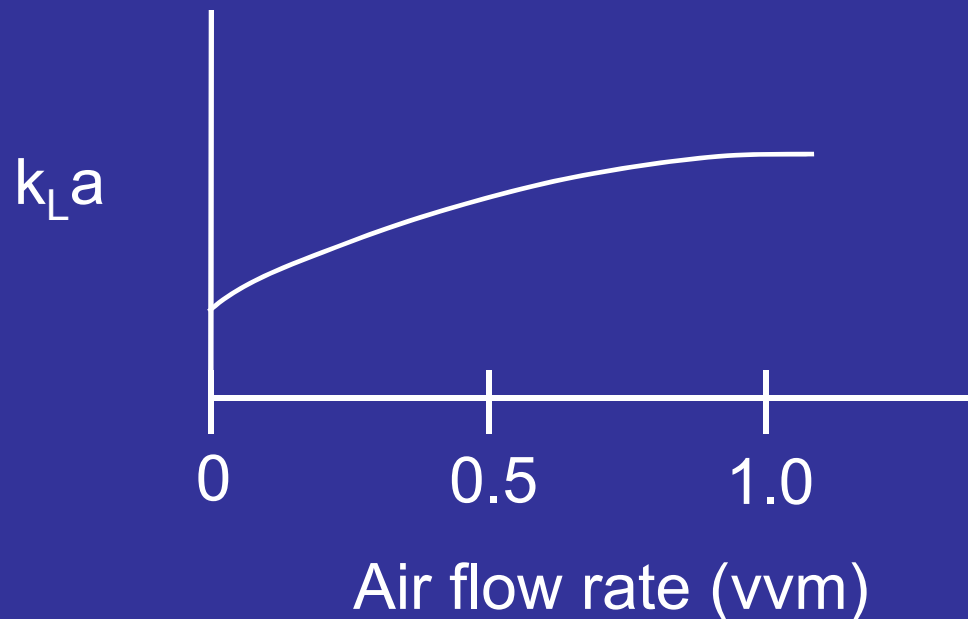
OTR (mmol/Lh)

$7.31 \times 10^5 \rightarrow$ comes from ideal gas law...

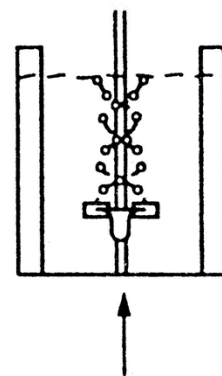
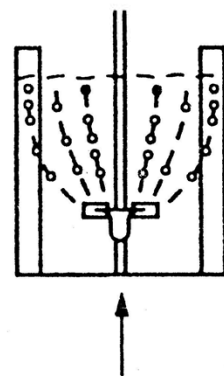
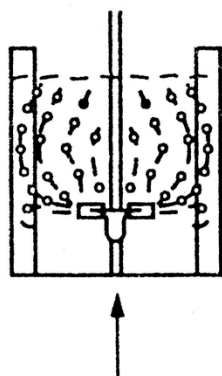
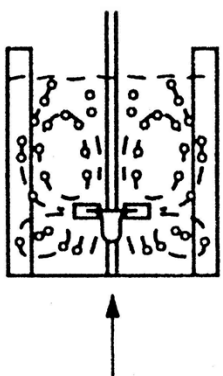
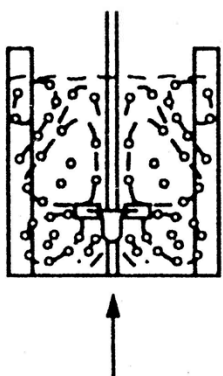
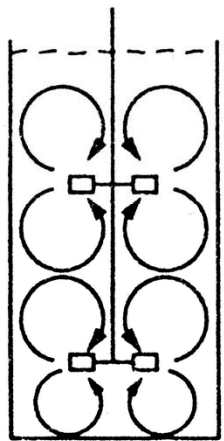
$$= 60 \times 1000 / 0.0820575$$

8. Factors affecting OTR and $k_L a$ (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 →

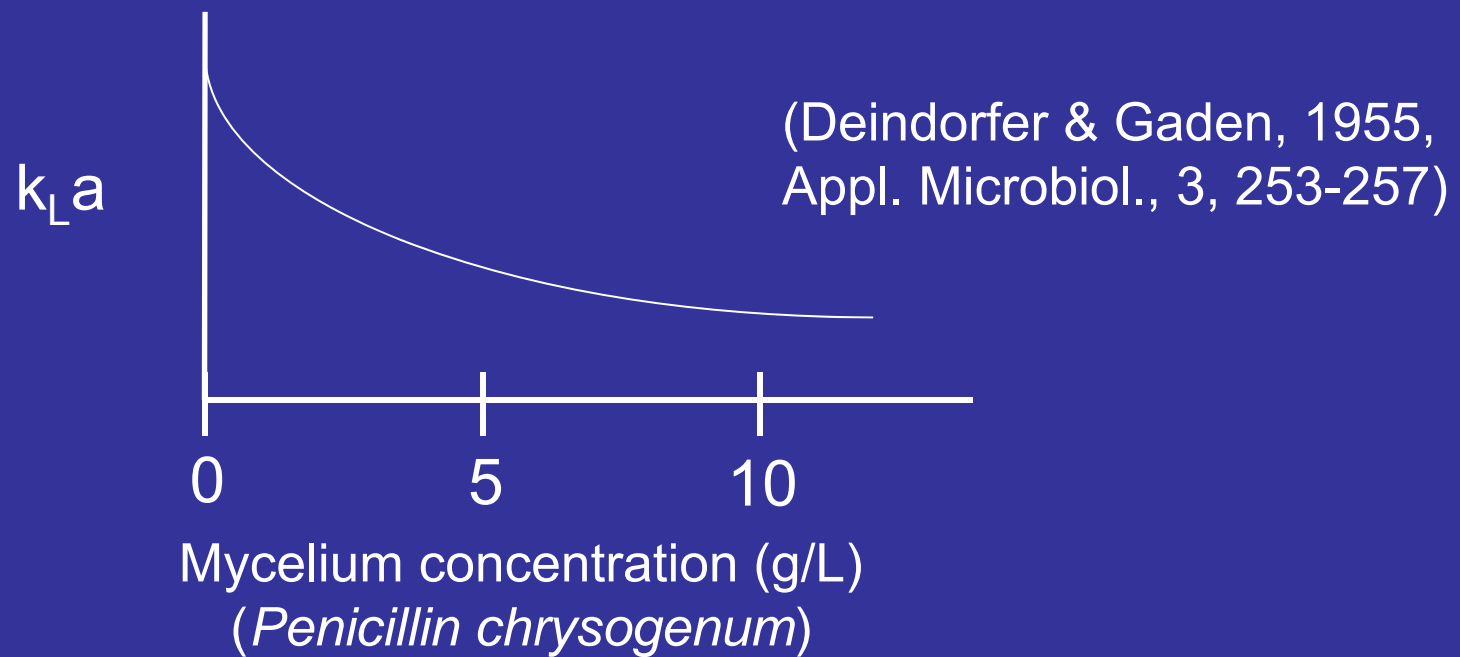
b. Agitation (more later...)

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.

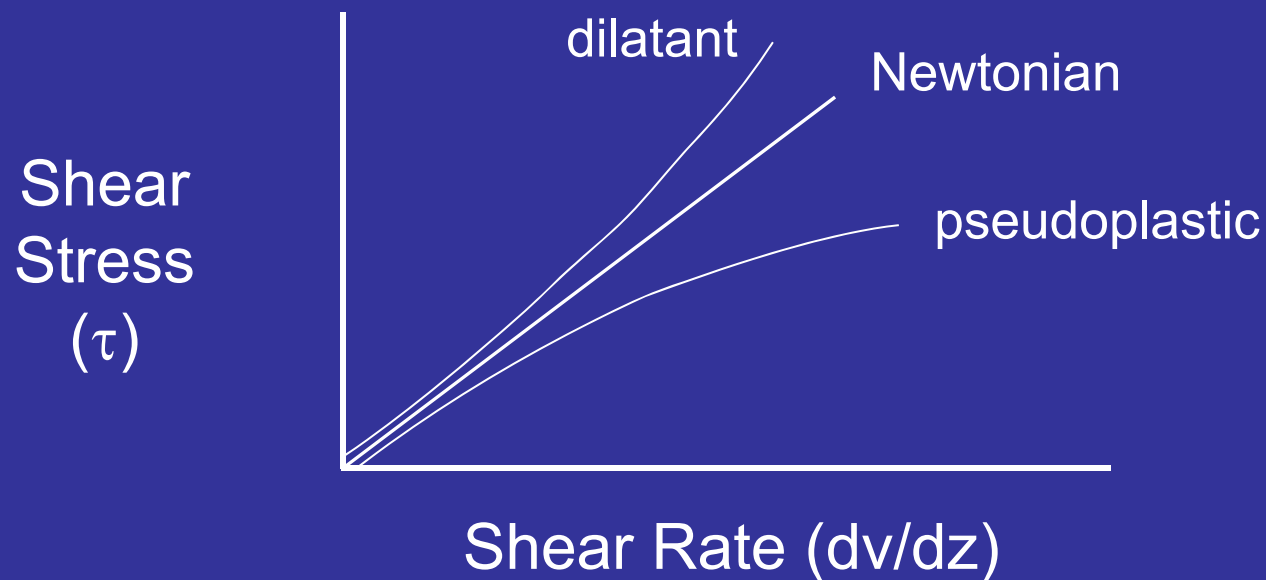
c. Viscosity

$$k_L a \propto \frac{1}{\sqrt{\mu}} \quad (\text{Buckland et al., 1988})$$



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

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



d. Air enrichment

$$\text{OTR} = k_L a (c_{\text{O}_2}^* - c_{\text{O}_2}^l)$$

Increasing concentration of oxygen in air proportionally increases the equilibrium liquid phase oxygen concentration ($c_{\text{O}_2}^*$).

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).