# Physical Processes Affecting Bioreactions



## Mass Transfer

- Gas ($O_2$; $CO_2$)
- Solutes (nutrients; extracellular products)
- Heat (not considered)

$$\text{Rate of Transfer} = \frac{\text{Driving Force}}{\text{Resistance}}$$

**The driving force is determined by the difference in potential between the transfer points.**



## Oxygen Transfer

**The requirement for oxygen:**

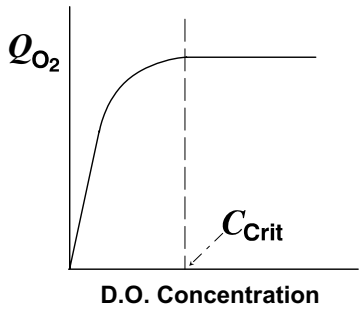
- $Y_{O/P}$ g $O_2$ consumed (g product formed)$^{-1}$
- $Y_{O/X}$ g $O_2$ consumed (g cells formed)$^{-1}$



## The Requirement for Oxygen 2

$Q_{O_2}$ Specific rate of $O_2$ uptake

$C_{crit}$ Critical level of dissolved oxygen

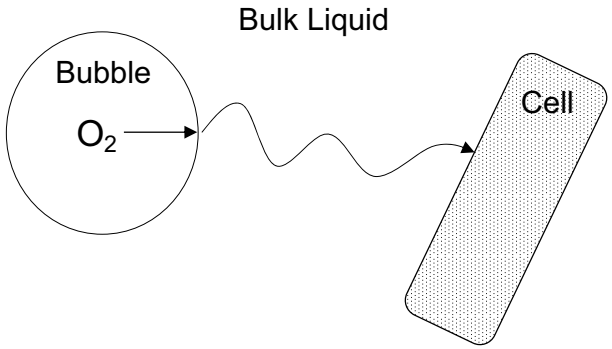



Maximum biomass production is achieved by maintaining

[D.O.] > $C_{crit}$

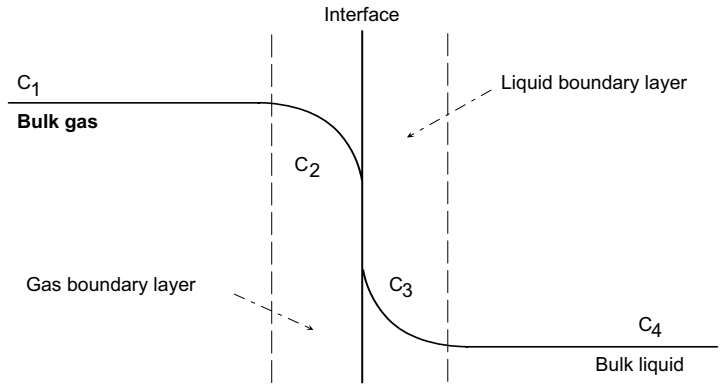
However,
where product ≠ biomass,
[D.O.] is controlled to be < or > than $C_{crit}$.

## Schematic Representation of Oxygen Transport From An Air Bubble to the Interior of a Microorganism






## The Twin Film Theory of Mass Transfer




**Concentrations at a gas/liquid interface**



## Oxygen Transfer Rate

**Rate of $O_2$ Transfer from air bubble to the liquid phase:**

$$\frac{\mathrm{d}C_L a}{\mathrm{d}t} = K_L a(C^* - C_L)$$

Where $C_L$ is the concentration of D.O. in the fermentation broth (*m*moles dm$^{-3}$).

$t$ is time (h).

$\frac{\mathrm{d}C_L}{\mathrm{d}t}$ is the change in [$O_2$] over a period of time, *i.e.* $O_2$ transfer rate (*m*moles $O_2$ dm$^{-3}$ h$^{-1}$).

$K_L$ is the mass transfer coefficient in liquid (cm h$^{-1}$). Reciprocal of resistance to OTR. is $\frac{D}{\delta}$ where D = molecular diffusivity of $O_2$ in a film; $\delta$ = film thickness.

$a$ is the gas/liquid interface area per liquid volume (cm$^2$ cm$^{-3}$).

$C^*$ is the saturated [D.O.] (*m*moles dm$^{-3}$). $(C^*-C_L)$ is the driving force across resistances.



## Volumetric Transfer Coefficient, $K_L a$

- $K_L$ and $a$ are difficult to measure separately.
- $K_L a$, the Volumetric Transfer Coefficient is used.
- **Is a measure of the aeration capacity of a bioreactor.** The larger the $K_L a$, the larger the aeration capacity.

## Volumetric Transfer Coefficient, $K_La$ 2

- **[D.O.] reflects the balance of $O_2$ supply and demand.**
- **If $K_La$ of a bioreactor < oxygen demand, then [D.O.] will decrease below $C_{crit}$.**
- **If $K_La$ > oxygen demand, then the converse is true and [D.O.] may be as high as 70-80% of saturation.**



## Determination of $K_La$

### 1. Sulphite Oxidation Technique

- **Cooper, Fernstrom & Miller, 1944.**
- $Na_2SO_3 + \frac{1}{2}O_2 \xrightarrow{Cu^{++}or\ Co^{++}} Na_2SO_4$
- **As $O_2$ enters solution, it is immediately consumed so that**

  **OTR = $K_La$. $C^*$ *i.e.* $C_L$ = 0**
- **Procedure**
  i. **Test solution is aerated and agitated as desired.**
  ii. **Samples are removed and excess iodine is added.**
  iii. **Back titration with standard sodium thiosulphate.**
  iv. **The volumes of thiosulphate are plotted against sample time. OTR is obtained from the slope of the plot.**



## Determination of $K_La$ 2

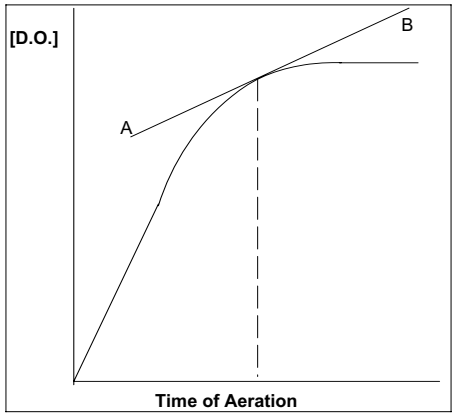
- **Advantages of the Sodium Sulphite Oxidation Technique.**
  i. **Simple. Accurate (under perfectly clean conditions).**
  ii. **Bulk liquid sampled** - minimises problems due the variation in conditions in a bioreactor vessel.
- **Disadvantages**
  i. **Time consuming.** May take up to 3 h per determination depending on aeration and agitation conditions.
  ii. **Inaccurate in the presence of surface-active contaminants** *e.g.* amino acids, proteins, fatty acids, lipids.
  iii. **Rheological differences between solutions of sodium sulphite real fermentation broths.**
  iv. **Prohibitive cost of sodium sulphite in industrial scale bioreactors.**



## Determination of $K_La$ 3

### 2. Gassing out techniques

- **The increase in [D.O.] is monitored during aeration and agitation.**




- **OTR will decrease as $C_L$ approaches $C^*$** (because driving force decreases).
- **OTR at any time = slope of tangent of the plot of [D.O.] against time of aeration.**
- **Method requires the initial reduction of [D.O.] to a low level.**
- **Two techniques of lowering.**
  * Static method.
  * Dynamic Method.

## Gassing out techniques 2

### a. Static Method

- Wise (1951)
- Gassing out with nitrogen.
- D.O. probe is used during aeration and agitation to monitor increase.
- $\frac{dC_La}{dt} = K_La(C^*-C_L)$

  Integration gives

  $\ln(C^* - C_L) = -K_La t$
- Thus, in the plot of $\ln(C^*-C_L)$ against time, a straight line is obtained, the slope of which is $-K_La$.



## Static Method 2

- Advantages of the Static Method of Gassing Out

  i. Rapid (normally taking 15 minutes).
  ii. Fermentation broth and/or biomass can be used.

- Disadvantage

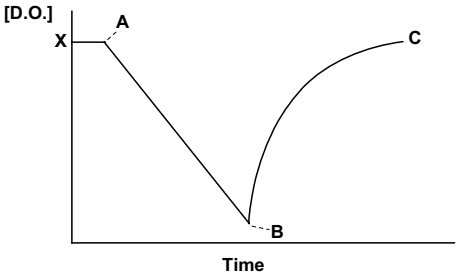
  i. Response time of membrane electrodes. May require the use of correction factors.
  ii. Membrane-less electrodes would require the use of electrolytes in the fermentation broth.
  Large amount of nitrogen required to deoxygenate industrial-sized bioreactors.
  iii. Point sampling. May not represent bulk liquid.



## Determination of $K_La$ 4

### b. Dynamic Method

- Taguchi & Humphrey (1966).
- Culture used to deoxygenate the test solution prior to aeration (AB).




- When aeration and agitation is resumed (B), [D.O.] increases until Point X is reached.
- Over BC,
  $\frac{dC_L}{dt} = K_La(C^* - C_L) - xQ_{O_2}$

  Where $X$ = [Biomass],
  $Q_{O_2}$ = Specific Respiration Rate
  ($m$Moles $O_2$ $g^{-1}$ Biomass $h^{-1}$)



## Dynamic Method 2

- Re-arranging,
  $$C_L = \frac{-1}{K_La}\left(\frac{dC_L}{dt} + xQ_{O_2}\right) + C^*$$

  Where, values of $\left(\frac{dC_L}{dt} + xQ_{O_2}\right)$ are obtained from tangents to the curve BC, at various values of $C_L$.
- Thus, a plot of $C_L$ against $\left(\frac{dC_L}{dt} + xQ_{O_2}\right)$ will yield a straight line, the slope of which will equal $-\frac{1}{K_La}$.

## Determination of $K_La$ 5

### 3. Oxygen Balance Technique

**Measures the amount of $O_2$ transferred into a solution in a set time interval.**

- $\text{OTR} = \frac{7.32 \times 10^5}{V_L}\left(\frac{Q_i P_i y_i}{T_i} - \frac{Q_o P_o y_o}{T_o}\right)$ Wang *et al.* (1979).

Where $V_L$ = the broth volume in $dm^3$,
$Q$ = flow rates in $dm^3\ min^{-1}$,
$P$ = Pressure at atm. absolute,
$T$ = temperature in $^oK$,
$y$ = mole fraction of $O_2$,
$i$ = inlet of bioreactor,
$o$ = outlet of bioreactor,
$7.32 \times 10^5$ is the conversion factor.

- $K_La$ can be determined from the relationship

  OTR = $K_La\,(C^*-C_L)$ where $C$ is measured using a membrane D.O. electrode.



## Oxygen Balance Technique 2

- **Advantages**

  i. **Measurement during a fermentation.**

- **Disadvantages**

  i. **Requires expensive equipment.**
  ii. **Point sampling.** Overcome by taking several samples at different points and using an average value.
  iii. ***C*** may not be the same throughout a large bioreactor.** Can be corrected for by assuming plug flow conditions and obtaining a logarithmic mean value.



# Fluid Rheology

**Newton's Law of Viscous Flow**

$$F = \mu A \frac{dv}{dx}$$

Where $F$ = viscous force opposing motion at the interface between two liquid layers.
$A$ = area
$\frac{dv}{dx}$ = velocity gradient across the layers.
$\mu$ = the fluid viscosity.

$$\mu = \frac{F/A}{dv/dx}$$

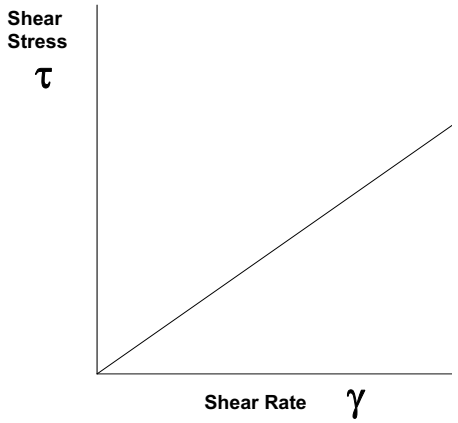
Where F/A = shear stress ($\tau$)
$dv/dx$ = shear rate ($\gamma$)

**Therefore, viscosity is the ratio of shear stress to shear rate.**



## Newton's Law of Viscous Flow 2

### 1. Newtonian Fluids

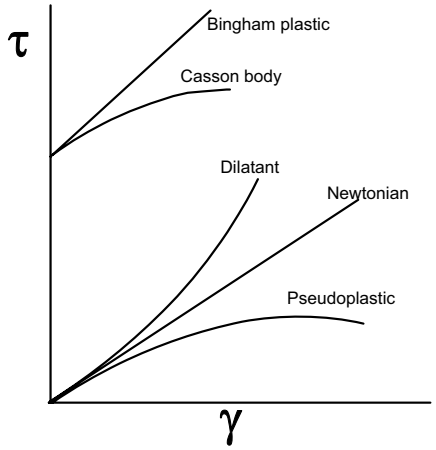



Rheogram of a Newtonian Fluid

- **The plot of $\tau$ against $\gamma$ of Newtonian fluids yields a straight line.**
- **Therefore, Newtonian fluids have constant viscosity regardless of shear rate *i.e.* $\mu$ will not vary with agitation rate.**

## Newton's Law of Viscous Flow 3

**2. Non-Newtonian Fluids**




**Rheogram of fluids of different rheological properties**

- **Newton's Law of Viscous Flow is not obeyed.**
- **The viscosity of non-Newtonian fluids varies depending on the shear rate and therefore agitation rate.**



# Factors Affecting $K_La$ In Bioreactors

## 1. Degree of agitation

### Agitation

a. **increases surface area for transfer by bubble formation.**

b. **increases bubble path length.**

c. **delays bubble coalescence.**

d. **decreases thickness of the liquid film at the gas/bubble interface by creating turbulence in the fermentation broth.**

**Agitation can be measured by the amount of power consumed in stirring the contents of a bioreactor.**



## Degree of agitation 2

**The relationship between $K_La$ and power consumption has been described in various ways:**

$$K_La = k\left(\frac{P_g}{V}\right)^{0.95} V_s^{0.67}$$ **Cooper *et al.*, 1944**

**Where** $P_g$ **= power absorption in an aerated system.**

$V$ **= liquid volume in the vessel.**

$V_s$ **= superficial air velocity *i.e.***

$$\frac{\text{Vol. air flow rate}}{\text{Cross-sectional area of the vesse}}$$

$k$ **= constant**

**Thus, $K_La$ is claimed to be almost directly proportional to the gassed power consumption per unit volume.**



## Degree of agitation 3

**However,**

- **The relationship proposed by Richards (1944) may depend on the size of the bioreactor (Bartholomew, 1960) as follows:**

| Scale | Value of exponent on $P_g/V$ |
|---|---|
| Laboratory | 0.95 |
| Pilot plant | 0.67 |
| Production plant | 0.50 |

- **Other variables have not be included in the relationship *e.g.* impeller speed and size, culture rheology**

**Thus,**

$$K_La \propto (P_g/V)^{0.4} V_s^{0.5} N^{0.5}$$ **Richards (1966)**

**where $N$ = impeller rotational speed**

# Degree of agitation 4

$$K_L a = k \frac{P_g^{0.33}}{V} V_s^{0.56}$$ Taguchi *et al.,* 1968

was proposed for pseudoplastic broths.

$$K_L a = k P_g^{0.46}$$ Steel and Maxon, 1962

was proposed for Bingham plastic rheology.

Therefore, while it is not possible to derive a relationship which is applicable to all situations, derivation may be possible within certain limits.

Quantitative relationships between power consumption and operating variables may be useful in:

- Estimating power requirements in bioreactor design.
- Providing similar degrees of power consumption (= agitation = $K_L a$) in vessels of different size.