SEMESTER 7
C15 APPLIED BIOTECHNOLOGY

Unit-IV Applications of Bioprocess Engineering: (10 Lectures)
Agitation and aeration: requirement in industrial processes, concept of volumetric oxygen transfer coefficient and its determination (Kla), Factors affecting Kla values; Uses of microbes in mineral beneficiation and oil recovery. Introduction to food technology; Elementary idea of canning and packaging, Sterilization and pasteurization of food products.

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- On successful completion of the course the students should have understood the basics of fermentation technology and learnt the concept of different metabolite production by microbes in industrial setup.

THEORY

Unit-I Microbial Cell Growth and Death Kinetics: (10 Lectures)
Screening and Improvement of industrially important microorganisms, Microbial Growth and Death Kinetics, Media for Industrial Fermentation, Air and Media Sterilization. IKS: Contribution of Indian Scientists.

Unit-II Operation and Control of Bioreactors: (10 Lectures)
Types of Fermentation Processes: Analysis of batch, fed-batch and continuous bioreactors, stability of microbial bioreactors, analysis of mixed populations, specialized bioreactors-pulsed,

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PRACTICALS

1. To plot Microbial growth curve for shake flask culturing using turbidity method.
2. Prepare a standard curve of reducing sugar by 3,5-Dinitrosalicylic acid method
3. To produce invertase enzyme and find its activity from Baker's Yeast
4. Preparation of standard curve of Ethanol
5. Quantitative estimation of ethanol produced during Yeast fermentation
6. Production of Penicillin and assaying its activity
7. To get familiarized with the lab scale fermenter (bench top fermenter)
8. To determine dissolved oxygen concentration in tap and aerated water.
9. To determine the volumetric transfer coefficient (K la)
10. Estimation of BOD in a given waste water sample
11. Centrifugation studies during settling of yeast cells.
12. Yeast cell disruption by mechanical methods.

SUGGESTED BOOKS

1. Bioprocess Engineering, Shular M &Kargi F, Prentice Hall
2. Biochemical Engineering Fundamentals, Bailey JE &Ollis DF
3. Bioprocess Engineering Principles, Doran, PM, Academic Press, California

Most industrial microbial processes are aerobic, and are mostly carried out in aqueous medium containing salts and organic subtances; usually these broths are viscous, showing a non-Newtonian behavior. In these processes, oxygen is an important nutrient that is used by microorganisms for growth, maintenance and metabolite production, and scarcity of oxygen affects the process performance. Therefore, it is important to ensure an adequate delivery of oxygen from a gas stream to the culture broth. Consequently, accurate estimtion of the oxygen transfer rate (OTR) at different scales and under different operational conditions has a relevant role for the prediction of the metabolic pathway for both growth and production of any wished metabolite in the aerobic culture it is of critical importance for the selection, design and scale-up of bioreactors.

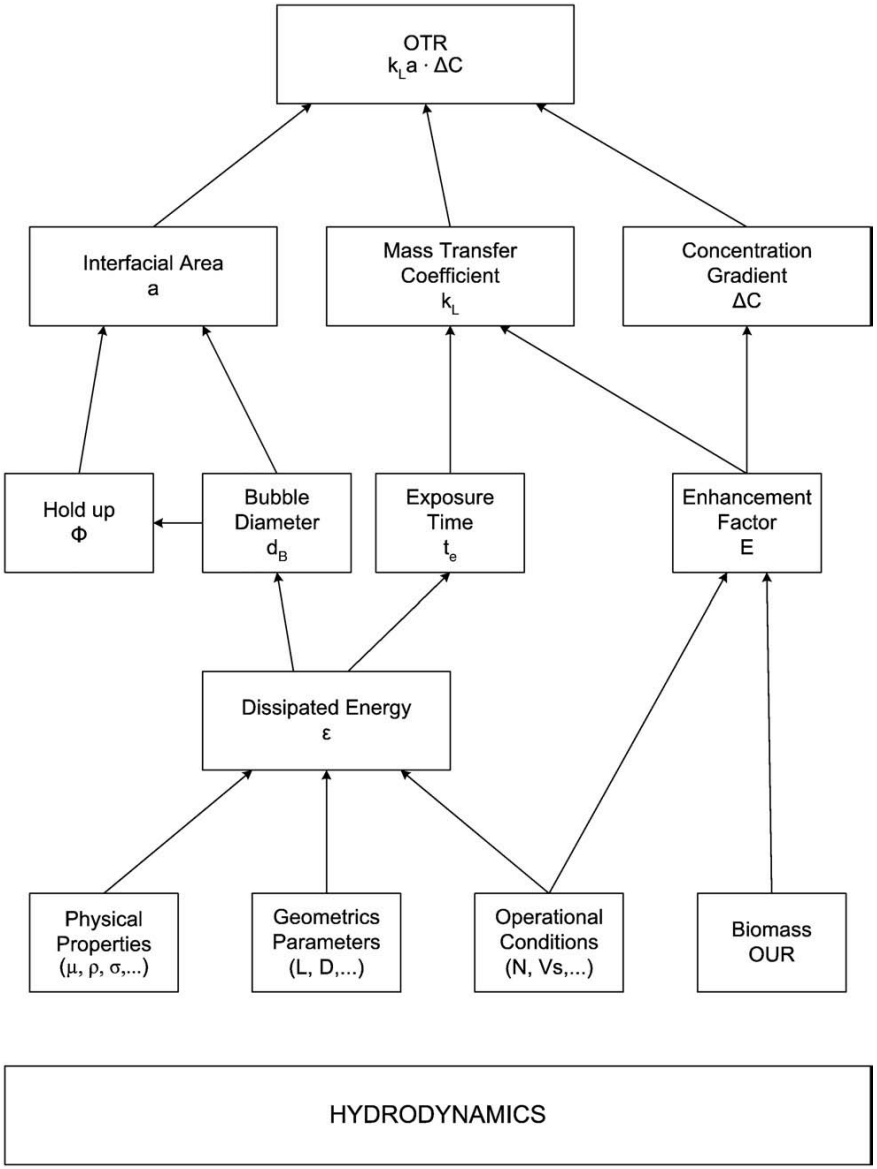
What is kLa?

The volumetric mass transfer coefficient (kLa) is a parameter that determines the rate at which a gaseous compound (e.g.O2orCO2) can transfer between the gas phase and the liquid phase. The 'kL' represents the rate of molecular diffusion through the gas-liquid interface and the 'a' represents the area of this interface per liquid volume. Together, these parameters form the kLa, which is often described in units per time and can be utilized to define the limits of any given bioprocess with respect to gas transfer (OTRmax).

The maximum value of the concentration gradient is limited due to the low solubility of most gases associated to aerobic fermentation, notably oxygen. Therefore, the maximum mass transfer rate from the gas to the liquid in the bioreactor can be estimated by the product $kLa \cdot C*$, being $C*$ the saturation concentration in the liquid phase.

The bioprocesses are usually conducted under previously optimized conditions (temperature, pH, pressure, mixing, concentrations ofbiomass and nutrients), with an operational mode previously chosen (batch, fed-batch, resting cell, continuous).

The overall mass transfer rate OTR is influenced by a high number of parameters (physical properties of gas and liquid, operational conditions, geometrical parameters of the bioreactor) and also by the presence of biomass, that is, the consumption of oxygen by the cells.



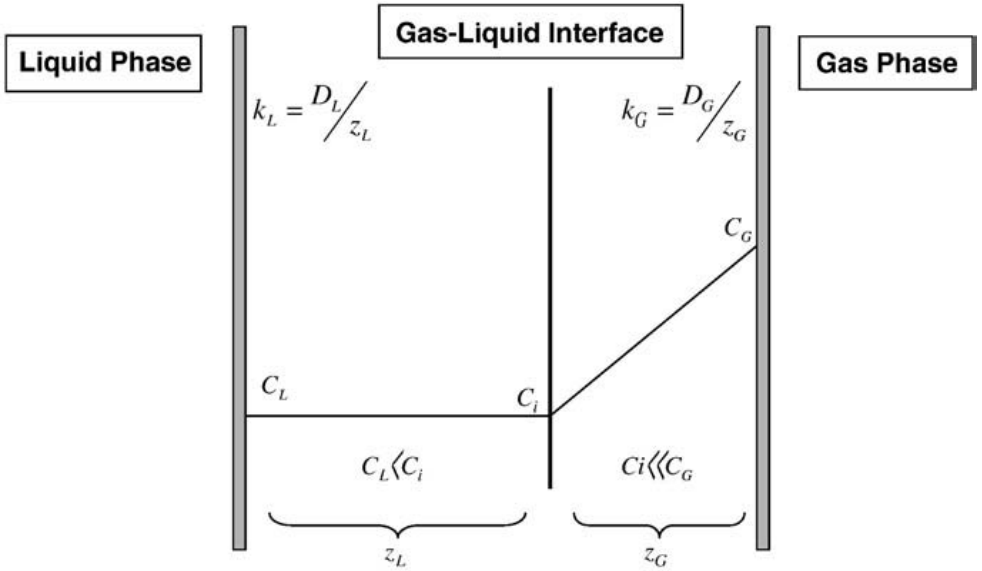
The simplest theory on gas–liquid mass transfer is the two film model and usually the gas–liquid mass transfer rate is modeled according to this theory.
the flux through each film as the product of the driving force by the mass transfer coefficient, according to:

$$J^0 = k_G \cdot (p_G - p_i) = k_L \cdot (C_i - C_L)$$

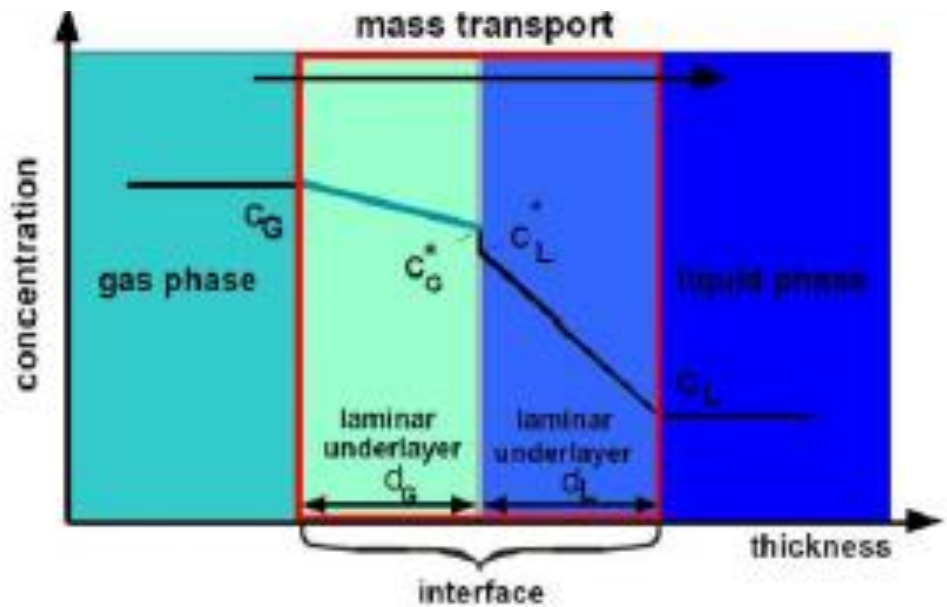
being J^0 the molar flux of oxygen (mol·m−2 s−1) through the gas–liquid interface; k_G and k_L, are the local mass transfer coefficients; p_G is the oxygen partial pressure in the gas bubble; and C_L, the dissolved oxygen concentration in the bulk liquid; index i refers to values at the gas–liquid interface.
Since the interfacial concentrations are not directly measurable and considering the overall mass transfer coefficient, it can be rewritten:

$$J^0 = K_G.(p_G - p^*) = K_L\ (C^* - C_L)$$

where p∗ is the oxygen pressure in equilibrium with liquid phase;
C* is the oxygen saturation concentration in the bulk liquid in equilibrium to the bulk gas phase, according to Henry's law (p∗=HC*); K_G and K_L are the overall mass transfer coefficients.



$$1/K_L = 1/H \cdot k_G + 1/k_L$$



Experimental determination of the volumetric mass transfer coefficient (kLa)

The determination of kLa in bioreactors is essential in order to establish aeration efficiency and to quantify the effects of the operating variables on the provision of dissolved oxygen. A number of methods have been developed to determine the oxygen transfer rate in bioreactors . Some of these methods are applied to others compounds as well, but others are specific for oxygen transfer measurement. When selecting a method, several factors must be taken into account :

i. the aeration and homogenization systems used,

ii. the bioreactor type and its mechanical design,

iii. the composition of the fermentation medium and

iv. the possible effect of the presence of microorganism.

The mass balance for the dissolved oxygen in the well-mixed liquid phase can be established as:

$$\frac{dC}{dt} = OTR - OUR$$

where dC/dt is the accumulation oxygen rate in the liquid phase, OTR represents the oxygen transfer rate from the gas to the liquid, described according to Eq. (4), and OUR is the oxygen uptake rate by the microorganisms;

Measuring methods of kLa without biological consumption of oxygen In the absence of biomass or with non-respiring cells, when biochemical reactions do not take place, OUR=0. In this case, Eq. can be simplified to:

$$\frac{dC}{dt} = k_L a \; C^* - C$$

For aerobic fermentation the maximum value of the concentration gradient is limited due to the low solubility of oxygen.

Therefore, the maximum mass transfer rate from the gas to the liquid in the bioreactor can be estimated by $k_L a \, .C_i$

as C_L^* is the saturation concentration in the liquid phase.

$$\int_{C_{L1}}^{C_{L2}} \frac{1}{\left(C_L^* - C_L\right)} dC = k_L a \int_0^t dt$$

$$\ln \frac{C^* - C_2}{C^* - C_1} = -k_L a \cdot (t_2 - t_1)$$

Hence a plot of of

$$\ln\left(\frac{C_L^* - C_{L1}}{C_L^* - C_{L2}}\right)$$

vs. t should result in a straight line of slope $k_L a$.

Measurement method		$k_La.10^2$ [s^{-1}]	Assay time	Scale applied	Assumptions/Drawbacks
Chemical	Sulfite oxidation	0 - 0,3	Hours	Laboratory scale	The rate of reaction is assumed to be zero order in sulfite. Alteration of driving force, diffusion coefficient, and coalescence properties; complex kinetics boundary layer reduction. This method is fairly labor intensive.
	Absorption of CO_2	0 - 0,1	Minutes	Laboratory scale	Assumptions about kinetic reaction must be made. Possible alteration of the driving force. Change of the coalescence behavior.
	Dynamic measure of pH	0 -0 ,03	Half an hour	Any scale	Assumptions about kinetic reaction must be made. Salt addition does not alter the mass transfer rate of CO_2.
	Hydrazine oxidation	0-0,5	Minutes	Pilot plant	Hydrazine does not accumulate. No chemical enhancement.
	Bio-oxidation of catechol	< 0,8	Minutes	< 100ml	Available of oxidative enzyme; limited to small scales.

Physical	Dynamic	0 - 0,1	Minutes	>100 ml	A nonrespiring system can be employed to simulate the fermentation broth. The response time of the electrode, τ_r, is a critical parameter. Gassing time can be significant at larger scales
Biological	Biological Dynamic gassing out	0 - 0,1	Minutes	Any scale	High DO concentration is necessary. Nongassing period must be short and OUR independent of DO concentration. Invasive probes are necessary and response time must be considered. Hydrodynamic changes may disturb the microbial metabolism.
	Biological dynamic method with high OUR	0 - 0,1	Minutes	Any scale	OUR is independent from DO concentration. Invasive probes are necessary and response time must be considered
	Gas phase analysis	0 - 0,3	Hours	>100 ml	For large scales, the assumptions of well-mixed gas and liquid phase may not be valid. This method may not be the best choice in case of small bioreactors, where the difference between *F*in and *F*out may be very small because of the short contact time The accuracy depends on the precision of oxygen analyzer