BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE PILANI, PILANI CAMPUS
I Semester 2022 – 2023 : Comprehensive Examination
CHE F 421: Biochemical Engineering: 21.12.2022 : Total duration- 3 hour (9:00 AM - 12:00 PM)
Closed Book – 40 Marks + Open Book – 40 Marks

---

1. **Instructions: Make necessary assumption, wherever needed with proper justification.**
2. **Close Book and Open Book Question Paper is given together**
   Close book time : Blanks + 7 Questions - 90 Minutes and Open book – 4 Questions - 90 Minutes

CLOSE BOOK

Blanks [5]

1. Logistic growth model describes ___ **substrate based** growth.
2. Size of the gas bubbles and their **dispersion** throughout the column are critical to reactor performance.
3. Restriction of enzyme mobility in a fixed space is known as **enzyme immobilization.**
4. Micronutrients are nutrients required in less than __**10^{-4}**_Molar.
5. van der Waals forces and **hydrogen bonding** are responsible for the formation of ES complexes.

---

1. Answer the following questions. Enzymes [2+1+3 = 6]

a) Usually, immobilization results in a loss in enzyme activity and stability. However, in some cases, immobilization may cause an increase in enzyme activity and stability due to more favorable microenvironmental conditions. Why?

Because enzymes often have more than one functional site that can bind the surface, an immobilized enzyme preparation may be very heterogeneous. Even when binding does not alter enzyme structure, some enzyme can be bound with the active site oriented away from the substrate solution and toward the support surface, decreasing the access of the substrate to the enzyme. Retention of activity varies with the method used.

b) Uncompetitive inhibition occurs when the inhibitor does not bind to the free enzyme and instead binds to the already formed enzyme substrate complex and makes the complex inactive. What is the net effect of uncompetitive inhibition on Vm and Km values.

The net effect of uncompetitive inhibition is a reduction in both Vm and Km values.

c) The double-reciprocal (also known as the Lineweaver-Burk) plot is created by plotting the inverse initial velocity ($1/V_0$) as a function of the inverse of the substrate concentration (1/[S]). Explain the genesis of the concept of enzyme inhibition nature of plots by referring the graph.

The V_{max} can be accurately determined and thus K_M can also be determined with accuracy because a straight line is formed. The nature of kinetic experiments conducted and the information sought from reversible enzyme inhibition data are as follows:

1. **Monitor initial velocity "v" by varying the concentration of one substrate at different fixed concentrations of the inhibitor. If the enzyme reaction in question involves more than one substrate, then the concentration of all other substrates (other than the one whose concentration is varied) is fixed.**

2. **The v → [S] data are plotted in the double reciprocal format (double reciprocal plots) to generate a series of curves – one for each fixed concentration of the inhibitor. These patterns are analyzed qualitatively**
3. **Gradual changes in the slope and/or intercepts, as a function of the fixed inhibitor concentration, are noted. An inhibitor may affect the first-order rate constant (Vmax/KM which is reflected in slope changes) or the zero-order rate constant (Vmax as reflected in intercept changes) or both.**
4. **On quantitative analysis of slope and intercept changes, various kinetic constants including K_I values are evaluated. Depending upon whether the slope/intercept increases as a linear function of [I] or not, the inhibition may also be classified as linear, hyperbolic, or parabolic**

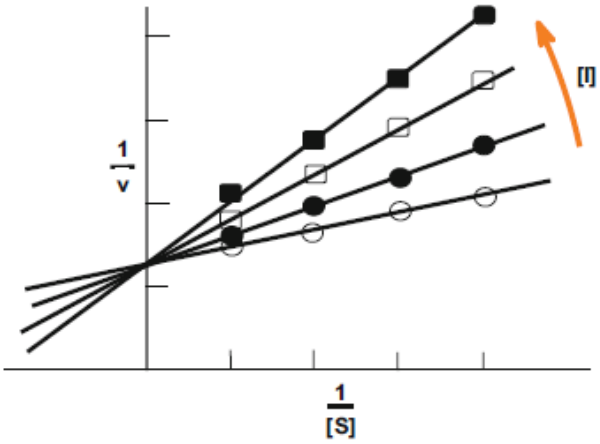

Fig. 22.2 Double reciprocal plots for the competitive inhibition of the enzyme with *S* as the varied substrate

2. Answer the following questions. Bacterial Stoichimetry [2 + 2 = 4]

a) The Kerbs cycle shows that anabolism is exactly reversal of catabolism, that may not be always accurate. Why ?

1. Some of the enzymes carrying out catabolism, may be different from those involved in conversions in reverse direction
   - This would be the case, when enzymes, which has resulted in breaking molecules may not be most efficient in putting them back together
   - And thus leading to evolution of different enzymes for carrying out 2 different processes

2. Also catabolic & anabolic processes, may take place in different places in cell

3. Also organisms need for building blocks, for synthesis is independent of substrate used for energy

- ***Thus number of enzymes, required for synthesis of a given cellular component may be different than number required for degradation, hence different pathways for catabolism & anabolism***

b) Porges et al (1956) suggested way for prediction of stoichiometry of bacterial reactions. Mention three basic requirements to predict the stoichiometry of bacterial reactions?

- Empirical formula for cells
- Framework for describing how the electron donor substrate is partitioned between energy generation and synthesis
- Means to relate the proportion of the electron donor substrate that is used to synthesize new biomass to the energy gained from catabolism and the energy needed for anabolism

3. Lithotrophic microorganisms are employed to oxidize ammonium in a wastewater to nitrate under aerobic conditions in order to reduce the oxygen consumption by nitrification in a receiving stream. If the concentration of ammonium in the wastewater, expressed as nitrogen, is 22 mg/L. Assume f_s equals 0.10 and that inorganic carbon is used for cell synthsis. The water – oxygen reaction is represented as ¼ O_2 + H^+ + e^- = ½ H_2O . The cell synthesis reaction is :

1/5 CO_2 + 1/20 NH_4^+ + 1/20 HCO_3^- + H^+ + e^- = 1/20 $C_5H_7O_2N$ + 9/20 H_2O

The ammonium – nitrate half reaction is : 1/8 NO_3^- + 5/4 H^+ + e^- = 1/8 NH_4^+ + 3/8 H_2O del G = – 35.11 kJ / electron equivalent. **[7]**

a) How much oxygen will be consumed for nitrification in the treatment of 1000 m^3 of wastewater ?
b) What mass of cells in kg dry weight will be produced ?
c) What will be the resulting concentration of nitrate – nitrogen in the treated water ?

4. Answer the following questions. Cell Growth. [1+2+3 = 6]

a) Why does the age of the inoculum culture has a strong effect on the length of lag phase ?

Older the inoculum culture ; the lag phase is prolonged.

b) Why does OUR becomes an important parameter for the industrial scale fermenters?

OUR discusses the oxygen uptake rate by microorganisms. It correlates the volumetric mass transfer coefficient, which is an important parameter while scaling up the reactor.

c) The chemostat is a widely-used apparatus in the study of microbial physiology and ecology. With a very logical steps and with all basic chemostat equations; prove that the specific growth rate is equal to the dilution rate.

5. From the graph of Monod Chemostat Model, explain the graph for dilution rate with substrate concentration and cell concentration by explaining the terms such as *D*max and *D*washout. **[4]**

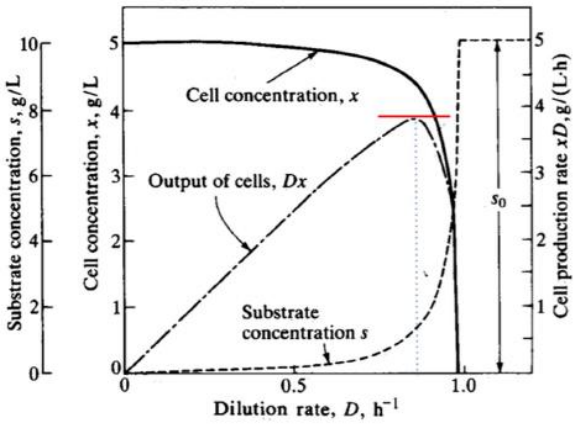

Figure 4.6. Sample graph illustrating the dependence of effluent concentration s, cell concentration x, and cell production rate xD, on continuous culture dilution rate D as computed from the Monod chemostat model. Modified from (Prpich, 2019).

- For very slow rate, D→ 0 ; S tends to 0 as all substrate is consumed but the value of X tends to be maximum
- From the given fig ; it is evident that X decreases with D but S increases with the increasing D
- Productivity (XD) increases with D
- But at a certain value of D ; XD reaches the maximum indicating the maximum value of D ,called as Dmax
- Above certain value of Dmax ; cell concentration reduces to 0 and corresponding D is known as Dwashout
- It is to be noted that near the washout ; reactor is very sensitive to variation in D ; leading to unsteady state situation
- Dmax can be evaluated as in the plot of XD vs D
- But mathematically, D max, dilution rate for maximum cell output can be evaluated by setting d(XD)/dD = 0 in the equation

6. Comment on that a bio separation process must combine with high selectivity and high throughput. **[3]**

7. A simple, batch fermentation of an aerobic bacterium grown on methanol gave the results as shown in the table. Calculate : **[5]**

a. Maximum growth rate (μ_{max})
b. Yield on substrate ($Y_{X/S}$)
c. Saturation constant (K_s)
d. Specific growth rate (μ_{net}) at t = 10 h

Time (h)	X (g/l)	S (g/l)
0	0.2	9.23
2	0.211	9.21
4	0.305	9.07
8	0.98	8.03
10	1.77	6.8
12	3.2	4.6
14	5.6	0.92
16	6.15	0.077
18	6.2	0

OPEN BOOK

1. Michaelis–Menten kinetics are used to describe intracellular reactions. Yet [E0] = [S0]. In in vitro batch reactors, the quasi-steady-state hypothesis does not hold for [E0] = [S0]. The rapid equilibrium assumption also will not hold. Explain why Michaelis–Menten kinetics and the quasi-steady-state approximation are still reasonable descriptions of intracellular enzyme reactions. **[5]**

2. Pseudomonas sp. has a doubling time, td = 2.5 hrs when grown on acetate. The saturation constant for this substrate is K_s =1.2 kg/m^3 and the yield coefficient, $Y_{x/s}$ = 0.42. For a chemostat operating with a feed of 35 kg/m^3 (S_o) of the estimate. Calculate the following : **[10]**

a) A cell concentration when the dilution rate. D is one half of D_{max} output
b) Substrate concentration when the dilution rate, D, is 0.75 D_{max}
c) Maximum dilution rate for maximum output of cells
d) Cell productivity, XD at D = 0.75 P_{max} output

3. Referring to the article 'Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview" Most industrial microbial processes are aerobic, and are mostly carried out in aqueous medium containing salts and organic substances; usually these broths are viscous, showing a non-Newtonian trend. In these processes, oxygen is an important nutrient that is utilized by microorganisms for growth, metabolite production, and scarcity of oxygen affects the process. **[3 + 3 + 4 = 10]**

a) Why does the accurate estimation of oxygen important at different scales for the overall process?
b) Bioprocesses involve *simultaneous transport and biochemical reactions of several chemical species*. What are the rate controlling steps in the overall biological processes?
c) The simplest theory on gas–liquid mass transfer is the two film model (Whitman,1923) and usually the gas–liquid mass transfer rate is modeled according to this theory (see Fig. 3), describing the flux through each film as the product of the driving force by the mass transfer coefficient, according to:

$$J^0 = k_G \cdot (p_G - p_i) = k_L \cdot (C_i - C_L) \qquad (1)$$

And kla estimation is explained by the given graph. Explain the concept of kla calculation for the design of overall aerobic bio-based scale up process.

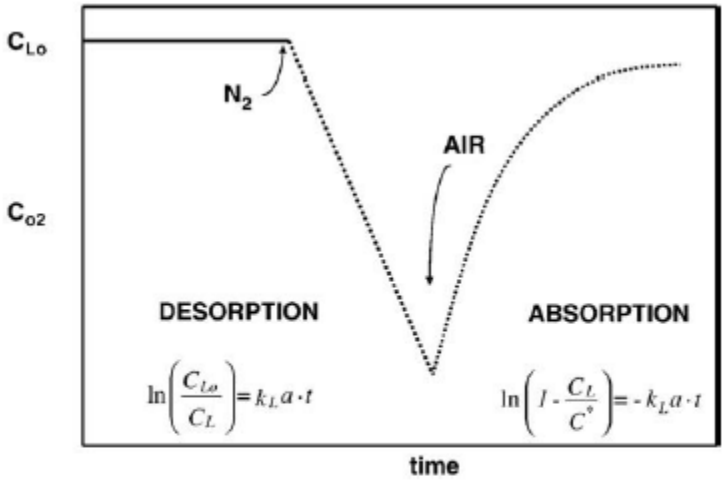

Fig. 4. Schematic description of the dynamic technique desorption–absorption of oxygen for inert condition measurements.

4. The growth of a microorganism with a wide range of substrate concentration was studied in a Chemostat of 1 m^3 volume with sterile feed. The flow rate (F) and the inlet concentration were varied in the range of 30 to 60 kg/m^3. The outlet concentration of substrate at the steady state of fermenter was measured. The data obtained : **[15]**

F m^3/h	0.2	0.25	0.35	0.50	0.70	0.80	0.50	0.60	0.70
So kg/m^3	30	30	30	30	30	30	60	60	60
S kg/m^3	0.5	0.7	1.1	1.60	3.3	10	30	22	15

A substrate inhibition model was suggested in the following form :

$$\mu = \mu_{\max}\left[1+\frac{Ks}{S}+\frac{S}{K_I}\right]$$

a) Determine the kinetic parameters of the Model (µmax, Ks and Ki) - At lesser substarte concentration and higher substrate concentration.
b) If Yx/s = 0.46 kg/kg ,what was the steady state concentration of cell mass (X) where the flow rate is 0.20 m3/h
c) Explain the fact that the substrate inhibition in a continuous culture may lead to instability ?