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Course Information

Course code and title	Bioreactor design and Analysis
Department	Biotechnology
Course type	Undergraduate
Program level	Year III
Contact hours	48 hours

Section 1:

Prerequisites: Bioprocess calculations.

Knowledge of Bioprocess Engineering and Biochemical Thermodynamics

Section 2:

Syllabus:

1. Fundamentals and classification of Bioreactors: bioreactor design- overview of bioreactor, its developments and types based on presence and absence of oxygen, type of process, type of microbe agent, mode of operation and method of generating microbes- mammalian cell culture, plant cell culture & environmental applications, Components of bioreactors and importance.
2. Bioreactor operations and scale up: Batch bioreactors- cell death in bioreactors, endogenous metabolism, Plug flow reactors, fed batch reactors, chemostat- Chemostat in series, comparison of productivity in batch and continuous culture, large scale commercial bioreactors- packed bed, fed batch reactors. Oxygen mass transfer in bioreactors – microbial oxygen demands; methods for the determination of mass transfer coefficients; mass transfer correlations. Scale up criteria for bioreactors. Power requirements in mixing under aerated and non aerated conditions.
3. Biochemical aspects of bioreactor analysis for cells and enzymatic reactions: Batch reactor- calculation of batch time, quantitative evaluation of batch processes, sources of non-ideality; continuous flow bioreactors- Gas-liquid mass transfer in bioreactors, concept of K_La and its measurement, aeration and agitation system in reactors . Design of immobilized enzyme reactors – packed bed, fluidized bed and membrane reactors mean residence time,

washout condition; recycle bioreactors; combination of bioreactors. Heat transfer in bioreactors.

Textbooks:

1. Michael L. Shuler and Fikret Kargi, Bioprocess Engineering: Basic Concepts, 2nd Edition, 2002, Prentice Hall.
2. Octave Levenspiel, Chemical Reaction Engineering, 3rd Edition, 1999, Wiley.

References:

1. James E. Bailey and David F. Ollis, Biochemical Engineering Fundamentals, 2nd Edition, 1986, McGraw Hill.
2. H. Scott Fogler, Elements of Chemical Reaction Engineering, 4th Edition, 2005, Prentice Hall
3. S. Liu, Bioprocess Engineering: Kinetics, Biosystems, Sustainability, and Reactor Design, 2013, Elsevier
4. James Lee. Biochemical Engineering

Section 3:

Course Learning Outcome (CLO):

CLO1: Apply various bioreactor designs and operational modes to increase productivity.

CLO2: Analyze biochemical aspects of bioreactor for various cells and enzymatic reactions.

Section 4:

Pedagogical Plan: Learning outcomes (LO): Mapped based on the syllabus.

Students should be able to...

- LO1: Compare kinetics and reaction rates for various bioreactor designs, based on operational mode and type of substrate.
- LO2: Differentiate and estimate productivity in commercial bioreactors- packed bed, fed batch reactors.
- LO3: Compare and derive various methods of effective mass-transfer correlation and mass-transfer coefficients.

- LO4: Apply concept of Gas-liquid mass transfer in bioreactors and measure $K_L a$ and power consumption in aeration and agitation system reactors.
- LO5: Analyze immobilization techniques in reactors and measure mass transfer resistance based on the design.

Section 5:

S.No.	LO	Brief Description
1	<p>LO1: Compare kinetics and reaction rates for various bioreactor designs, based on operational mode and type of substrate.</p>	<p>Teaching Learning Activity(TLA):</p> <ul style="list-style-type: none"> • Instructional strategy: Active Cooperative Learning (ACL) • Session 1 • Instructional material: Reading material on classification of Bioreactors- mode of operation <i>and various types of substrates.</i> • Activity/task: • Activity/Task 1 (15 minutes): Students should work in group to assemble the various components of bioreactor and based on operational modes compare various types and justify its application. • Activity/task 2 (15 minutes): Teacher select volunteers from two groups to share their work with the class. Each group provides feedback and debate the application of the bioreactor. • Activity 3 (15 minutes): Teacher provides feedback and presents the key points of the content. • Session 2: • Activity/Task 1 (15 minutes): Students should work in group to calculate reaction kinetics-How reaction rate is influenced by

<p>2.</p>	<p>LO2:Differentiateand estimate productivity in commercial bioreactors- packed bed, fed batch reactors.</p>	<p>reaction conditions such as substrate, product, and enzyme concentrations. Formula sheet is provided.</p> <ul style="list-style-type: none"> • Activity/task 2 (15 minutes): Teacher select volunteers from two groups to share their work with the class. Each group provides feedback on the answer and approach. • Activity 3 (15 minutes): Teacher provides feedback and presents the sample solution. <p>Teaching Learning Activity(TLA):</p> <ul style="list-style-type: none"> • Instructional strategy: Case Based Learning (CBL) • Instructional material: Reading material on commercial packed bed and fed batch Bioreactors with various types of substrates- microbes, mammalian cell and plant cells. • Activity/task: 1 (20 minutes): Students should work in group to solve a case/scenario, for example: <p>Discuss stages of Industrial production of Penicillin- Preparation, Innoculation, cultivation and downstream processing.</p> <ul style="list-style-type: none"> • Activity/task 2 (15 minutes): Teacher select volunteers from two groups to share their work with the class. Each group provides feedback. • Activity 3 (15 minutes): teacher provides feedback and presents
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<p>3</p>	<p>LO3: Compare and derive various methods of effective mass-transfer correlation and mass-transfer coefficients.</p>	<p>the key points of the content.</p> <ul style="list-style-type: none"> • Session 2: • Students work on numerical problems to estimate productivity in commercial bioreactors. <p>Teaching Learning Activity(TLA):</p> <ul style="list-style-type: none"> • Instructional strategy: Flip classroom • Instructional material: Lecture /reading material (PDF of PPT) on bioreactor analysis explaining each parameter for the kinetics of the bioreactors. Various sources of non-ideality; combination of bioreactors-Semi-continuous bioreactors including batch-fed and fed-batch. • Activity/Task 1 (15 minutes): In class activity- where students solve different types mathematical problems in group. • Activity/Task 2 (15 minutes): Students use the white board to share it with the class where teacher is the facilitator. • Activity 3 (15 minutes): Teacher takes questions from each group to address any queries from the material which was provided prior to the class.
<p>4</p>	<p>LO4: Apply $K_L a$ for scale up criterion, in gas-liquid mass transfer and power consumption in aeration and agitation system</p>	<p>Teaching Learning Activity(TLA):</p> <ul style="list-style-type: none"> • Instructional strategy: Use of

5.	<p>reactors.</p> <p>LO5: Analyze immobilization techniques in reactors and measure mass transfer resistance based on the design.</p>	<p>technology (TPACK)- Geo-algebra for visualization using graphs to see demonstrate correlation of K_{La} for scale up criterion and its relationship with power consumption.</p> <ul style="list-style-type: none"> • Instructional material: Various parameters on geoalgebra to measure K_{La}. Narrated power point presentation on aeration and agitation system. <p>Activity/task:</p> <ul style="list-style-type: none"> • Activity/Task 1: Students should work individually/group to on the problem (see formative assessment questions) using geoalgebra • Activity/task 2 (15 minutes): Teacher select volunteers from two groups to share their work with the class. Each group provides feedback. • Activity 3 (15 minutes): Teacher provides feedback and presents the key points of the content. <p>Teaching Learning Activity(TLA):</p> <ul style="list-style-type: none"> • Instructional strategy Use of technology (TPACK)-: Use of technology- use Matlab or other programming tool to compute equations for immobilisedenzyme reactors. • Instructional material: reading material or PPT on immobilized
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		<p>enzyme reactors- based on various techniques..</p> <p>Activity/task:</p> <ul style="list-style-type: none"> • Task 1: Students should work individually/group to compute equations using programming tool and demonstrate the reaction mechanism, represented by the Michaelis-Menten equation and how it varies by adding enzyme and substrate concentration (see formative assessment questions). • Activity/task 2 (15 minutes): Teacher select volunteers from two groups to share their work with the class. Each group provides feedback. • Activity 3 (15 minutes): Teacher provides feedback and presents the key points of the content.
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Section 6

Formative assessment: Sample questions for each LO:

LO1: Compare reaction rates, kinetics for bioreactor designs, based on various operational mode and substrate type.

Q1: A carbohydrate (S) decomposes in the presence of an enzyme (E). The Michaelis-Menten kinetic parameters were found to be as follows:

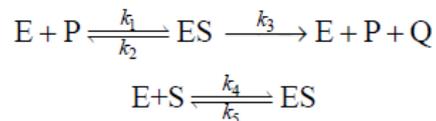
$$KM = 200 \text{ mol/m}^3$$

$$r_{\text{max}} = 100 \text{ mol/m}^3 \text{ min}$$

a. Calculate the change of substrate concentration with time in a **batch reactor** the initial substrate concentration is 300 mol/m^3 .

b. Chemostat (**continuously stirred-tank reactor**) runs with various flow rates were carried out. If the inlet substrate concentration is 300 mol/m^3 and the flow rate is $100 \text{ cm}^3/\text{min}$, what is the steady state substrate concentration of the outlet? The reactor volume is 300 cm^3 . Assume that the enzyme concentration in the reactor is constant so that the same kinetic parameters can be used.

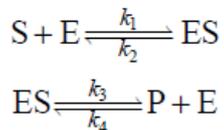
Q2: The kinetic model of lactose hydrolysis by *Aspergillus niger* lactase can be described as follows (Scott et al., 1985):



where S, P, Q, and E are lactose, galactose, glucose, and free enzyme.

- Derive the rate equation for the production of galactose.
- Does galactose inhibit the reaction competitively or noncompetitively?

Q3: When glucose is converted to fructose by glucose isomerase, the slow product formation step is also reversible as:



Derive the rate equation by employing the Michaelis-Menten.

LO2: Differentiate and estimate productivity in commercial bioreactors- packed bed, fed batch reactors.

Q4: You are going to cultivate yeast, *Saccharomyces cerevisiae*, by using a 10 m³ fermenter your company already owns. You want to find out the amount of ethanol the fermenter can produce. Therefore, a chemostat study was carried out and the Monod kinetic parameters for the microorganism grown in the glucose medium at 30°C, pH 4.8, were found to be: $K_S = 0.0025$ g/L and $\mu_{max} = 0.25$ h⁻¹. The ethanol yield ($Y_{P/S}$) is 0.44 (g/g) and cell yield ($Y_{X/S}$) is 0.019 (g/g). The inlet substrate concentration is 50 g/L.

- What flow rate will give the maximum total ethanol production in the continuous fermenter and what is the maximum ethanol production rate?
- If you want to convert 95 percent of the incoming substrate, what must the ethanol production rate be for the continuous fermenter?

LO3: Compare and derive various methods of effective mass-transfer correlation and mass-transfer coefficients.

Q5: Derive the relationship between the overall mass-transfer coefficient for liquid phase K_L and the individual mass-transfer coefficients, k_L and k_G . How can this relationship be simplified for sparingly soluble gases?

LO4: Apply concept of Gas-liquid mass transfer in bioreactors and measure K_La and power consumption in aeration and agitation system reactors.

Q6: A cylindrical tank (1.22 m diameter) is filled with water to an operating level equal to the tank diameter. The tank is equipped with four equally spaced baffles whose width is one tenth of the tank diameter. The tank is agitated with a 0.36 m diameter flat six-blade disk turbine. The impeller rotational speed is 2.8 rps. The air enters through an open-ended tube situated below the impeller and its volumetric flow rate is $0.00416 \text{ m}^3/\text{s}$ at 1.08 atm and 25°C .

Calculate the following properties:

$P_m = 697 \text{ W}$; $H = 0.02$; $k_La = 0.0217 \text{ s}^{-1}$.

- Power requirement
- Gas hold-up
- Sauter-mean diameter
- Interfacial area
- Volumetric mass-transfer coefficient

Q7: To measure k_La , a fermenter was filled with 10 L of 0.5 M sodium sulphite solution containing 0.003 M Cu^{++} ion and the air sparger was turned on. After exactly 10 minutes, the air flow was stopped, and a 10 mL sample was taken and titrated. The concentration of the sodium sulfite in the sample was found to be 0.21 mol/L. The experiment was carried out at 25°C and 1 atm.

Calculate the oxygen uptake and k_La .

LO5: Analyze immobilization techniques in reactors and measure mass transfer resistance based on the design.

From Q7 and Q8, analyze the mass transfer resistance with two techniques for immobilized enzyme, based on their effectiveness.

Q8: An enzyme is immobilized by copolymerization technique. The diameter of the spherical particle is 2 mm and the number density of the particles in a substrate solution is 10,000/L. Initial concentration of substrate is 0.1 mole/L. A substrate catalyzed by the enzyme can be adequately represented by the first-order reaction with $k_0 = 0.002 \text{ mol/Ls}$. It has been found that both external and internal mass-transfer resistance are significant for this immobilized enzyme. The mass-transfer coefficient at the stagnant film around the particle is about 0.02 cm/s and the diffusivity of the substrate in the particle is $5 \times 10^{-6} \text{ cm}^2/\text{s}$.

- If the internal mass transfer resistance is negligible, what is the concentration of the substrate at the surface of the particle? What is the effectiveness factor for this immobilized enzyme?

b. If the internal mass-transfer resistance can be described as the distributed model and the external mass transfer resistance is not negligible, what is the concentration of the substrate at the surface of the particle? What is the overall effectiveness factor considering both internal and external mass-transfer resistance?

Q9: An enzyme is immobilized uniformly in a gelatin slab (thickness L and area A). One side is in contact with substrate solution (C_{sb}) and the other side is in contact with a glass plate. The mass transfer coefficient on the surface of the gelatin is k_s .

a. Derive the equation for the substrate concentration with respect to x when the substrate is catalyzed by zero-order reaction (k_0). Assume that the substrate is transferred by molecular diffusion in the x direction only and the gelatin slab is thick enough to catalyze all the substrate while it diffuses into the slab. The substrate concentration at the surface of the slab in contact with the solution is C_{S0} .

b. What is the critical thickness (x_c) at which all substrate is consumed?

c. What is the substrate concentration at the surface of the slab (C_{S0})?

Q10: The typical oxygen demand for yeast cells growing on hydrocarbon is about 3 g per g of dry cell. Design a 10-L stirred fermenter (the diameter and height of fermenter, the type and diameter of impeller) and determine its operating condition (impeller speed and aeration rate) in order to meet the oxygen demand during the peak growth period with the growth rate of 0.5 g dry cell per liter per hour. You can assume that the physical properties of the medium is the same as pure water. You are free to make additional assumptions in order to design the required fermenter.

Section 7:

Assessment Plan: Mapped for each LO's.

LO	Type of assessment	Frequency	Assessment task	Learning Verification
LO1	Formative	In class for LO 1	Students work in groups for the assigned class activity. Activity/Task 1 (15 minutes): Students should work in group to assemble the various components of bioreactor and based on	Learning verification: Teacher provide answer to each question given as class activity

			<p>operational modes design three types and evaluate its application.</p> <p>Example of In class problem: Check above section 6- Q1 and Q2.</p> <p>Minute card from students (Feedback): What did they learn and what concepts did they find very challenging. Reflection: Their experience on class activity and the challenges.</p>	
LO2	Formative	In class for LO2	<p>Activity/Task 1 (20 minutes): Students should work in group to solve a case/scenario, example mentioned in pedagogical section. Numerical Q3, section 6.</p> <p>Minute card from students (Feedback): What did they learn and what concepts did they find very challenging. Reflection: Their experience on class activity and the challenges.</p>	Learning verification: Teacher provides answer for each question
LO3	Formative	In class for LO3	<p>Mathematical calculations to analyze biochemical</p>	Learning verification: Teacher provides answer for each question

			aspects of bioreactor. Section 6. Q4	
L04	Formative	In class for L04	In class activity - using geoalgebra- calculate K_{LA} Section 6- Q6 individual verification by comparing the correct response provided by the instructor. Group activity: students are given group assignment to design mathematical models using geoalgebra and present it as a group.	
L05	Formative	In class L05	In class activity - using Matlab- section 6- Q7 AND Q8, individual verification by comparing with the correct response provided by the instructor.	Learning verification: Teacher provides answer for each question given as class activity
	Summative	TEST PAPER I: sample questions given below	Hard copy	Evaluate the hard copy and grade Weightage for this portion: xx %

Sample Questions:

Time: 3 hours

No. of pages: 4

Max marks: 50

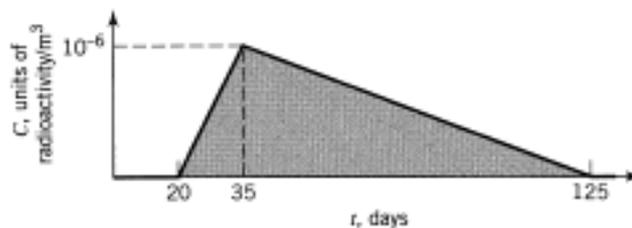
PART A (Attempt all Questions: Total 20 marks)

Derive the following

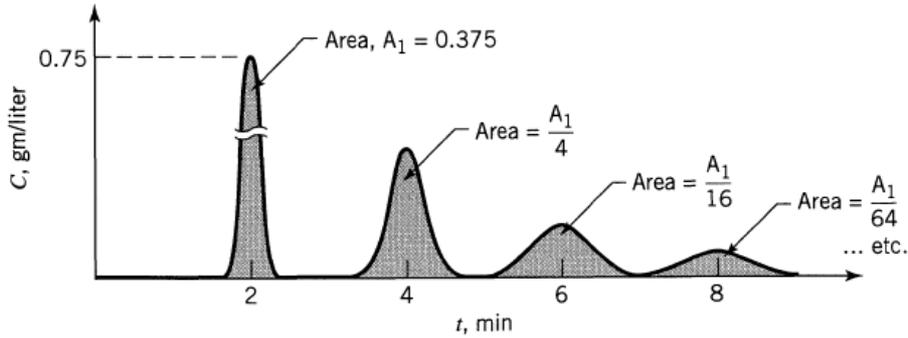
1. In a bacterial culture undergoing balanced growth, how is the doubling time related to the specific growth rate and the division rate of the culture?
(2marks)
2. In a steady state CSTR, how is the specific growth rate of culture related to the dilution rate?
(3marks)
3. Derive an expression for optimum cell concentration and substrate concentration for maximum productivity in a steady state CSTR.
(5marks)

Multiple Choice Questions

1. A batch of radioactive material is dumped into the Columbia River at Hanford, Washington. At Bonneville Dam, about 400 km downstream the flowing waters ($6000 \text{ m}^3/\text{s}$) are monitored for a particular radioisotope ($t_{1/2} > 10 \text{ yr}$) and the data of Fig. are obtained.



- a) How many units of this tracer were introduced into the river? (2 marks)
(i) 25613 (ii) 33589 (iii) 27216 (iv) 14563
2. A large tank (860 liters) is used as a gas-liquid contactor. Gas bubbles up through the vessel and out the top, liquid flows in at one part and out the other at 5 liters/s . To get an idea of the flow pattern of liquid in this tank a pulse of tracer ($M = 150 \text{ g}$) is injected at the liquid inlet and measured at the outlet, as shown in Fig.



- a) The mean of the C_{pulse} curve (\bar{t}) from the figure is (2 marks)
- (i) 1.5min (iii) 7.8min
(ii) 5.3 min (iv) 2.7min
- b) What is the liquid fraction in the vessel? (2 marks)
- (i) 7% (ii) 43% (iii) 74% (iv) 93%

3. The concentration readings in table given below represent a continuous response to a pulse input into a closed vessel which is to be used as a chemical reactor.

Time (min)	t	Tracer output concentration C_{pulse} (gram per liter fluid)
0	0	0
5	3	3
10	5	5
15	5	5
20	4	4
25	2	2
30	1	1
35	0	0

- a) The mean residence time is (2 marks)
- (i) 10min (ii) 15 min (iii) 20 min (iv) 25 min
- b) The total amount of tracer introduced (g. min/L) is (2 marks)
- (i) 50 (ii) 75 (iii) 100 (iv) 125

PART B

Attempt any three questions (Total 30 marks)

1. A pilot-scale fermenter of diameter and liquid height 0.5 m is fitted with four baffles of width one-tenth the tank diameter. Stirring is provided using a

Scaba6SRGT curved-blade disc turbine with diameter one-third the tank diameter. The density of the culture broth is 1000 kg/m^3 and the viscosity is 5 cP. Optimum culture conditions are provided in the pilot-scale fermenter when the stirrer speed is 185 rpm. Following completion of the pilot studies, a larger production-scale fermenter is constructed. The large fermenter has a capacity of 6 m^3 , is geometrically similar to the pilot-scale vessel, and is also equipped with a Scaba 6SRGT impeller of diameter one-third the tank diameter.

- a. What is the power consumption in the pilot-scale fermenter?
 - b. If the production-scale fermenter is operated so that the power consumed per unit volume is the same as in the pilot-scale vessel, what is the power requirement after scale-up?
 - c. For the conditions in (b), what is the stirrer speed after scale-up?
 - d. If, instead of (b) and (c), the impeller tip speed ($5\pi NiDi$) is kept the same in the pilot and production-scale fermenters, what is the stirrer speed after scale-up?
 - e. For the conditions in (d), what power is required after scale-up?
(10 marks)
2. A 15 m^3 chemostat is operated with dilution rate 0.1 h^{-1} . A continuous steriliser with steam injection and flash cooling delivers sterilised medium to the fermenter. Medium in the holding section of the steriliser is maintained at 130°C . The concentration of contaminants in the raw medium is 10^5 ml^{-1} ; an acceptable contamination risk is one organism every 3 months. The Arrhenius constant and activation energy for thermal death are estimated to be $7.5 \times 10^{39} \text{ h}^{-1}$ and $288.5 \text{ kJ gmol}^{-1}$, respectively. The steriliser pipe inner diameter is 12 cm. At 130°C the liquid density is 1000 kg m^{-3} and the viscosity is $4 \text{ kg m}^{-1} \text{ h}^{-1}$. Assuming perfect plug flow, determine the length of the holding section.
(10 marks)
3. *Lactobacillus casei*s propagated under essentially anaerobic conditions to provide a starter culture for manufacture of Swiss cheese. The culture produces lactic acid as a by-product of energy metabolism. The system has the following characteristics:
 $Y_{x/s} = 0.23$; $K_s = 0.15 \text{ kg m}^{-3}$; $\mu_{\max} = 0.35 \text{ h}^{-1}$; $m_s = 0.135 \text{ kg kg}^{-1} \text{ h}^{-1}$
- A stirred fermenter is operated in fed-batch mode at quasi steady state with a feed flow rate of $4 \text{ m}^3 \text{ h}^{-1}$ and feed substrate concentration of 80 kg m^{-3} . After 6 h, the liquid volume is 40 m^3 .
- a. What was the initial culture volume?
 - b. What is the concentration of substrate at quasi-steady state?
 - c. What is the concentration of cells at quasi-steady state?
 - d. What mass of cells is produced after 6 h fed-batch operation?
(10 marks)

4. A two-stage chemostat system is used for production of secondary metabolite. The volume of each reactor is 0.5 m^3 ; the flow rate of feed is 50 L h^{-1} . Mycelial growth occurs in the first reactor; the second reactor is used for product synthesis. The concentration of substrate in the feed is 10 g L^{-1} . Kinetic and yield parameters for the organism are:

$$Y_{X/S} = 0.5; K_S = 1 \text{ kg m}^{-3}; \mu_{\max} = 0.12 \text{ h}^{-1}; m_s = 0.025 \text{ kg kg}^{-1} \text{ h}^{-1}; q_p = 0.16 \text{ kg kg}^{-1} \text{ h}^{-1}$$

$Y_{P/S} = 0.85$. Assume that product synthesis is negligible in the first reactor and growth is negligible in the second reactor.

- Determine the cell and substrate concentrations entering the second reactor.
- The overall substrate conversion?
- What is the final concentration of product? (10 marks)

5. Mushroom tyrosinase is immobilised in 2 mm spherical beads for conversion of tyrosine to DOPA in a continuous, well-mixed bubble column. The Michaelis constant for the immobilised enzyme is 2 gmol m^{-3} . A solution containing 15 gmol m^{-3} tyrosine is fed into the reactor; because of the high cost of the substrate, the desired conversion is 99%. The reactor is loaded with beads at a density of $0.25 \text{ m}^3/\text{m}^3$; the entire enzyme is retained within the reactor. The intrinsic v_{\max} for the immobilized enzyme is $1.5 \times 10^{-2} \text{ gmol s}^{-1} \text{ m}^{-3}$ beads. The effective diffusivity of tyrosine in the beads is $7 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$; external mass-transfer effects are negligible. Immobilisation stabilises the enzyme so that deactivation is minimal over the operating period. Determine the reactor volume needed to treat 18 m^3 of tyrosine solution per day.

Given: the reaction follows first order kinetics; Thiele modulus for a spherical bead following

$$\phi = \frac{R}{3} \sqrt{\frac{k_1}{D_A}}$$

order kinetics is

Appendix (To be added)

1. TPACK
2. Blooms Taxonomy
3. Pedagogical Techniques
4. Assessment and Evaluation
5. Rubrics