

10.492 – ICE Topics: Biocatalysis

Fall 2004

Homework #3

Due Friday, Dec 3rd at the beginning of class. Solutions should be written and submitted on your own paper. All pages should be stapled together.

- 1) The following questions refer to the indene → indandiol bioconversion as presented in the reference Buckland et al. (1999) *Metabolic Engineering*. 1(1):63-74.
 - a) It was determined that the EE upgrade observed in the *P. putida* process was the result of selective conversion of the *cis*-(1R, 2S)-indandiol. What was the experiment and result used to confirm this hypothesis?
 - b) Although *P. putida* strains could produce the desired product(s), an additional option was sought and found in the form of a *Rhodococcus*. What were the advantages and drawbacks of using *Rhodococcus* sp. instead of *P. putida*?
 - c) The *Rhodococcus* B-264-1 strain suffered from substrate (indene) toxicity. What was the initial solution to this problem, and why was it not optimal? What was the final solution?
 - d) It was stated that this process could be run with indene present during the growth phase, or could be added after cell mass had accumulated during a bioconversion phase. Which do you think is a better option and why?
 - e) A free enzyme process was deemed unfeasible for this bioconversion. What characteristics of the conversion led to this conclusion?
- 2) In the case of substrate inhibition, the reaction kinetics indicate that the substrate concentration should be kept at a low level to achieve the highest reaction rate. In 10.37, you learned that a CSTR configuration provides the lowest average reactant concentration in a reactor. However, it is not economically feasible to run a traditional CSTR (*ie*, one in which the outlet stream is equivalent in content and concentration to the composition inside the reactor) for an enzyme bioconversion process. Why is this true? What are two alternatives to the traditional CSTR that you could use instead?
- 3) The following data were obtained for porous immobilized enzyme particles of different sizes. Enzyme was immobilized uniformly throughout the particles. (Hint: You will need to use Fig 4.21 in your handout on immobilized enzyme kinetics for this problem.)

Particle radius, mm	1	0.1	0.01
v_o (observed rate), $\mu\text{mols}/\text{cm}^3\text{catalyst}\cdot\text{min}$	200	1000	1000
s_o , mM	100	100	100

- a) What is the significance of observed reaction rates being the same with the 0.1 or 0.01 mm particle?
 - b) Assuming that $Da \ll 0.1$ (*ie*, no external mass transfer effects) and $\beta \approx 0$, what is the internal effectiveness factor for the largest catalyst particles?
 - c) Estimate the effective diffusivity of the substrate through the catalyst pores.
- 4) You have been assigned to develop a process for the conversion of a ketone to a chiral amine using a purified transaminase enzyme (EC Class 2). Describe the steps you will take to design this process, including how you will do the following: select a catalyst, optimize the reaction conditions, determine whether your enzyme is substrate- and/or product-inhibited, and maximize product yield. LIMIT YOUR ANSWER TO ONE PAGE.

(over)

- 5) Recall from 10.37 that one parameter used to define the operation of a reactor is the mean residence time or space time, τ , defined for a batch or plug flow reactor as follows:

$$\frac{V}{F_0} = \tau = [S_0] \int \frac{dX}{v}$$

where V is the reactor volume, F_0 is the flow rate, X is conversion, and v is the reaction rate.

- Assuming Michaelis-Menten kinetics with no inhibition, determine the equation for τ as a function of the M-M parameters and extent of conversion.
 - Determine the equation for τ in the case of substrate inhibition.
 - Calculate the reactor residence time required to achieve 95% conversion of a substrate with initial concentration of 10 mM with and without substrate inhibition, given $V_{\max} = 3.2 \times 10^{-5}$ M/min, $K_M = 5 \times 10^{-4}$ M, and $K_I(K_{S2}) = 1.2 \times 10^{-3}$ M.
 - What working volume reactor would you need to convert 100 g of a compound with molecular weight of 131 g/mol in a batch reactor?
- 6) You have been assigned to develop a process for the conversion of a ketone to a chiral alcohol (EC Class 1) using a yeast whole cell process. Describe the steps you will take to design this process, including how you will do the following: select a catalyst, optimize the growth and conversion conditions, determine whether the conversion is substrate- and/or product-inhibited, determine whether you have significant by-product formation, and maximize product yield. **LIMIT YOUR ANSWER TO ONE PAGE.**
- 7) You have identified a bacterium that can perform a desired bioconversion, with constitutive gene expression and no catabolite repression. There is no purified enzyme alternative, so you decide to run a whole cell process. You conduct several experiments to optimize the growth and conversion steps and obtain the following data (DCW = dry cell weight):

Growth Phase

$T_{\text{opt}} = 35^\circ\text{C}$, $\text{pH}_{\text{opt}} = 7.2$
 Lag time = 2 hr
 Specific growth rate = 0.35 hr^{-1}
 Initial cell density = 0.15 g DCW/L
 Max cell density = 13.5 g DCW/L

Conversion Phase

$T_{\text{opt}} = 35^\circ\text{C}$, $\text{pH}_{\text{opt}} = 8.0$
 Sp. Activity = 5.5×10^{-6} g substrate/(mg-DCW*min)
 Max conversion = 98%
 Yield of R+S enantiomers = 93%
 EE(S) = 97%
 Substrate solubility = 22.6 g/L

You are asked to provide 10 kg of the S-enantiomer to the isolation group for further processing. You have one 100-L (working volume) vessel to use for both the growth and conversion phases. Detail the process you would design and run. Include the number of batches, the batch cycle time, the total process time, mass substrate added per batch, and mass S-product obtained per batch. Assume that there is no substrate or product/by-product inhibition, and that the vessel turnaround time (*ie*, the amount of time you need for cleaning, sterilization and set-up between batches) is 4 hours.