**10.492 – ICE Topics: Biocatalysis**
Fall 2004

Homework #2
*Due Friday, Nov 19th* *at the beginning of class*. Solutions should be written and submitted on your own paper. All pages should be stapled together.

1) We spoke in class about choosing an enzyme from an initial screen based on conversion and EE. Ideally, we'd like an enzyme to deliver the target EE (*eg*, >95%) in the conversion step; however, it is possible to perform a so-called "EE upgrade" in the isolation steps that follows the conversion. EE is upgraded through a crystallization step in which a racemate crystallizes. Thus, the downside to an EE upgrade is that it is accomplished by removing *both* the desired and undesired compounds in equal amounts, which reduces the overall yield of the desired product. The objective of this problem is to see the impact of EE upgrades on product yield.

   (a) Derive an equation that expresses the amount of material that must be removed from each enantiomer as a function of the starting EE (*ie*, the EE at the end of the bioconversion step), the upgraded EE (*ie*, the EE following the crystallization step), and the total product concentration (*ie*, the sum of both enantiomers) at the end of the bioconversion step.

   (b) Assume the total product concentration is 100 g/L, with 100% conversion. What is the yield of the *R*-enantiomer if the EE following the bioconversion step is 75%?

   (c) What is the yield if the EE is upgraded to 80%? 85%? 90%? 95%? 99%? (Summarize your answers in a table that lists the upgraded EE, the amount of product removed in the upgrade step, the amount of each enantiomer remaining, and the *R*-enantiomer yield.)

   (d) Under what conditions would you *not* be able to upgrade the EE?

2) In class (and in the class notes), we derived rate expressions for the cases of *competitive*, *uncompetitive*, and *non-competitive* inhibition. In all three cases, the final rate equations were expressed in the Michaelis-Menten form. We know that the M-M parameters can be obtained from Lineweaver-Burke or Eadie-Hofstee plots.

   (a) For each form of inhibition, sketch both Lineweaver-Burke and an Eadie-Hofstee plots indicating how the linear fits (*ie*, the plotted lines) will change as a function of increasing the inhibitor concentration.

   (b) Assume that you have a case of product inhibition in your bioconversion. Describe the experiments you would conduct to determine both the type of inhibition and the dissociation constant for the enzyme-inhibitor (or enzyme-substrate-inhibitor) complex, $K_I$.

3) Discuss the pros/cons of using a purified enzyme over a whole cell for a bioconversion. In considering only the bioconversion step, which process is likely to be more expensive and why? In considering purification of the desired product, which is likely to be more expensive and why? In looking at the three enzyme classes on which we've been focusing (EC 1-3), which one is most likely to benefit from using a whole cell over a purified enzyme?

4) *Content for problem 4 removed due to copyright reasons.*

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5) *Content for problem 5 removed due to copyright reasons.*