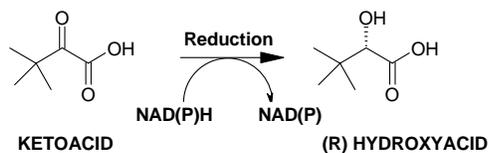**10.492 – ICE Topics: Biocatalysis**
Fall 2004

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?

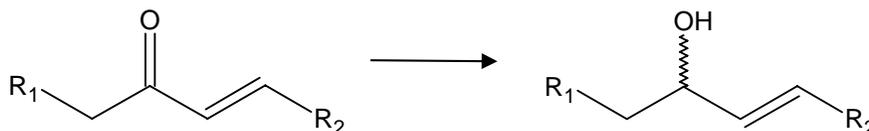4) Let's once again consider the reaction from
   Homework #1, Problem 5:

After some development work on a purified enzyme, you find that the cost of the catalyst is too high for an economically viable large-scale process. So, you want to investigate the possibility of using a whole cell catalyst from your pre-existing reduction library for the bioconversion. Your library consists of 10 yeast strains. You inoculate (seed) a small amount of growth medium in each of a set of test tubes with an aliquot of frozen cells and let the cultures grow up overnight. The next morning, you measure the optical density of the cells at 600 nm (OD_{600}), then you charge each well with 1 g/L substrate. After 16 hours, you measure the substrate and product concentrations and obtain the following data (values given are material concentrations in g/L):

Organism	OD_{600}	Ketoacid	R-Hydroxyacid	S-Hydroxyacid
Yeast1	1.52	0.52	0.300	0.150
Yeast2	0.54	0.88	0.100	0.020
Yeast3	0.95	0.24	0.230	0.470
Yeast4	1.13	0.25	0.110	0.630
Yeast5	0.32	0.75	0.229	0.004
Yeast6	0.21	0.67	0.005	0.290
Yeast7	0.17	0.80	0.150	0.010
Yeast8	1.84	0.90	0.005	0.092
Yeast9	0.28	0.10	0.730	0.110
Yeast10	0.46	0.38	0.450	0.001

For each culture, calculate the conversion, the specific activity (normalized to OD_{600}), and the EE. Which is your top candidate to take forward for process development? (Courtesy of Merck & Co., Inc. Used with permission.)

5) You have identified a yeast strain that performs the following reaction with high conversion and EE:

You want to isolate the S-enantiomer, but when you do further analysis on the reaction, you find the following additional products as well:

Draw a diagram that shows the possible routes from the one substrate to each of the products and indicate the EC class for each step. What's the maximum number of enzymes that could be involved in this reaction? What's the minimum number of enzymes likely to be involved? (Note that the "squiggly" line means that both enantiomers are formed. For this problem, we'll treat both enantiomers as a single product.) (Courtesy of Merck & Co., Inc. Used with permission.)