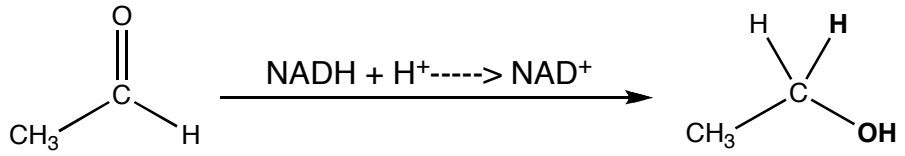
Energy Workshop: Chapter 6: Kinetics

Nicotinamide adenine dinucleotide (NAD) is a coenzyme that acts as an electron carrier in redox reactions. One of its forms is the reduced form, NADH, formed when a hydride ion, H^-, reacts with the oxidized form, NAD^+. This coenzyme helps dehydrogenase enzymes do their job. One "job" of alcohol dehydrogenase is to make ethanol from acetaldehyde in fermentation reactions performed by the cell in the absence of oxygen. The reaction is shown below:



Acetaldehyde Ethanol

The bold H atom and OH group designate where the 2 H atoms in the reaction showed on the arrow (intermediate reaction) go

1) Kinetics experiments show that in the presence of dehydrogenase, the rate of reduction of acetaldehyde to ethanol increases as you increase the concentration of the substrate, acetaldehyde. Eventually, the rate of the reaction reaches a maximum, where further increases in the concentration of the substrate have no affect on the reaction rate. Why is this the case?

2) Provide a graph of the rate increase of ethanol production vs. NAD^+ concentration with continuous addition of acetaldehyde (i.e. acetaldehyde concentration is constantly increasing.) What do you find?

3) On the same graph, provide the relationship of reaction rate vs. acetaldehyde concentration for both the enzyme catalyzed reaction, and the reaction without a catalyst, assuming that it is a first order reaction? Assume that the concentration of acetaldehyde is greater than NADH for the enzyme catalyzed reaction.

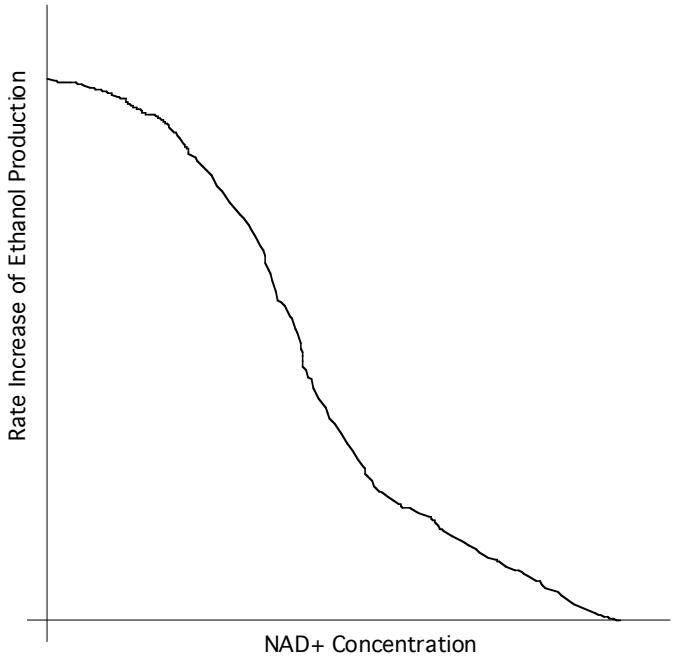
4) You are in your lab and want to increase the temperature of the enzyme-catalyzed reaction to produce more ethanol. What should you take into account before heating your system up?

5) If you just need the NADH for this reaction to proceed, why does it have to be a coenzyme for dehydrogenase, an enzyme consisting of a very large amount of amino acids, for the reaction to proceed catalytically?

Solutions to Energy Workshop: Chapter 6: Kinetics

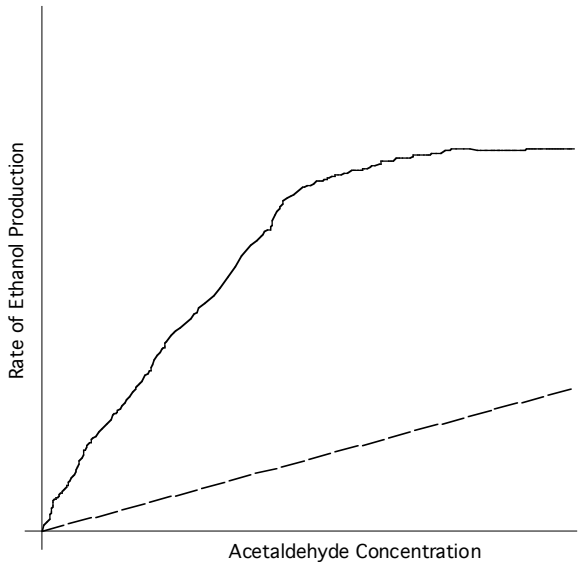
1) **Kinetics experiments show that in the presence of dehydrogenase, the rate of reduction of acetaldehyde to ethanol increases as you increase the concentration of the substrate, acetaldehyde. Eventually, the rate of the reaction reaches a maximum, where further increases in the concentration of the substrate have no affect on the reaction rate. Why is this the case?**
   Answer:
   This is the case because at the maximum, enough acetaldehyde is added such that all the dehydrogenase is in use catalyzing the reaction. So, the reaction remains constant because once an acetaldehyde molecule is converted to ethanol, the dehydrogenase used is available once again for catalysis. This saturation of reactants is very common in enzyme-catalyzed reactions.

2) **Provide a graph of the rate increase of ethanol production vs. NAD^+ concentration with continuous addition of acetaldehyde (i.e. acetaldehyde concentration is constantly increasing.) What do you find?**
   Answer:
   In this problem, we assume that the reaction begins with all NADH as the catalyst (i.e. when no dehydrogenase is being used) and goes all the way to NAD^+. The rate increase will attain a maximum at the very beginning of the reaction because all the NADH is not yet converted to NAD^+. However, the rate increase will go to zero because all the NADH is being used and NAD^+ is at its maximum. With no catalyst, there is no more rate increase.



3) **On the same graph, provide the relationship of reaction rate vs. acetaldehyde concentration for both the enzyme catalyzed reaction, and the reaction without a catalyst, assuming that it is a first order reaction? Assume that the concentration of acetaldehyde is greater than NADH for the enzyme catalyzed reaction.**
   Answer:
   Here, the dotted line is the uncatalyzed reaction and the solid line is with a catalyst. Without a catalyst, ethanol production linearly increases with the more acetaldehyde added. However, the slope of the line is quite small, because the rate constant is very small (i.e. the reaction rate is quite slow.) For the enzyme-catalyzed reaction, the rate of ethanol production increases quickly with NADH added. However, a maximum rate is attained. This rate is where all the enzymes in the reaction vessel is currently occupied by acetaldehyde molecules. After this point, ethanol production is constant, assuming the

catalyst can regenerate itself instantly after reaction. As an aside, one would say to just increase acetaldehyde concentration in the non-catalyzed reaction and the rate of production will eventually surpass the maximum of the catalyzed reaction. This would be foolish because the reaction rate is SO MUCH higher in the first place with an enzyme that it would not be feasible to use that much reactant for the same reaction rate. You can find the amount of ethanol produced by merely finding the area under the curve (intergration.) The area is much greater for the enzyme-catalyzed reaction than the non-catalyzed reaction.



4) **You are in your lab and want to increase the temperature of the enzyme-catalyzed reaction to produce more ethanol. What should you take into account before heating your system up?**
Answer:
First, you have to make sure the dehydrogenase enzyme can survive intact at the temperature you choose. Temperature changes can weaken some of the intramolecular interactions (van der Waals, hydrogen bonds, etc.), which would change the conformation of the enzyme, which makes it unable to host the NADH that helps catalyze the reaction. Also, you have to make sure the acetaldehyde can react at this temperature (i.e. you choose a temperature below the boiling points of the reactants (and products, too).) Lastly, you should look up to see if at higher temperatures, the acetaldehyde will not react with itself to produce non-ethanol products (i.e. polymers, larger alcohols, branched molecules, etc.)

5) **If you just need the NADH for this reaction to proceed, why does it have to be a coenzyme for dehydrogenase, an enzyme consisting of a very large amount of amino acids, for the reaction to proceed catalytically?**
Answer:
NADH has to be a coenzyme because it needs the dehydrogenase enzyme as a host to conform it to the correct molecular arrangement. NADH alone will not be able to catalyze the reaction because its structure is unfavorable for reaction with acetaldehyde. With the help of the large dehydrogenase molecule, the various interactions (stretched covalent bonds, increased hydrogen bonding with non-needed areas of the molecule, etc) make the NADH position itself ideally for the reaction.