Lecture #1 – Course Overview, Enzyme Classification System


1. Review Syllabus/Course Requirements

2. Survey class to determine the range of biology background, especially molecular biology.

3. Definitions and course objectives

Defining biocatalysis –

*Catalyst* – A substance, usually used in small amounts relative to the reactants, that modifies and increases the rate of a reaction without being consumed in the process.

*Biocatalyst* – A catalyst of biological origin → an *Enzyme*

Broadly speaking, biocatalysis then encompasses any enzymatically-catalyzed reaction, whether the enzyme has been purified or is part of a whole (microbial) cell, since the “working” parts of cells are enzymes. This can include, for example,

- Fermentation of yeast to convert sugars to ethanol for production of alcoholic beverages
- Growth of filamentous fungi to produce antibiotics
- Cheese production – uses a variety of microorganisms to convert milk to cheese
- Baker’s yeast (*Saccharomyces cerevisiae*) to convert sugars to CO$_2$ to make leavened bread
- Others from the class?

All of these involve multi-step pathways for converting one or more substrates into one or more products, including biomass, energy (through re-dox reactions) and waste (CO$_2$). We will focus much more narrowly, and follow a working definition of biocatalysis as the *use of an enzymatically-catalyzed reaction to convert a single starting compound to a single product, without consumption of the substrate for production of biomass, energy, or waste.*

Refer to Handout on industrial biotransformations for examples of large scale biocatalysis.
Note that this narrow definition puts “biocatalysts” in direct conflict with synthetic chemists. So, we may have as a corollary, that “Biocatalysts” are individuals who seek to limit the dominance of traditional organic chemists in the field of synthesis!

Scientists and engineers have a wide variety of both purified enzymes (partially or fully) and active whole cells to use as biocatalysts. The objective of this course in biocatalysis is to provide an overview of the following:

- The advantages and limitations of using biocatalysts for synthesis, and the criteria that one may choose for using these over inorganic catalysts
- The differences between using purified enzymes and whole cells as the biocatalyst
- Understanding the biological properties that lead to limitations/challenges for process design
- Understanding the chemical/physical properties that lead to limitations/challenges for process design and the differences from the biological ones
- Finding engineering solutions to these limitations/challenges
- Exploring biological solutions to these limitations/challenges

We will also emphasize the process development aspect of design. That is, how you determine and conduct needed experiments to help you finalize a design.

4. **Advantages of biocatalysis and when to use enzymatic reactions**

The most often cited advantages of biocatalysis are as follows:

1. **Selectivity/Specificity**
   - Substrate selectivity – ability to distinguish a particular compound from among a mixture of chemically related compounds, *eg* proteases acting following a specific amino acid sequence
   - Stereoselectivity – ability to act on a substrate or produce a product of one enantiomeric or diastereomeric form, *eg* resolution of racemic mixtures
   - Regioselectivity – ability to act on one location in a molecule, *eg*, hydroxylations
   - Functional group selectivity – ability to act on one functional group selective even when other groups may be more chemically reactive

2. **Mild reaction conditions** – most enzymes operate in aqueous solution, at mild temperatures and pH’s, and at atmospheric pressure. Chemical catalysts often require organic solvents, high temperatures, extremes of pH and high pressure. Enzymes can therefore result in lower energy and materials cost.
3. Environmentally friendly, ie, “green chemistry” – proteins are naturally biodegradable, aqueous solution avoids solvent waste, lower energy costs = lower emissions

4. High catalytic efficiency – high turnover numbers, ie, substrate molecules catalyzed per molecule of enzyme, resulting in less catalyst required to complete the conversion

Biocatalysis has the broadest use for the production of chiral compounds. Generally speaking, chiral chemistry is difficult to achieve chemically and economically. To produce chiral compounds with high selectivity, expensive catalysts that are usually required in large quantities are typically used. This makes large-scale production difficult. An alternative to highly selective synthesis is racemic synthesis followed by chiral chromatography for separation. Again, while this works fine on a small-scale, it is prohibitively expensive for large-scale production.

Limitations/when not to use biocatalysts – easy answer is when chemists can do it better! This is more likely to be true for non-chiral compounds and for very small (low molecular weight) molecules, where chemical synthesis is likely to be cheaper. Also, although enzymes do perform synthetic reaction steps, biocatalysts are usually not used for addition reactions since the substrate range is typically not wide enough to accommodate many different reactions. (Note that for addition reactions, the enzyme pocket must be able to hold and properly orient two molecules so the degree of flexibility for these reactions is likely to be less.) Enzymatic synthesis is also difficult for compounds with very low aqueous-phase solubility, since many enzymes are unstable in organic solvents. Finally, recall that enzymes are often unstable at extremes of temperature and pH. So, if the substrate and or product are extremely acidic or basic (ie, unstable in the presence of neutralizing salts), enzymes are also unlikely to be effective for chemical synthesis.

Enzymes are most likely to be effective when the substrate is similar to a substrate that is naturally-occurring, ie, one that the enzyme would encounter in its natural environment. Let’s look at the various kinds of enzymes that exist and how they

5. The Enzyme Classification System

Six classes of enzymes – refer to Handout for Review.

Examples:

Class 1. Oxidoreductases – will hear these referred to as oxidases, reductases, hydrogenases; common conversion is ketones/keto-acids to chiral alcohols

(a) EC 1.1.1.27 – lactate dehydrogenase
pyruvate +NADH + H+ → lactate + NAD+

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\begin{align*}
\text{pyruvate} & \xrightarrow{\text{EC 1.1.1.27}} \text{lactate} \\
\end{align*}
\]
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(b) EC 1.4.1.9 leucine dehydrogenase (reductive amination)
trimethylpyruvate + NADH + NH4+ → L-tert-leucine + NAD+ + H2O

Class 2. Transferases – aminotransferases (transaminases),
(a) EC 2.6.1.x transaminase
acetophenone + L-alanine → phenylethylamine + pyruvate

(b) EC 2.1.1.20 glycine-N-methyltransferase
glycine + S-adenosyl-methione → methylamino-acetic acid + S-adenosyl-cysteine

Class 3. Hydrolases – lipases, nitrilases, proteases
(a) EC 3.1.1.x lipase
ethyl 2-methyl-5-thien-2-ylpentanoate + H2O → 2-methyl-5-thien-2-ylpentanoic acid + ethanol

(b) EC 3.5.5.1 nitrilase
3-hydroxypentanedinitrile + 2H2O → 4-cyano-3-hydroxybutanoic acid

Class 4. Lyases
(a) EC 4.1.1.12 L-Asp-β-decarboxylase
aspartate → alanine + CO2
Class 6. Ligases

(a) EC 6.5.1.1 DNA ligase (ATP-dependent)

Consult your neighborhood bio book!

Of these six classes, three are primarily used for biocatalysis as we’ve defined it: oxidoreductases, transferases, and hydrolases. Class 4 reactions are more easily achieved chemically, Class 5 are not generally used in synthesis, and Class 6 are almost exclusively for biological processes.

Remember that the biocatalyst can be a free (purified) enzyme or a whole cell. Even in the latter case, however, the active ingredient in the conversion is some enzyme that has yet to be purified. We’ll discuss both free enzymatic and whole cell bioconversions, starting first with the enzyme cases.