10.492 - Integrated Chemical Engineering (ICE) Topics: Biocatalysis
MIT Chemical Engineering Department
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Lecture #5 – Using Whole Cells as Biocatalysts: Why/When, Growth vs Conversion (Screening)

Handouts: (1) Economist article, (2) Buckland paper (*Metabolic Engineering*)

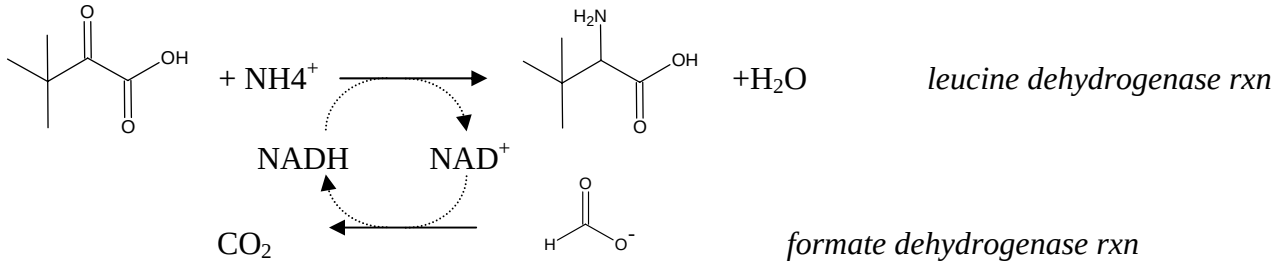
So far in our discussions of enzymes and enzyme kinetics, we've assumed that the biocatalyst to use for a particular conversion has been a purified enzyme. For example, in talking about screening enzymes for a reaction, we discussed setting up a series of identical reactions in which only the identity of the enzyme catalyst was changed. Realize, however, that all enzymes come from some biological source, be it of bacterial, plant, or mammalian origin, to name a few. For simpler organisms like bacteria or fungi, you often have the option of using the entire cell as the biocatalyst without going through the purification process. In this case, you will need to account for both growth of the "catalyst" and conversion of the substrate in your design process. Let's first discuss the advantages/limitations of using whole cells as catalysts.

1. Why and when to use whole cell catalysts

Following are some of the advantages/pros and disadvantages/cons of using whole cells as the catalyst versus using purified enzymes. Note that we are typically interested in using bacteria or yeast (*ie*, non-filamentous fungi) because they are easiest to culture.

Advantages/Pros	**Disadvantages/Cons**
• Only limited by the number of organisms that can be cultured, while enzymes are limited by the number that are commercially available.	• Processes are more complicated (so dev't takes longer), since you must design for cell growth (*ie*, enzyme manufacturing phase) and substrate conversion (*ie*, production phase)
• "Biomass is cheap", *ie*, the cost of fermentation is usually not too high, and you're not paying the purification costs associated with the purchase of an enzyme.	• Processes are "messier" – the vessel may contain cells and spent growth medium in addition to products and residual substrates (affects downstream purification)
• You don't need to provide exogenous co-factor for re-dox reactions since the cells will recycle co-factor with existing machinery (true even if cells aren't growing).	• Much more likely to have unwanted by-products since many other enzymes in addition to your desired enzyme will be present in the "catalyst"
• Also for re-dox reactions, you don't need to provide a complimentary reaction to recycle the co-factor that is converted (example below).	• If the enzyme is not secreted, it requires that the cells be lysed prior to the conversion, or that substrate be transported across the cell wall.

4th point under advantages – Note that one of the advantages of whole cell catalysts is that you don't need to provide exogenous co-factor to the reaction. In a purified enzyme reaction that requires co-factor, you can add an additional enzyme to re-cycle the co-factor. As an example, consider the following reductive amination:



In this case, the purified enzyme process is still simpler (and note that the formate is supplied as ammonium formate, so we provide the amine donor with the substrate that enables recycling of the co-factor), but now you have to consider the cost of developing and running a process with two enzymes.

For *rapid* process development of a *single-step* reaction, purified enzymes would be an advantage in the short term, especially in the initial screen. For larger-scale and long-term process development, you would also want to consider process economics since purified enzymes can be quite expensive. (Chemical catalysts can also be expensive, especially for chiral synthesis.)

2. "Bioprospecting"

One implied advantage of purified enzymes is that they are (or easily can be) well-characterized. The package insert will tell us what the operating and optimal pH's and temperatures are, and will probably also include the *specific activity* for one or more substrates (to be defined shortly).

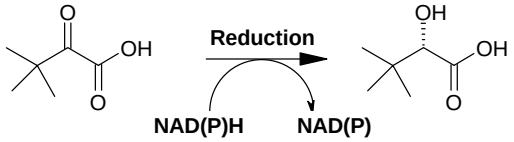
Although whole cells can provide a great diversity of enzyme activities, they will necessarily be much less characterized since the activities (*ie*, enzymes) will not be purified. So the question becomes: where do you look for sources of whole cell catalysts? Typically, there are two choices: (1) a pre-existing library and (2) randomly generated libraries.

Pre-existing libraries usually consist of a set of organisms that have been characterized to some degree, but for which detailed enzymatic activities are unknown. For example, we know that yeasts are a good source of alcohol dehydrogenases. (This is because yeast are good fermenters, *ie*, alcohol producers.) So, you may decide to build a library of yeast strains to test them for dehydrogenase activity. In companies where there is a lot of work in Biocatalysis, they will have their own "culture collections" that are partially characterized in this way. The American Type Culture Collection is a US-based repository of various strains that can be purchased by academic or industrial labs. Some

of the cultures deposited here have been partially characterized for enzyme activity as well. For example, a strain of *Candida albicans* from ATCC has the following among its list of applications:

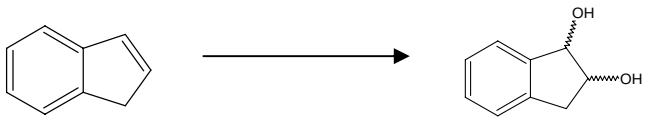
- produces D-arabinolactone oxidase
- produces aspartic proteinases [aspartyl proteinases]
- produces lanosterol synthase [2,3-oxidosqualene lanosterol cyclase]
- produces polyamine oxidase

So, you may be able to use resources such as this to choose a set of organisms to screen as well. A well-characterized library can be similarly efficient as purified enzymes. For example, consider the following bioconversion (Courtesy of Merck & Co., Inc. Used with permission.):



For this conversion, ~250 organisms and 10 enzymes were screened. 10 organisms and 1 enzyme produced the desired product. Therefore, the "hit rate" for the microbes was 10/250 or 4%, while the hit rate for the enzymes was 10%, a similar order of magnitude. (Note, however, that whole cell libraries are typically larger since they are less characterized.)

The other source for strains to screen is a randomly-generated, or *environmental* library. In this case, researchers would typically look to culture whatever they may find, *eg*, in neighborhood soil, on a rotten piece of fruit in your refrigerator, near an oil refinery, etc, to then test the organisms for activity. In this case, however, it is helpful to look for bugs to screen in an environment that might contain compounds similar to the ones you want to convert. If the cells have found a way to metabolize these compounds, they may have an enzyme that works on your substrate. As an example, consider the conversion of indene to indandiol:



An organism capable of producing the desired product in this case was isolated from toluene-contaminated soil, a good place to look since an organism known as *Pseudomonas putida* was known to do this chemistry when grow in the presence of toluene.

3. Whole cell screens

Assuming that we now know where to look for a whole cell catalyst, the question becomes, how do we actually screen, *ie,* how do we set up our identical reactions to determine whether a particular organism will do the conversion we want? Remember that for whole cells, we first have to produce a sufficient amount of the enzyme of interest to get conversion. So, we need to grow the cells. If we have a pre-existing library, it will usually exist as a frozen culture. A small amount of the frozen material is then used to start new cultures growing. If we have environmental samples, these must be grown up to obtain enough material to be able to store the cells.

Let's assume that we're starting from a pre-existing library. We will also assume that we have a growth medium that allows us to propagate (*ie*, grow) all of the cells in the library. One easy way to do this is in a 96-well plate. Each well can contain a different organism, so that we have a convenient way to look at a large library. The typical growth time is an overnight culture, but the degree to which each culture will grow over this fixed time period will vary. Once the growth phase is completed, each well will then receive a fixed amount of substrate, and the conversion phase can begin. This may last from a few hours to overnight. Then just as with the purified enzymes, we can measure the residual substrate and the product(s) to evaluate the performance of each strain.

Remember, though, that the activity of an enzyme will depend on how much enzyme is present (by V_{max}). The *specific activity* is defined as the amount of material converted per unit time per mass of enzyme, for example:

$$Activity = \frac{moles \cdot substrate}{mg \cdot protein \times \min}$$

We can add the same amount of enzyme to each reaction to be sure that we're comparing specific activity across all of the samples. In whole cell conversions the *starting amount* of catalyst is usually not the same in each well. So, while for a purified enzyme we can fix the catalyst amount and know that we are comparing specific activity among the different samples, the whole cell screen gives us varying amounts of cells. In order to then compare specific activity, we must normalize based on cell mass. We can define a whole cell based specific activity as follows:

$$Activity = \frac{moles \cdot substrate}{mg \cdot cells \times \min},$$

where the cells may be expressed as wet weight, dry weight or some proxy such as the optical density, *ie,* absorbance of visible wavelength light which is proportional to cell density.

Additionally, whole cell catalysts are much more likely to produce undesired by-products that are not simply the undesired enantiomer, because there's not just a single enzyme in your system. Other enzymes may convert your substrate to a product that cannot be

converted back to anything you desire, and which, therefore, has an impact on your overall yield. [refer to ME article] In this example, the starting substrate is acted upon by what is believed to be 4 different enzymes in the first step, producing 4 different products. Only two of these are useful for going forward, and the others are "lost" mass for us. In evaluating our whole cell conversions then, we can calculate conversions and EE's, but we also need to consider the overall mass balance (or yield of undesired product) to determine the most suitable organism to carry forward for process development. Remember as well that for whole cell conversions, we need to develop a *growth* process and a *conversion* process. In the next lecture, we'll discuss some aspects of developing both processes.