Lecture # 12, 13, 14 – Putting It All Together: Process Design for Bioconversions

Handout: Process Design Guidelines

This lecture is intended as a review of the material we've covered in class to-date, with the objective being to understand a methodology for overall process design for bioconversions. We have not, and will not now, address any isolation process design. The one note to make here is that if you are designing the reaction phase, you should do so in consultation with those responsible for the isolation steps, so that you will not inadvertently design a process to make a product that can't be easily isolated!

1. The Basics

There are three questions that need to be answered for any process design: *(1) How much? (2) How long? (3) How large?*

How much…
- material do you need to supply to your customer, 100 g, 1 kg, 10 kg?
- product can you obtain per unit of reaction volume (substrate/product solubility)?

How long…
- does one batch take to run in your process per unit of reaction volume?
- do you have to make your delivery?

How large…
- is(are) the reaction vessel(s) that you have at your disposal for the reaction?
- of a vessel can you practically and safely operate?

The answers to these questions determine the nature of your process and the configuration of the final design. Your ultimate goal is to deliver at least the requested amount of material on time using the resources you have available. Let's look back at our design development process to see how we address these issues.

2. A Design Strategy

Your basic strategy will be as follows, for both purified enzymes and whole cells:

1. Select a catalyst
2. Establish a baseline process
3. Optimize the process
4. Determine how to run the process to meet a target delivery

(Handout provided with the following table)

Let's look in a little more detail at each of these steps:

1. Select a catalyst (screening)	
Enzyme Process	**Whole Cell Process**
• ~10-20 enzymes, based on commercial availability • Set-up identical reactions, keep enzyme loading (mass) constant • Choose enzyme with good conversion, high selectivity	• ~100-200 strains for pre-existing library, ~10X more for environmental library • Add same amount of substrate, normalize to cell mass • Choose cell with good specific activity, high selectivity, (if possible) low by-products

- Remember that the number of catalysts you have to screen with whole cells will typically be larger than for a purified enzyme because you will generally have less confidence in the performance of these versus a more well-defined system.

- For a whole cell process, some medium optimization may be a part of the initial screening process. Remember that glucose should be the limiting substrate in the medium to account for the possibility of catabolite repression.

- The amount of substrate you add for the selection is usually a low concentration (≤ 1 g/L) so you're not likely to see inhibition or toxicity in the screening stage.

- Given two strong candidates at this stage, you may take both forward for further development.

2. Establish *baseline* process	
Enzyme Process	**Whole Cell Process**

• Optimize pH, temperature
• Determine amount of enzyme needed to achieve target conversion (*eg*, 95%) in target time (*eg*, 3 hrs)
  ➤ Initial read on kinetics
• IDENTIFY CHALLENGES (usually manifest as failure to achieve target conversion and/or time, or as a "loss of material" due to inability to close mass balance)
	• Optimize *growth* phase (pH, temperature, medium) for target activity
	• Optimize *conversion* phase (pH, temperature)
	• Determine amount of time needed to achieve target conversion with fixed catalyst (max cell density)
	  ➤ Initial read on kinetics
	• IDENTIFY CHALLENGES (same)

- A baseline process usually starts with "seeing what you have." Hence, the first thing to do is scale-up your screening reaction to see how it behaves.

- You will also get your initial read on the kinetics from this scaled-up reaction. Your objective is to achieve conversion within a certain amount of time, and

initially, your "kinetics" don't have to be any more complicated than that. For a free enzyme process, you can always add more catalyst (until you hit the solubility limit and/or induce aggregation). For a whole cell process, you will typically run at the maximum achievable cell density and simply adjust the time.

- o Note that concentration of cell mass is a possibility, but it's better to avoid this if possible.

- For optimization of the whole cell growth process, don't forget to consider inducible gene expression and catabolite repression.

- Note that "identifying challenges" does not mean fully characterizing them. For example, you don't need to determine the type of inhibition and the inhibitor dissociation constant, Ki, to know that you have product inhibition. You only need to add product and see if your reaction rate slows. Similarly, you don't have to use mass spec to identify all of the by-products of a whole cell conversion. You only need to see if your substrate-product mass balance closes to know whether by-products exist.

- It's standard practice to optimize the pH and temperature, for purified enzyme or whole cell, growth or conversion. We just briefly mentioned medium optimization for the growth phase of whole cells. The equivalent to purified enzymes is buffer optimization. Different salts (ions) will affect enzymes differently, so it's worth looking at this as well.

3. Optimize process	
Enzyme Process	**Whole Cell Process**
• Address challenges (*eg*, low solubility, substrate/product inhibition, yield limits) • Determine kinetics for optimized process (Michaelis-Menten type expression or specific activity) • Determine operational limits (max achievable substrate charge and product titer)	

- Steps 2 and 3 are not as clearly divisible as what's represented here. You may not actually identify challenges until you've begun what you consider to be the optimization step, and you may have already determined the operational limits as part of your "baseline" process.

- You should also only address your challenges to the extent necessary to achieve your objectives. For example, while it's important for you to understand enzyme kinetics and how to interpret data, it's not always necessary for you to calculate V_{max}, and K_m.

- At the end of Step 3, you should have identified each of your design challenges, and either addressed them or decided that they cannot be remedied. You also want to have established your operating range and your operational limits. The question here is, how far can I push this process if needed?

4. Design for delivery	
Enzyme Process	**Whole Cell Process**
• Address questions of *how much, how long, how large?* • Determine reactor parameters, *eg*, batch reactor time to achieve target conversion of fixed substrate amount (delivered as one charge or fed) for given vessel size • Determine total cycle time (growth and/or conversion time + vessel prep time) • Determine total processing time required for delivery	

- This is the final stage of the design process, which is to determine how you're actually going to run the process to provide the material that's been requested of you. Remember, your ultimate goal is to deliver at least the requested amount of material on time using the resources you have available.

- You would ideally like to achieve a substrate concentration on the order of 100 g/L (this is representative of typical chemical conversions), but remember that your maximum substrate concentration is either the solubility limit or the limit at which you don't have significant inhibition effects (or an effect on protein stability). You will have established these limits in the optimization steps.

- If you know the starting concentration of your process, you can calculate a time or (volume) for conversion based on a "unit" volume (or time). From 10.37, the design equations are as follows:

  Batch or Plug-Flow Reactor   (2.1) $V\big/F_0 = \tau = [S_0]\int \dfrac{dX}{v}$

  CSTR   (2.2) $V\big/F_0 = \dfrac{X_{in} - X_{out}}{v}$ $V = \dfrac{F_0(X_{in} - X_{out})}{v}$

  For a PFR, the time is a space time, in which concentration varies as a function of position (distance) in the reactor. For a Batch Reactor, concentration varies a function of actual time. The flow rate for BR and PFR is a volumetric flow rate. For a CSTR, the flow rate is usually defined as a mass or molar flow rate, and if this is set, the reactor volume is what needs to be determined. In all cases, note that the reaction rate, or velocity, v, is the rate of production formation.

- Since the space time depends on a starting substrate concentration, you only need to know the total mass to determine the volume (or flow rate for a fixed volume) needed for conversion. If it's not a continuous process, you will need to calculate the number of runs (for batch or fed-batch reactors) necessary to achieve your target mass.

- You can also use the specific activity to do an algebraic determination of required conversion time. This is analogous to a reaction velocity and is sufficient if the rate doesn't change appreciably with substrate concentration (*ie,* large K_m). Recall that the specific activity can be defined as follows:

$$(2.3) \quad Activity = \frac{moles \cdot substrate}{mg \cdot protein\_or\_cells \times \min}$$

A specific activity can be obtained for enzymes as well and used to determine the reaction time and reactor volume needed. Given a certain enzyme or cell concentration, you can convert this to an activity per unit volume, and then determine the time needed to achieve your conversion based on the production target.

- Your total batch cycle time should include growth time (for a whole cell process), conversion time, and vessel prep/turnaround time.

Keep in mind that these are guidelines. What you will actually need to do will change with each process. Also, remember that the objective of your academic training is to gain as much of an understanding as possible of the different factors that influence a process. In practice, you will want to use only those "tricks" in your bag that are needed to achieve your objectives.

Happy Designing!