Modelling the Production of Bacterial Cellulose in a Bubble Column Bioreactor

SoundBio iGEM

Introduction

Bacterial cellulose (BC) is a widely used polymer that has applications in fields ranging from biomedicine to manufacturing. The SoundBio iGEM 2019 team is building a bubble column bioreactor that produces BC. In order to optimize said production and gain insight into the bioreactor's operation, a group using a bioreactor similar to ours might find it useful to mathematically model the bioreactor's operation. **In this guide, we will discuss how to design and implement a mathematical model for a bacterial cellulose-producing bubble column bioreactor.**

Model Options

Any team trying to build a mathematical model for a bioreactor of our design has four high-level options to choose from. This section analyzes these options.

Metabolic Control Analysis Model

Metabolic control analysis (MCA)[2] [7] is a mathematical framework, typically used for modelling metabolic pathways, that explains how changes in the concentrations of metabolic species affect specific network parameters. It depends on variables called control coefficients, which represent the degree of control that each enzyme exerts on a metabolic pathway flux, and elasticity equations/coefficients, which represent how an enzyme responds to local changes in its environment. Lastly, control equations are used to relate the elasticity coefficients to the control coefficients. To use these equations for modelling a bioreactor, there are a couple things to keep in mind.

1. You would extract the information for equations that describe the process performance of the bioreactor using an MCA approach. This would involve creating an equation for the **control coefficient** that relates the changes in a kinetic parameter to the change in mass of BC produced. In the below equation, *x* represents the control coefficient, *s* represents the mass of BC produced at time *t*, *p* represents the manipulated control parameter in the bioreactor at time *t*.

$$x(t) = \Delta \frac{ds}{dp}$$

2. In order to apply MCA to the bioreactor, you'd need to express the bioreactor as a reaction network of the transport processes -- what is going in and out of the bioreactor.

Since our bioreactor design uses a constant substrate concentration in the feed, the concentration of substrate entering the bioreactor would be D * Sf where Sf is the constant substrate concentration in the feed and D is the flow rate (volume/time) per unit volume of the reactor. Likewise, the expression for the flow out of the bioreactor is D * S.

3. Optimization- In order to optimize the metabolic control analysis model, several differential mass transfer equations must be made with respect to a performance index. Gradients in the performance index (with changes to the parameters of the mass transfer equations) can be found and applied with a gradient descent algorithm to achieve an optimum. In this case, you'd use the mass of BC produced as the performance index. Constraints must also be set based on theoretical knowledge of the bioreactor, which keep the parameter values in ranges that are physically reasonable.

Gradient Model

A gradient model is a mathematical model based on mass transfer equations and gradient equations. Such a model could be used to understand the relationships between mixing, oxygen and substrate concentration, hydrodynamics, and the process performance of the bioreactor.

- **Oxygen gradients** - Based on our own experiments and evidence from the literature[5], it is clear that oxygen plays a significant role in BC production. Therefore, a gradient-based model would require an equations that express the oxygen gradient, such as the one below[4].
    - $[O_2]$represents the oxygen concentration in the liquid phase in a given region of the reactor, C represents the convection flow in or out of that region (the divergence of the vector of liquid flow from that point * the oxygen concentration there), OTR represents the oxygen transfer rate from gas to liquid phase and OUR represents the oxygen uptake rate (by cells).

$$\frac{d[O_2]}{dt} = C + OTR + OUR$$ [4]

- **Carbon source/substrate gradients** - Bacterial cellulose is built by the cells using sugars/carbon sources from their media. This is an additional factor that affects BC production. Based on the media used, it is possible to create a carbon source gradient equation of the carbon source concentrations vs. the biomass yield or time. If the gradients for the carbon at different parts of the bioreactor - where the oxygen is sparged and the gas outlet - are very different, it is likely that there is insufficient mixing in the bioreactor and changes to the bioreactor should probably be made.
- **Axial dispersion gradients** could also be created (see below)

This model can be effectively paired with computational fluid dynamics (CFD), which can also involve gradient equations. Including these equations in the model could give insight into mixing and mass transfer conditions in the bioreactor.

In order to optimize the gradient model, we suggest the use of stochastic gradient descent. In this method, using multiple updates from each training example (data from the bioreactor while

running), the algorithm updates the parameters and the measures the performance index (rate of bacterial cellulose growth). Then the parameters are changed based on the gradient of each update. However, frequent parameter updates make the optimization technique rather costly, in terms of both resources and time.

Axial Dispersion Model [6]

An axial dispersion model could be used to model mixing and energy transfer in the reactor. In such a model, the balance equation of a component in the liquid state is described as:

Convection = Dispersion + Reaction + Absorption - Evaporation

The column is assumed to have imperfect mixing and plug flow (uniform and non-turbulent), but with axial dispersion (dispersion around the upward axis) to explain the deviation from plug flow.

The following equation describes the energy balance in the reactor:

Convection = Dispersion - Cooling - Evaporation - Reaction

It presents a system of second order partial differential equations and ordinary differential equations, that can be solved by using finite difference method (increasing a variable in small time steps) to convert the PDEs to ODEs, and then using an ODE solver (like the ODE15s solver in matlab) to get the resulting function.

Modified Tank-in-Series Model

This model was initially designed for internal loop airlift reactors, but could potentially have an application in bubble column reactors. The model works by breaking up the entire riser and downcomer sections into multiple stacked continuous stirred tank reactors that feed into each other. It tracks the substrate, biomass, and product via a series of ordinary differential equations.

This model could be considered due to its relative simplicity and high accuracy. Unfortunately, since our bubble column reactor doesn't have well-defined riser and downcomer sections, applying this model to our design might lead to inaccurate results.

Recommendation

We recommend the use of **gradient modelling** for modelling the bioreactor. The optimization technique allows training to occur while the bioreactor is running, rather than collecting lots of data from the bioreactor and then feeding that data into the computer. Also, the CFD aspect to this model will give relevant information about mixing in the bioreactor.

Model Implementation

Methodology

1. After considering your available time and resources, choose one of the four models listed above. An axial dispersion or tank-in-series model would likely require the least amount of time because of their relatively few and simpler equations. A gradient model, conversely, would require more parameters and thus be more costly.
2. Next, determine the model's parameters (see guide below). Again, consider your resources in determining whether or not it is necessary to omit some of the parameters. For example, for a gradient model, you could omit the equations involving bubble sizes because bubble size might not affect the growth of BC significantly.
3. You could implement the model either through an iterative, numerical approach, or by finding an analytical solution. Since finding such a solution could be complex, we recommend the former; working through the model's equations in small time steps could yield close-to-perfect results while permitting flexibility and simplicity. (see Software Tools for info about possible software)
4. Once the model has been created, you should iterate on and refine it through comparisons to experimental data.

Software Tools

This model could be built in Matlab or Python (though any language would work). Additional libraries or tools such as Tellurium or Antimony could be helpful, especially if the model is implemented numerically. For modelling computational fluid dynamics, you could use ANSYS or Simscale (which outsources the heavy processing).

Parameter Guide

Below are some (not all!) of the parameters your model should consider and advice on measuring them:

- ➔ **General Parameters:** The following values can be measured by sensors either for testing purposes, or for model-building. Note that all of these measurements will be limited to the immediate vicinity of the sensor. These three parameters have been shown to greatly affect the production of BC.
  - Co (measured by a Gravity analog DO sensor)
  - pH (measured by a Gravity analog pH sensor)
  - Temperature (measured by a DS18B20 sensor)
- ➔ **Oxygen Gradient Parameters [3]:**The equations describing oxygen gradients involve parameters that can be determined experimentally via the gas-out gas-in method.
  - KLa (*volumetric mass transfer coefficient*, (1/hr)
  - Co* (*oxygen concentration of liquid when saturated* (mg/L))

- Qo2*X (Qo2, rate of oxygen use (mg/s) per unit biomass (g), multiplied by X, biomass (g); aka *volumetric microbial oxygen uptake rate*) -
    - The gas out period is set up with a sealed container, such that no O2 is entering or leaving, and the only O2 loss rate is by Qo2*X, or bacterial respiration. After finding Qo2*X by measuring the rate of oxygen concentration over time (using a dissolved oxygen sensor), gas-in nitrogen to remove all oxygen from the media.
    - The equation:

      $\ln((Cl0-Cl)/(Cl0-Cs))=KLa*(t-ts)$

      where subscript s is when the gas in period started, and 0 is the t=0 (before gas in) describes the gas-in period, when the sparger and oxygen flow are reintroduced. Calculate KLa more accurately by plotting the left-hand side over time since gas-in, to find a line of constant slope KLa.
    - Then, the *rate of change of O2 concentration over time* is

      $(dCo/dt) = KLa(Co^*-Co) - Qo2*x$

    - With the data from the gas in period, Co* can be calculated. [3] (Note: make sure biomass is stagnant at this point, or the Qo2*X term could change.)
- *Bubble size (db)*, which can be determined using computational fluid dynamics via the approach used by Bach [4], 46.

Note: Software sensors, which involve mathematical methods of estimating parameters, can be explored when a particular parameter is very costly to measure or is too hard to measure.

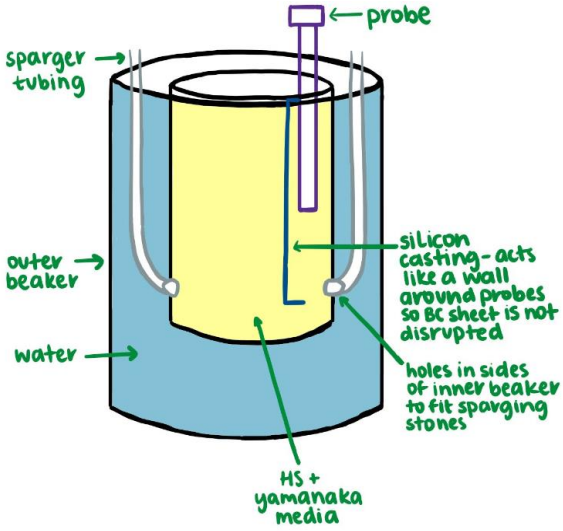
- For example, moving a DO sensor upwards in the tank slowly can yield a graph that can be used to verify the oxygen gradient (across z position) predicted by the model.

➔ **Axial Dispersion Related Parameters:**

- Eotvos Number - calculated using gravity, difference between the densities of the two fluids, surface tension of the liquid, and the surface diameter
- **εgas** or gas holdup is the fraction of gas in the bioreactor (by volume), which can be calculated using the Eotvos number (and the values used to find it), bubble fugacity, cross-sectional area of the reactor and bubble velocity [6].
- Dax, the axial dispersion coefficient could be calculated (in multiple ways) from the diameter of the reactor, the velocity of the gas, and the gas holdup.[6]
- If a packed bed reactor is being used, then **εpor** is the fraction of empty space to obstructions (i.e. it is 1 for columns without packed beds)

Bioreactor Specs

Our bioreactor is a bubble column reactor without a packed bed or any cascades. Temperature is controlled via a water bath surrounding the main tank. Sparging ring(s) are set at the edge of the base of the inner tank, and are used to supply oxygen. Temperature, dissolved oxygen, and pH probes are put in through holes in the inner tank. The bacterial cellulose should collect in a pellicle at the top of the inner tank.



Open Questions + Things to Consider

- How exactly does mass and energy transfer affect the growth of bacterial cellulose?
- How accurate would the application of a tank-in-series model to bubble column reactors be? What parameters/equations would have to be changed?

Contact Us

If you have any questions, please contact us at igem@sound.bio!

References

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