Laboratory Experiment #2
***E. coli* Batch Culture**
BCHE 8210

Introduction

In lecture we discussed batch growth of microbial cells. This experiment involves a side-by-side comparison of cell growth under two different batch-growth conditions. In the first experiment ("Bioreactor A") the medium will contain 3.5 g/L NH_4Cl and 12 g/L glucose. This medium composition will result in the carbon/energy source being depleted first. In the parallel second experiment ("Bioreactor B") the medium will contain 1.5 g/L NH_4Cl and 12 g/L glucose. This medium composition will result in the N source being depleted first. The calculations are as follows:

Bioreactor A:

- The medium contains 12 g/L glucose and 3.5 g/L NH_4Cl. This is 65.4 mM N.
- The biomass yield on N ($Y_{X/N}$) is about 8 g/g.
- Therefore, this quantity of N would result in a dry cell ("DCW") concentration (X) of 7.3 g/L.
- 65.4 mmol N/L × mol/1000 mmol× 14 g N/mol N × 8 g cells/g N = 7.3 g cells/L
- The general rule of thumb is that 1 OD unit is equal to 0.35 g cells/L. So, 7.3 g cells/L = 20.9 OD.
- The biomass yield on glucose ($Y_{X/Glu}$) is roughly 0.4 g/g.
- Therefore, this quantity of glucose would result in an X of 4.8 g/L (an OD of 13.7).
- Thus, the cells will run out of glucose first, and the medium is carbon limited.

Vessel B:

- The medium contains 12 g/L glucose and 1.25 g/L NH_4Cl. This is 23.4 mM N.
- The biomass yield on N ($Y_{X/N}$) is about 8 g/g.
- Therefore, this quantity of N would result in an X of 2.6 g/L, or an OD of 7.5.
- The quantity of glucose is unchanged, and able to provide an X of 4.8 g/L (an OD of 13.7).
- Thus, the cells will run out of nitrogen first, and the medium is nitrogen limited.

Carbon- and Nitrogen-Limitation

As we discussed, cells consume substrates ("S") for three possible purposes: for maintenance, for biomass, and for the generation of products. Each of these terms has units of volumetric rate (e.g., g/Lh).

$$-r_S = m_S X + \frac{\mu X}{Y_{X/S}} + \frac{Q_P}{Y_{P/S}} \quad (1)$$

In this experiment, we can identify two different substrates. If we consider carbon (or actually glucose) to be the substrate, then we can make this equation more specific:

$$-r_{Glu} = m_{Glu} X + \frac{\mu X}{Y_{X/Glu}} + \frac{Q_P}{Y_{P/Glu}} \quad (2)$$

If we consider nitrogen to be the substrate, then we can write this equation for nitrogen. In the case

of N, *E. coli* does not use ammonium as an energy source, and thus the maintenance term is zero. Also, *E. coli* does not generate significant products from nitrogen, so the product generation term will also be zero. Thus, for nitrogen:

$$-r_N = \frac{\mu X}{Y_{X/N}} \quad 3$$

We will not measure nitrogen during the course of the experiments. However, if we know the quantity of nitrogen present initially in the medium, and the cell concentration at the time the nitrogen is depleted, we can calculate the value for the cell yield coefficient on N ($Y_{X/N}$). There is no difference between "true" yield and "observed" yield here because N does not contribute to maintenance, and usually not to products.

When cells deplete either N or C, they no longer have enough material to construct new cells. At this time in either case the second term becomes zero (and which means $r_N = 0$ when any nutrient like N or C is depleted). Of course, when cells have depleted the carbon source, $r_S = 0$, and each of the terms of Equation 2 is zero. However, when the cells have depleted N but the energy source remains available, they will typically still consume it, using the energy to generate products if their metabolism allows it, and to satisfy maintenance requirements. Thus, after N is depleted and glucose remains:

$$-r_{Glu} = m_{Glu}X + \frac{Q_P}{Y_{P/Glu}} \quad 4$$

If the cells are generating a product from the carbon source, then how much of the carbon source goes towards product is difficult to distinguish from how much of the carbon source is going to maintenance (essentially, one cannot determine m_{Glu} and $Y_{P/Glu}$ independently). The best we can do without additional experimentation is estimate one from a literature value of the other.

Oxygen

We have not spoken a great deal about oxygen during the lectures. Oxygen is really just another substrate. Because oxygen does not go directly toward a product in the same way that carbon goes toward a product, Equation 1 for oxygen becomes:

$$-r_O = m_O X + \frac{\mu X}{Y_{X/O}} \quad 5$$

The term r_O is usually referred to as the Oxygen Uptake Rate, or OUR. As described in Lab #1, the OUR tends in the short time scale to balance the OTR ("Oxygen Transfer Rate"), which is the ability of the agitation and gas delivery process to transfer oxygen to the culture. Thus, we may write:

$$k_L a(c_{O2}^* - c_{O2}) = m_O X + \frac{\mu X}{Y_{X/O}} \quad 6$$

During the course of this lab, we are going to measure the dissolved oxygen concentration (c_{O2}). We will also be measuring the OD of the culture, which is directly proportional to the biomass concentration. As noted above, the general rule of thumb is that 1 OD unit is equal to 0.35 g cells/L. There are a couple ways to use this equation. Probably most interesting is if you have values for the

other parameters (k_La, c^*_{O2}, m_O, Y_{XO}), along with the measured value of X and μ at a given time, then we can predict the value of dissolve oxygen (c_{O2}).

But, more generally, let us simply consider what might happen during the course of nitrogen-limited or carbon-limited processes. During cell growth, the value of X increases. Thus, by Equation 6, the value of c_{O2} must decrease. The only way to prevent this result is to increase the c^*_{O2} and/or k_La (and you should know how to do that now). So, from the inoculation to the time the cells quickly become limited, the carbon-limited and nitrogen-limited processes appear identical. But, what happens when either the carbon source is depleted or the nitrogen source is depleted?

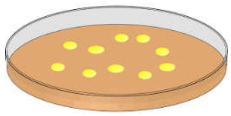
In a carbon-limited batch process, when the carbon source is depleted, as discussed above, cell growth ceases and the maintenance requirement ends. If both terms on the right hand side of Equation 6 are zero, then $c_{O2} = c^*_{O2}$. We therefore expect the DO to decrease during a carbon limited batch process until very close to the point at which the carbon source is depleted, when the DO should increase to near 100%. The only exception to this rule is when the cells have accumulated a "product" which can also be consumed as a second carbon source after the depletion of the original carbon source.

In a nitrogen-limited batch process, when the nitrogen source is depleted, the cells are still consuming the carbon for maintenance and potentially for the generation of products (Equation 4). Maintenance requirements are fulfilled by the generation of electrons (and the generation of products can generate electrons also). Thus, the term m_{OX} in Equation 6 is non-zero. We expect the DO to increase at the point at which the nitrogen source is depleted because of the termination of cells growth, but some oxygen will still be required for maintenance. Thus the DO will not increase to 100%, but the exact value that it reaches will depend on the biomass concentration X (see Equation 6). The more cells in a given volume, the more oxygen is required to support their maintenance and the lower the DO.

Procedure

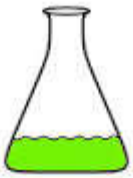
Set-Up

1. Assembly of the Bioreactor
    a. Use a standard volume of water (1.555 L final volume after 55 mL inoculum)
    b. Use a standard temperature (37°C)
    c. Use an agitation of 800 rpm and an air flowrate of 3.0 L/min
2. Calibration of the Dissolved Oxygen Electrodes
3. The general inoculation train will be as follows:



Petri dish containing
E. coli MG1655

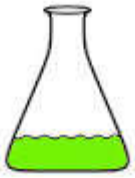
12 pm Day 1



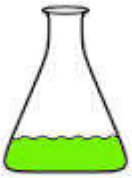
10 mL in
125 mL baffled flask
LB medium
T = 37°C

10 pm Day 1

5 mL

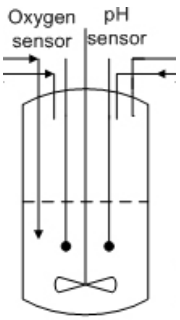


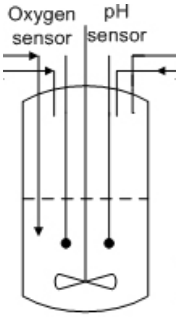
5 mL



50 mL (+ 5 mL) in two
250 mL baffled flasks
MXWT Medium
(5 g/L glucose)
pH = 7.0
T = 37°C

9:00 am Day 2





1.50 L (+ 0.055L)
bioreactor
MXWT Medium
(12 g/L glucose)
pH = 7.0
T = 37°C

4. Use NaOH for pH control.
5. Carefully prepare and label vials for liquid samples which will be frozen for later HPLC analysis (e.g., A-1, A-2,…, B-1, B-2,…)

Sampling

You should periodically measure DO, OD and take samples for the HPLC. You do not have to measure all these parameters at each "sample time". For example, because merely requires viewing a digital read-out, you can record DO every 5 minutes over a certain period of time for one vessel, but not take any samples for OD and HPLC for that time, or not record any measurements for the other vessel during that interval.

The exact schedule for sampling and recording DO measurements depends on the health of the inocula. My estimate is that the processes will require about 8 hours (*but biological experiments don't always go as expected*). You can record the DO often. For sampling (OD and HPLC), although an initial sample at the time of inoculation (t = 0) is always helpful to confirm the initial concentration of glucose (and N if we were measuring it), the most valuable samples in either case will be around the time that each culture becomes nutrient-limited. It is also possible to take more samples for potential HPLC analysis than you ultimately analyze. My general recommendation is an interval of 2 hours at the beginning for sampling, and decreasing to 45 minutes – 1 hour when "things get going."

Finally, although it is valuable to observe the processes in their entirety, I understand if your schedule demands your presence elsewhere for a portion of these experiments.

One student will have to prepare the medium and inoculum on Day 0.

Report

BE ORGANIZED. In addition to the comments above,

1. Usually batch results are plotted on a single figure (for each experiment) with time on the x-axis, and concentrations, DO and OD on the y-axis.

2. Discuss what happened. Do the results match our predictions?

3. Using literature values for appropriate parameters, make any calculations that you can.