1 Introduction

This experiment provides an introduction to biological processes and techniques by demonstrating the transfer of reaction engineering knowledge learned in nonbiological systems to reactor design for microorganism growth. The increasing understanding of biological systems and recognition of their importance in chemical processing indicate the value of familiarity with biological processes. The growth of *Escherichia coli*\(^1\) under aerobic conditions is a simple experiment for which the kinetics of growth may be compared with theoretical behavior.

The experiment is patterned after commercial processes for cell culturing in which an initial charge of the organism in a nutrient solution is allowed to multiply and grow, as in for instance brewing of beer. After an induction period, the *E.coli* growth is rapid and follows kinetics indicative of autocatalysis. As the initial amount of the nutrients is depleted, the growth rate decreases, falling to zero eventually. The cell mass is monitored during the experiment, and the results compared with theoretical predictions. *E.coli* is used because it grows rapidly enough to preclude contamination by other organisms during the experiment.

The growth of *E.coli* is carried out over an entire lab period until the required data has been collected or until it is obvious that growth has ceased.

2 About *E. coli*

Bacteria can be used to produce a number of commercial products including lactic acid (lactobacillus), L-glutamate (micrococcus glutamicus), butanediol, vitamin B\(_{12}\), and certain amino acids. *E. coli* is arguably the most thoroughly studied bacterial species and is therefore often used as a model of generic bacterial properties. Frequently, it is described as a “Gram negative rod”, referring to the fact that it does not take up the Gram stain which makes cells look purple in the microscope, and it is shaped like a short rod, approximately 2 by 0.5 µm. The dry weight of the cell approximately consists of ~5% DNA, 10% RNA, 70% protein, 10% lipids and 5% polysaccharides. *E. coli* can grow both aerobically and anaerobically. Under anaerobic conditions, growth of *E. coli* is relatively slow, converting simple sugars to lactic acid, acetic acid, ethanol, CO\(_2\) and H\(_2\) in a process called mixed acid fermentation (for example, the bacterium acetobacter is used to produce vinegar from ethanol, itself produced by fermentation). Under aerobic conditions, growth is much faster with doubling times as low as 20 minutes; the process is simply called respiration and the metabolic products are primarily acids and aldehydes together with CO\(_2\).

*E. coli* can synthesize enzyme systems capable of utilizing many different nutrients. It can also make the enzymes capable of synthesizing all 20 amino acids from simpler precursors, although it grows faster if amino acids are provided. Therefore the growth medium for *E. coli* can be relatively simple. For example, the Bacto Nutrient Broth used in some cultures consists only of

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\(^1\) The common way to indicate species names is to give both the genus and the species name in full when the organism is first mentioned in the body of the text. The genus name is capitalized while the species name is not and the entire name is italicized. Subsequent use of the name use an abbreviated form with the first capital letter of the genus followed by a period and the species name, all italicized. When used in the title, both the full and the abbreviated forms are seen.
beef extract (a carbon source, also providing vitamins, certain minerals, and amino acids) and peptone (partly digested proteins). Other more complex media with amino acids and certain minerals and vitamins may result in faster growth rates.

The particular strain used in this assignment is *E. coli* ATCC 23736. ATCC is the acronym for American Type Culture collection.

### 3 Theoretical Background

Several general reviews of the application of engineering techniques to biological processes are available (1). The kinetics of reactions in microorganisms is treated in books by Hinshelwood (2), Aiba et al. (3), and Bailey and Ollis (4).

#### The Classical Growth Curve

A growth curve is a graph of the amount of “biomatter” as a function of time. The biomatter is usually measured as dry weight concentration or as cell number concentration. In this experiment, we will use dry weight. The classical growth curve for a batch reactor is sketched in fig. 1. The concentration of biomass or cell number is indicated by *X*. Notice that it is a semi-log plot.

Initially, there is almost no growth as the cells in the inoculums adapt to the growth conditions of the fresh medium. This period is known as the lag period. This is followed by a period of rapid growth known as the exponential growth phase during which growth is well modeled by \( X \sim e^{\mu t} \). Finally, as the nutrients become depleted, growth slows down and the stationary phase is entered during which growth is absent. If growth is monitored long enough, one may also see a death phase during which the cell mass and cell number concentrations decrease.

![Figure 1 The qualitative shape of the classical growth curve.](image)

By drawing the two asymptotes of the growth curve, as well as the tangent at the inflection point, one can identify crude measures of the lengths of the three growth phases, as demonstrated in fig. 2.
Figure 2 Growth curve with asymptotes and tangent at the inflection point added. The intersection point between the asymptote through time zero and the tangent is a rough indication of the duration of the lag phase. The other intersection point is a rough indication of the time at which exponential growth ceases and the culture enters the stationary phase.

The qualitative explanation of the classical growth curve given above assumes that the growth of the culture is determined by the concentration of a single growth limiting substrate and that this substrate is added to the reactor at the start of the experiment, not continuously during the growth of the culture. Deviation from this growth curve can be observed when this assumption is not valid. For instance, growth may be oxygen limited, but oxygen is added continuously to the reactor and a different growth curve is obtained in this case. A mathematical model of this growth curve can be derived using the tools described in the section **Oxygen Mass Transfer** below. Another common deviation from the classical growth curve is the diauxic growth curve.

**Unstructured models**

The simplest models of the growth curve are all *unstructured*, meaning that the biophase is modeled by a single variable, usually the cell mass concentration. More complex structured models use several variables to describe the biophase, primarily the concentrations of key metabolites in the cell such as proteins, carbohydrates, DNA, RNA etc. The discussion below is restricted to unstructured models.

The rate of biomass formation by growth will be indicated as $R_X$ which has units of biomass per time per volume. To indicate the autocatalytic nature of growth, it is usually written

$$R_X = \mu X$$

where $X$ is the biomass concentration and $\mu$ is the specific growth rate which has units of inverse time. Almost all unstructured models in the literature are models of $\mu$. A common simplifying assumption used in many of these models is the assumption of a single growth limiting substrate, i.e. the assumption that $\mu$ is only a function of the concentration of one of the nutrients. With this assumption, growth can be summarized by the reaction $S \rightarrow YX$ where $S$ indicates the growth limiting substrate. The stoichiometric constant $Y$ (on the substrate $S$) is called the yield. This reaction is obviously a simplification of the many metabolic reactions that occur during growth and the yield is therefore not a true constant but does depend to some extent on the growth conditions. However, experience has shown that $Y$ is approximately constant over a wide range of
conditions and can therefore be regarded as such without introducing serious modeling errors. It follows that the rate of substrate consumption (-\(R_S\)) and the rate of biomass production, \(R_X\) are related by

\[
\frac{-R_X}{R_S} = Y
\]

Eq. (2) can be integrated for a batch reactor to give

\[
(X(t) - X_0) = Y(S_0 - S(t))
\]

where \(S\) indicates the substrate concentration and a \(0\) subscript indicates initial values.

**The Monod model.**

The most commonly used model for \(\mu(S)\) is the Monod model (5)

\[
R_X = \mu(S)X = \frac{\mu_m S}{K_S + S} \cdot X
\]

where

\[
\mu_m = \text{maximum specific growth rate of the organism.}
\]

\(K_S = \text{Monod saturation constant.}\)

Written in this manner, Eq. (4) then resembles the rate expression for enzymatically catalyzed reactions. The biomass and substrate balance for a batch reactor become

\[
\frac{dX}{dt} = \frac{\mu_m S}{K_S + S} \cdot X
\]

\[
\frac{dS}{dt} = -\frac{1}{Y} \cdot \frac{\mu_m S}{K_S + S} \cdot X
\]

Combining Eq. (3) with Eq. (5.1) to eliminate \(S\) gives for a batch process

\[
\frac{dX}{dt} = \mu_m \frac{YS_0 - (X - X_0)}{YS_0 - (X - X_0)} \cdot X
\]

which may be integrated assuming constant volume to give a "growth curve" for a batch reactor, where the initial condition that \(X = X_0\) at \(t = 0\) and the constant volume assumption have been used.

\[
\mu_m t = \frac{K_S Y + S_0 Y + X_0}{S_0 Y + X_0} \ln \left( \frac{X}{X_0} \right) - \frac{K_S Y}{S_0 Y + X_0} \ln \left( \frac{S_0 Y + X_0 - X}{S_0 Y} \right)
\]

The Monod growth curve of Eq. (7) does not include the lag phase, or the death phase after depletion of the growth limiting substrate. Notice also that equation (7) does not provide an explicit
solution for \( X(t) \) which must be determined numerically by root finding for each value of \( t \).

If the reactor is operated continuously as a CSTR (a “chemostat” in bioengineering), the same rate expression can be substituted into the CSTR mass balance equations to yield the following steady state mass balances, substrate and biomass, respectively.

\[
D(S_0 - S) - \frac{\mu_m S X}{Y(S + K_S)} = 0 \tag{8}
\]

\[
DX_0 - DX + \frac{\mu_m S X}{S + K_S} = 0 \tag{9}
\]

In eqs. 8 and 9, \( D \) is the dilution rate, the ratio of liquid volumetric flow rate to liquid volume (called space velocity in ChE). Ref. 4 discusses the solution of these equations, and design choices for CSTR operation.

**Other models**

There are many alternatives to the Monod model in the literature, some are listed below.

Growth with substrate inhibition:

\[
\mu(S) = \frac{\mu_m S}{K_S + S + S^2/K_I} \tag{10}
\]

Moser’s equation (6):

\[
\mu(S) = \frac{\mu_m S^n}{K_S + S^n} \tag{11}
\]

Contois’ equation (7):

\[
\mu(S) = \frac{\mu_m S}{K_S \cdot X + S} \tag{12}
\]

Tessier’s equation (8):

\[
\mu(S) = \mu_m (1 - e^{-K_S S}) \tag{13}
\]

Blackman’s model (9):

\[
\mu(S) = \begin{cases} 
\mu_m, & S > K_S \\
\frac{\mu_m S}{K_S}, & S \leq K_S 
\end{cases} \tag{14}
\]

The logistic or Verhulst model:
\[ \mu(S) = k \left( 1 - \frac{X}{X_m} \right) \]  

(15)

The \( X_m \) in the logistic equation is the maximum biomass concentration which is often referred to as the total carrying capacity. It should be viewed as a model parameter to be fitted as should be the constant \( k \).

If the assumption of a single growth limiting substrate is not valid, the Monod model is often extended to include multiple substrate concentrations, \( S_i \) through \( S_N \), as (Hinshelwood (2))

\[ \mu(S) = \mu_m \cdot \frac{S_1}{K_1 + S_1} \cdot \frac{S_2}{K_2 + S_2} \cdots \frac{S_N}{K_N + S_N} \]  

(16)

4 Oxygen Mass Transfer

In aerobic growth, oxygen is supplied continuously to the reactor and for this reason the configuration is sometimes called semibatch. Oxygen can be considered a substrate in its own right and growth becomes oxygen limited when the oxygen concentration falls below the critical oxygen concentration for the organism. The critical \( O_2 \) concentration for \( E. \ coli \) is \( \sim 0.0082 \) mmol/L at \( 37^\circ C \). Because \( O_2 \) solubilities in most growth media are at least 0.8 mmol/L, this means we need only \( >1% \) \( O_2 \) saturation of the media to support rapid growth.

The simplest way of accounting for the effect of oxygen on growth is to determine the critical oxygen concentration, the dissolved oxygen concentration above which growth is not oxygen limited, and make sure the bioreactor is always operated at greater than the critical concentration. This is too simple an approach for most situations and the effect of dissolved oxygen concentration on growth and rates of oxygen transfer must be taken into account when modeling bioreactors. It turns out that the rate controlling resistance in oxygen transfer into liquid microbial media is the liquid side of the gas bubble. The biomass and oxygen balances become:

\[ \frac{dX}{dt} = \mu(C_L, X) \cdot X \]  

(17)

\[ \frac{dC_L}{dt} = k_l a (C^* - C_L) - \frac{\mu(C_L, X) \cdot X}{Y_{O_2}} \]  

(18)

where \( C_L \) is the dissolved oxygen concentration, \( C^* \) the dissolved oxygen concentration in equilibrium with the gas phase oxygen concentration, \( k_l a \) is the capacity coefficient, the product of the mass transfer coefficient and the gas-liquid interfacial area per reactor volume, and \( Y_{O_2} \) is a yield constant relating cell mass growth to \( O_2 \) consumption. It is customary to talk about \( k_l a \) as a single parameter and determine only this product. Several assumptions are implicit in this model. Oxygen is assumed to be the only growth limiting substrate and the gas phase is assumed well mixed and the bubbles are all assumed equal in size. In reality, a reactor will contain a distribution of bubble sizes with different mass transfer coefficients and different rise velocities, and the gas bubbles at the bottom will have a different gas composition than the bubbles at the top of the liquid.
Microbial growth rates are far slower than typical rates of oxygen transfer, so it is reasonable to apply the quasi steady state assumption to $C_L$ and let $dC_L/dt \sim 0$, giving the quantity $\mu X/Y_{O_2}$ as the rate of oxygen transfer $k_l a (C^* - C_L)$. This is called the respiration, oxygen absorption rate, or oxygen uptake rate per reactor volume. This rate can be simplified further if the dissolved oxygen concentration is much smaller than the dissolved oxygen concentration in equilibrium with the gas phase oxygen concentration in which case we can set $(C^* - C_L) \approx C^*$. The specific respiration rate $Q_{O_2}$ is the respiration rate per amount of biomass $\mu Y_{O_2}$. The value of $k_l a$ can be estimated from correlations or, better yet, measured directly for the reactor in question. A standard method for doing this is described below.

**The Static Method**

The static gassing out method is done on a sterile reactor, so it provides a value of $k_l a$ that characterizes the reactor vessel and medium. It obviously does not provide a value that reflects the effect of the microbial growth. In the gassing out step, the reactor is scrubbed with nitrogen to remove all the dissolved oxygen from the medium. In the second step (gassing in), air is once again passed through the reactor and the dissolved oxygen is monitored versus time. During the gassing in step, the dissolved oxygen balance becomes

$$\frac{dC_L}{dt} = k_l a (C^* - C_L) \quad (19)$$

which is easily integrated assuming $C_L$ is the only dependent variable, and then easily rearranged to a linear form relating a ratio of concentrations to time. A least square fit of this linear expression to experimental data of $C_l(t)$ provides an estimate of $k_l a$.

**5 Reaction Procedure**

Use a hot soap solution for cleaning the glassware and the reactor and rinse with lots of DI (deionized, distilled) water. Wear latex gloves when handling *E. coli* solutions or solid cultures. Wash your hands after removing the gloves.

Stock cultures of *E. coli* are maintained on solid medium in petri plates. A growth curve experiment starts by inoculating an Erlenmeyer flask containing sterile liquid medium with a sample from such a stock culture. The Erlenmeyer flasks that will be used for the reactor inoculums must be inoculated from petri plates on the morning of the experiment. The Erlenmeyer flask is placed in an incubator/shaker until the cell culture has grown up, as evidenced by the cloudy appearance of the content, after which it is used to inoculate the reactor. Ideally, all equipment, Erlenmeyer flasks and reactor vessel, must be sterilized by autoclaving them and all steps must be carried out aseptically to avoid contamination of the cultures and reactor content. However, in this experiment we will relax these requirements and not autoclave the reactor. This does not result in any problems because the reactor experiments will be done with such large inoculums that contaminants cannot grow up and manifest themselves during the course of the experiment.
**Media preparation**

Your assignment may specify a specific type of media, but in most cases an LB medium is used. The composition is given below. The antifoam is only used for the medium for the reactor.

<table>
<thead>
<tr>
<th>LB MEDIUM</th>
<th>Concentration, g/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (dextrose)</td>
<td>10.0</td>
</tr>
<tr>
<td>LB Powder</td>
<td>20</td>
</tr>
<tr>
<td>Antifoam</td>
<td>0.5 mL (a few drops)</td>
</tr>
</tbody>
</table>

**Shake flask cultures**

Prepare the specified media and distribute 100ml aliquots into 250ml Erlenmeyer flasks. Cover the mouths of the flasks with aluminum foil and sterilize in the autoclave on the liquids cycle. Store the sterilized flasks in the refrigerator until ready to use. Two groups will probably be running this experiment so all flasks must be labeled with content and group number. Do this **after** the flasks are autoclaved as the glue on the labeling tape is turned into difficult to clean sticky gunk when autoclaved.

**Reactor culture**

The reaction is carried out in a 1.3-liter batch reactor (New Brunswick BioFlo/CelliGen 115) which attaches to a piece of equipment (“the unit”) which contains all the electronics and hardware needed to operate the reactor. The reactor basically consists of 3 parts, a metal stand, a thermostated jacked which is used for temperature control, the glass reactors, and a top plate which bolts to the metal stand to create a liquid-tight environment. The top plate consists of metal baffles and the metal impellor (below the plate) which reside in the glass reactor during use and a series of connecting tubes and inlet ports (above the plate) by which the unit is connected to the electronics. The top plate is only removed from the unit when cleaning the reactor. If not clean, the reactor should be cleaned with soap and hot water and rinsed with lots of DI (deionized, distilled) water. When cleaning the top plate of the reactor make sure to close off any all tubing, especially those connected to air filters. Tubing left open during cleaning with result in water clogging the air inlet filter, which will prevent oxygen from entering the system once the reactor is reassembled. The top plate is held in place by 6 thumb screws and can easily be removed for cleaning and access to the inside of the reactor. The top plate contains several ports that are shown in the photo in fig. 3. The ports that are used in a given experiment will depend on the assignment but in most cases the following holds.
Figure 3 Bioreactor top plate with ports numbered according to the list below.

1) Thermowell. This is a metal tube closed at the bottom that is used to hold a thermometer or a thermocouple for measuring the temperature of the culture.
2) DO probe. Port for the dissolved oxygen probe.
3) Inoculation port. Used for manual addition of inoculum, anti-foam etc. to the reactor.
4) Sample port. Three outlet tubes to remove sample. In current configuration two of the outlet tubes are connected and unused. The third outlet is connected to the metal port that will be used for collecting samples for analysis (Figure 4A)
5) Condenser. Used to regulate temperature of the reactor air exhaust. Top of condenser is connected to a secondary container (Figure 4B). The condenser uses cooling water from a chill water bath to cool air exhaust (Figure 5B).
6) Inlet ports. 2 inlet ports/1 outlet port directly connected to the master control that supply/expel the cooling water used to regulate the temperature of the unit. The third inlet port is connected to the gas input and will be the source of oxygen/nitrogen.
7) Impellor connector. Connects to motor to power the dual impellors in the unit.
8) Thumb screws.

*)--- Closed Ports. Not used for these experiments
To start a reactor experiment, add the medium **without** the glucose in 1L aliquots in 2L Erlenmeyer flasks. Use the Pyrex flasks for this, the Kimax 2L flasks do not fit in the autoclave. Store the autoclaved flasks in the refrigerator until ready to use. The reason for not adding the glucose to the reactor medium is that glucose will partially caramelize when autoclaved, resulting in a medium with a less well defined glucose concentration. While this is not a great concern for the inoculum cultures, it is undesirable for the reactor experiments where reproducible growth curves require well defined media.

At the start of the reactor experiment, add glucose to the media, pour 900ml into the reactor and attached the head plate. Place washers over each of the bolts before tightening the thumb screws in a star shaped pattern. The DO probe can only fit in the reactor if it is aligned with the gap in the rings that hold the baffles together and it is a good idea to insert the DO probe in its port before tightening the thumb screws as the probe can bump against the baffles and some adjustment of the head plate may be required before tightening the screws. The baffles occasionally get stuck when being inserted into the glass reactor, so make sure that they have been pushed down far enough to not interfere with the impellor or the insertion of the DO probe. Be sure that the valve on the sample port is closed (white plastic clamp).

Place the thermocouple in the thermowell making sure that it is pushed all the way down and touches the bottom. Adding a few drops of water to the well is also recommended for good contact. Connect the water inlet/outlet tubes from the top plate to the master controller (8 in Figure 3). The tubing ports are all color coded so match like colors - green to green, orange to orange, blue to blue, etc. (Figure 5A). Connect the cooling water inlet/outlet tubing (9 in Figure 3) to the master controller. Connect the inlet gas flow (9 in Figure 3) to the master controller. Connect the exhaust from the top of the condenser to the secondary container (Figure 4B) to
prevent any excess liquid from clogging the outlet filter. Unclip each hose clip except for the sample port and the two unused outlet tubes. Attach the impellor motor, which is stored on the top of the unit, to the top of the fermenter. Turn on the chill water baths and check that the air regulator is around 10 psi. Finally, wrap the heat jacket around the glass bioreactor. Make sure this is on the bioreactor before starting to ramp up the temperature as it becomes very hot, very quickly.

Figure 5. (A) Inlet tubing (color coded) connecting the top plate to the master controller. (B) Chill water bath (C) Air pressure regulator

Turn on the unit using the white toggle switch to the lower right of the unit. The touch screen should turn on and you will be able to adjust the parameters for the reactor (Figure 6). Once all the connections/tubing have been set-up you may initiate priming the reactors. Tap on Agit to access the menu for impellor speed and turn on the impellor with an initial speed of 200-300 rpm. Tap on the Temp icon to access the temperature control and set the reactor temperature to 37°C. Tap on the Air (1) icon to initiate the flow of oxygen. The oxygen added to the system comes from the house air. The GasFlo setting is pre-linked to the Air (1) value, once you have specified an Air value of 100% the GasFlo will begin. Due to presets, the icon labeled O2 (2) is actually connected to the nitrogen cylinder. This value will be adjusted later to calibrate the DO probe and/or modulate the oxygen saturation in the system depending on the experimental parameters being tested.

For proper polarization, the DO probe must be connected and the unit powered up for at least 2 hours prior to acquiring data. Dissolved oxygen measurements are very temperature sensitive, so wait until the temperature is stable before calibrating the DO probe (if DO measurements are required). Calibrate the DO probe by tapping on the Calibration icon at the
bottom of the touch screen and then selecting DO (Figure 6). Calibrate the DO probe by setting the zero value to be 0% when nitrogen is flowing into the system and the 100% value to be when the system is saturated with oxygen from the air inlet. You will notice after an hour or so of operation that the condenser at the top of the reactor will have a significant amount of condensation. This is a good sign, as it shows that the cooling water entering the system is at a low enough temperature to achieve condensation. Finally inoculate the reactor by pouring the entire content of an Erlenmeyer culture into the reactor through the inoculation port. You will need to halt the gas flow (set Air (1) to off or 0%), then unscrew the inoculation port. This is to prevent any of the reactor contents from being expunged through the condenser. It is advised to use a funnel when pouring the cells to avoid spilling any of the bacteria on the top plate of the unit. Take samples from the reactor and measure their optical density at 650nm (OD$_{650}$). This is accomplished by unscrewing the glass container connected to the sample port (Figure 4A) and opening the white plastic clamp. The gas flowing into the reactor will allow sufficient pressure for the sample to be collected into the disposable cuvette. It is possible that no suspension exits the system even when you open the clamp. This is often due to a kink in the tubing from the clamp itself (from the repeated clamping/unclamping necessary to collect samples). If this happens, move the clamp to another

Figure 6. Touch screen display that controls all reactor function including impellor agitation (Agit), temperature (Temp), oxygen inlet [Air (1)], nitrogen inlet [O2 (2)], and gas inlet flow rate (GasFlo). Display is also used to monitor dissolved oxygen levels (DO).
place on the tubing (either closer to or further from the reactor exit port; Figure 3, 7). Use some of the remaining 100ml of media as a blank for these measurements. Stop when you have accumulated enough data for a good growth curve.

To disassemble the reactor, reverse the above procedure: Turn off the agitation, gas flow and temperature control then remove the agitator motor, the thermocouple and dissolved oxygen probe. It is important to turn the temperature control off before the thermocouple is removed from the thermowell. Otherwise the temperature controller will respond by increasing the temperature of the water even to the point of generating steam because the thermocouple will be at ambient temperature which is typically much lower than the set point for the fermenter. Put the probe in buffer solution or electrolyte (1 M KCl) solution when not in use – the DO probe is stored in a graduated cylinder attached to the unit (filled with 1 M KCl). Turn off the chill water bath. Next, fasten all hose clamps before disconnecting the remaining connecting tubes between the unit the master controller. Take care when disassembling the condenser as water will sometimes remain in these tubes. Then unscrew the 6 thumb screws and lift the entire metal internals from the glass vessel; the internals are cleaned separately. Before disassembly, note the orientation of the inlets, especially the DO and the water inlets/outlets.

**Biomass concentration**

Dry cell mass concentration is determined indirectly by optical density or absorbance measurements at 650 nm (OD$_{650}$). All these measurements are converted to dry weight concentrations using a calibration curve. At low biomass concentrations, OD is proportional to cell concentration (mass/vol) and the best data for the biomass concentration are obtained if all OD measurements are taken in this linear region. Concentrated samples must therefore be systematically diluted with sterile media before measuring their OD. As a rule of thumb, dilutions must be used if OD$_{650}$ is greater than 0.6.

**Glucose concentration**

The yield can be determined by fitting the growth curve data to the model of choice, or it can be determined independently of the growth curve by measuring both biomass concentration and the concentration of the substrate in question for several samples taken during the growth curve. Note that the yield is defined for all components in the medium, not just the growth limiting component. As a practical matter, we only have the facility to measure glucose concentrations and so can only measure the biomass yield on glucose.

Additional shake flask cultures may be prepared for experiments to determine the yield constant or you may be able to use samples taken from the reactor. The samples should be at least 20ml and you will need at a minimum 2 samples taken at different times. For both samples, measure the biomass concentration and the glucose concentration. The yield on glucose then is calculated as (basically a restatement of eq. 3)

\[
Y = -\frac{\Delta X}{\Delta S}
\]  

where $\Delta X$ and $\Delta S$ are the changes in biomass and glucose concentrations, respectively.
6 References


7 Appendix – Operating instructions

Note that not all of these instructions may be relevant to your particular assignment.

Reactor operation
1. Prepare growth medium by dissolving the chemicals as instructed in DI water in a clean flask or bottle; do not label the bottle as yet. The growth medium must be sterilized prior to use and stored in the refrigerator; the instructor or movie will demonstrate. Charge 100ml to each 250ml Erlenmeyer used for growing the inoculum and charge 900ml of growth medium to the reactor. Allow the reactor content to come to the assigned growth temperature before calibrating the DO probe.
2. Make agitation violent (at least 300 rpm at first). Add *E. coli* suspension from an active liquid shaker flask from the incubator. Clean the flasks; bleach/water followed by hot soap solution.
3. Let the reaction solution mix for 1 min., and then take an initial sample.
4. Take samples at periodic intervals. Continue sampling until sufficient data are collected in the growth region. Note possible need for an early start, or late finish.
5. When shutting down the reactor, first add an aliquot of bleach to the reactor kill the *E. coli*. After a brief wait, disconnect the reactor, discard contents in drain and wash it down. Clean the reactor with bleach/water, then soap and water, and rinse with lots of DI water.

Use of JASCO spectrometer
Some composition analysis for this experiment is by UV or VIS spectroscopy. The Jasco spectrometer is used here. The switch for the unit is on the right side. Be sure to turn on the spec at least one hour, preferably two, before using it as it takes a while to warm up and you will get garbage results if the instrument is cold. The sample cuvette goes in the front compartment, the reference cuvette in the rear. Avoid touching the cuvettes on the clear sides, the side through which the light passes. Log on to the PC adjacent to the spectrometer and click on the "Spectrum Manager” icon to bring up the window in Figure 7.
Figure 7. Opening window for the Jasco spectrometer software.

Click on “Fixed Wavelength Measurement” and wait patiently until the window in Figure 8 appears.

![Fixed Wavelength Measurement Window]

**Figure 8. User interface for fixed wavelength measurements.**

To set the wavelength at which absorption is measured, click “Measurements” and “Parameters.” to bring up the interface in Figure 9.

![Window for setting the wavelength at which absorption is measured]

**Figure 9. Window for setting the wavelength at which absorption is measured.**

Type the desired wavelength in the small textbox and press “Add”. Highlight all other wavelengths in the large window and press “Delete”. Press “OK” to finish and return to the user interface in Figure 4. Unless instructed otherwise, you should always measure the optical density of cell suspensions at 650nm. Start by placing cuvettes with sterile media in both front and back compartments, press “Blank” then “Auto Zero” and you are ready to measure your samples. **Turn the machine off** at the end of the day, to conserve the lamps.
**OD measurement**

1. Remove a sample from the reactor and place it in a disposable cuvette. Be sure to drain the sample loop of old content before taking the sample for OD measurement.
2. Determine the absorbance at 650 nm, using the Jasco spectrophotometer. It should be <0.6 to be in the linear range of the spectrophotometer. If not in the linear range, dilute the samples with sterile media or DI water. Note the need to keep exact records of dilutions.
3. Use **pure sterilized growth medium, from same batch as used in the reactor** as the reference solution.
4. Put all used cuvettes and glassware and their contents in bleach/water. Discard this solution in the drain and rinse test tubes with DI water.

**Using the VWR Spectrophotometer**

![VWR UV-1600 PC Spectrophotometer](image)
**Set up**

1. Turn on the spectrophotometer by pressing the power switch located on the back of the unit.
2. Wait for the spectrophotometer to complete self-check and warm up, altogether about 30 minutes. (The unit must complete the warm up cycle to produce accurate results)
3. Once the spectrophotometer is ready select photometry on the display and press enter.

4. The spectrophotometer should be set to 650 nm by default. If it is not press Go to $\lambda$ on the unit and set the wavelength to 650 nm.
**Blanking**
1. Place a cuvette filled with LB broth into one of the four sample slots. Align the sample port with the sensor by moving the pull handle on the front of the unit.

2. Close the compartment cover and press the blank button, the spectrophotometer now has a reference point to compare sample absorbance.
3. Keep the LB broth blank in the sample tray to re-zero the unit if necessary.

**Measuring Absorbance**
1. Press the Start/Stop button, this will bring you to the absorbance chart.
2. Place a cuvette filled with sample in a sample slot.
3. Align the sample cuvette with the sensor using the pull handle on the front of the unit.
4. Hit the start button and wait for the results.
5. Repeat steps 2 through 4, using a new cuvette each time, until the experiment is complete.
6. If the spectrophotometer leaves the absorbance table page it will need to be re-zeroed before samples can continued to be measured.

**Shutdown**
1. Dispose of any samples or LB broth blanks from the sample ports.
2. Turn off the spectrophotometer by pressing the power switch on the rear of the unit.

**Dry weight measurements**
The proportionality constant between OD and biomass concentration must be determined experimentally. Additional shake flask cultures may be prepared for these measurements. Samples of known OD and volume are filtered on pre-dried and pre-weighed filters. The cell pellet on the filter is washed with water to remove any media remains and the filter with cells is dried at a prescribed temperature until constant weight. The weight of the pre-dried filter is subtracted from the final weight to get the dry weight measurement of the sample. The dry weight measurement is sensitive to the drying temperature, so it is important to dry all samples at the same constant temperature. Unless instructed otherwise, use a drying temperature of 60°C.

**Spectrophotometric glucose analysis**
Each sample is filtered to remove cells and the filtrate is collected for later analysis. The easiest way to do the analysis is to collect all needed samples and analyze them all at the same time. Samples that are not analyzed right away should be stored in the freezer until ready to be analyzed. The analysis is done using a standard diagnostic kit from Sigma.

1. Thaw frozen samples and label test tubes as: BLANK, STANDARD, and SAMPLE1, SAMPLE2 etc.
2. To BLANK, add: 0.5 ml of distilled water (use auto pipette)
3. To STANDARD, add: 0.5 ml of a glucose Standard Stock solution (0.05 g/L).
4. To each SAMPLE, add: 0.5 ml of an appropriately diluted sample. Make dilutions so that the glucose concentration in the samples to be measured is no more than 5 times greater than the glucose Standard Stock solution concentration. For example, if the initial glucose
concentration in the fermentor were 10.0 g/L, the ratio of the concentrations is then:

\[
\frac{C_{\text{sample}}}{C_{\text{standard}}} = \frac{10 \text{ g/L}}{0.05 \text{ g/L}} = 200
\]

Thus a 50-fold dilution of the sample would reduce the concentration ratio to 4. Note that concentration and absorbance are related proportionally only if diluted to the "linear range”.

To each tube, add 5.0 mL of Enzyme Color Reagent Solution prepared as follows:

1. Add one capsule of PGO Enzymes to 100 mL of distilled water in an amber bottle.
2. To the enzyme solution, add 1.6 mL of o-dianisidine dihydrochloride solution. The o-dianisidine dihydrochloride solution can be made simply by adding 20 mL water to the powder and dissolving it in the original bottle.
3. Mix color reagent solution by inverting bottle several times.
4. Store all assay chemicals in the refrigerator. They are stable for ~1 month.

Finally,

1. Incubate all tubes at 37°C for 30 minutes in the incubator. During this period, the oxidase and peroxidase reactions of the glucose go to completion.
2. Read absorbance of STANDARD and SAMPLES at 540 nm using BLANK as reference solution using the Jasco spectrophotometer.

IMPORTANT: Do not leave BLANK, STANDARD and SAMPLES standing for more than 30 minutes after incubation. Make additional BLANKs and STANDARDs as necessary.