Exercise 12: Yeast Growth Curve

Monitoring growth rate of fission yeast strain

You will inoculate a fission yeast culture and monitor the growth of the fission yeast cells throughout the day.

Objectives:
1) Learn how to use a spectrophotometer
2) Learn how to monitor the growth of a fission yeast cells growing in liquid culture
3) Learn how to graph collected data to determine generation time

Materials needed
- 8 cuvettes
- Spectrophotometer
- Glass pipets
- Electronic pipettor
- Pipetman set
- Pipet tips
- Yeast culture [Note: You will be using the fission yeast strain you were originally assigned on the first day of class] WRITE DOWN THE STRAIN NAME: “SP____”
- Sterile 125-ml culture flasks
- YES media
- Semi-log graph paper
- 25°C air shaker/water bath

Background Info
(Adapted from http://www-saps.plantsci.cam.ac.uk/worksheets/scotland/double.htm)

A standard lab technique is monitoring the growth rate of a microorganism cultured in liquid media. By plotting the number of cells against time, one can generate a growth curve (Figure 1). As the number of cells increase in this culture, the cloudiness or turbidity of the culture increases. This turbidity is the result of the cells suspended in the liquid media scattering the light. The turbidity can be measured using the spectrophotometer.

Figure 1. Typical yeast population curve. This graph shows the growth of a yeast population cultured in a flask. Initially the culture shows exponential growth. However, population begins to level off (plateau) and reaches a stable value. This type of growth curve is called sigmoid or S-shaped.

Addition of fresh culture medium to the flask results in exponential growth until a new higher plateau is reached.

From http://www.ggause.com/gfg04.htm
A specific wavelength of light moves through a cuvette containing the cell suspension. If the cuvette contained only liquid media, the spectrophotometer can measure the amount of light that is absorbed by this “blank” cuvette. When cells are in the cuvette, the cells will scatter the light and the spectrophotometer will measure this scattering as in increase in absorbance. This scattering, or absorbance, increases as the cell concentration increases. Thus, the spectrophotometer can be a convenient tool to measure cell growth rates.

Figure 2 below shows a growth curve plotting cell number vs. time. However, for this exercise, you will be plotting absorbance vs. time on a graph. Doubling of absorbance indicates doubling of the number of cells and the time taken for this to occur can be read from the graph. You can use this plot to calculate the doubling time and growth rate constant using absorbance as the measure of growth.

**Inoculation of culture**

1. Sterilely transfer 20 ml of YES liquid media into a culture flask, and cap the flask. Label your flask with your team’s initials and the strain number (“SP_____”).
2. Obtain a blue-cap tube containing a fresh overnight culture of your assigned fission yeast strain.
3. Transfer 1 ml of the fresh fission yeast culture into the 20 mL YES media, and recap the flask.
4. Incubate the culture flask in the 25°C air shaker.
5. Record the OD$_{595}$ of the culture every hour.

**Determining Absorbance (OD$_{595}$) of Cell Culture (BioRad Spectrophotometer)**

1. Set the wavelength ($\lambda$) of the spectrophotometer to 595 nm.
2. Insert a cuvette containing 1 mL of YES media into the cuvette slot (Blank – cover the top of your Blank tube with parafilm and use it to blank the spectrophotometer for each reading.). Close the cuvette chamber. Read the Blank (should display 0 now).
3. Insert your cell suspension cuvette into cuvette slot.
4. Close the cuvette chamber.
5. Measure the Absorbance of your cell suspension.
6. Record the time you measure the absorbance of your cell suspension
7. Take readings every 1 hour.

Use Table 1 below to record your readings.
Note: If your absorbance reading is greater than 1.0, you must make a 1:10 dilution in YES media and repeat the reading.

**Table 1: Data collection of Absorbance readings**

| STRAIN: ____________ |

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time</th>
<th>OD$_{595}$</th>
<th>Dilution, if any</th>
<th>Final OD$_{595}$</th>
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<tbody>
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**Determining Growth Rate**
1. Plot the Final OD$_{595}$ vs the time (see Figure 2) on semi-log paper.

   X-axis: ________________________

   Y-axis: ________________________
2. Determine the growth rate (doubling time, generation time) of your strain.

   Growth rate: ________________________________