ABE 304
Bioprocess Engineering Laboratory

EFFECT OF TEMPERATURE ON CELL GROWTH & XANTHAN PRODUCTION

INTRODUCTION AND SCOPE

Fermentation is a biological process whereby microbes produce chemical compounds through the consumption of a food source. Fermentation processes can yield simple products such as ethanol or butanol, or more complex products such as polysaccharides. For a reliable fermentation process, the following materials are required: a chosen microorganism that produces high yields of the desired product, inexpensive raw materials, a short fermentation time, and an efficient purification process.

AIMS

- To determine the temperature dependence of *Xanthamonas campestris* growth and the production of xanthan gum from glucose.
- To determine the yield of xanthan gum as a function of temperature.

MATERIALS AND METHODS

Materials required

- Cell culture media (Shu & Yang, 1990)
- 3 fermentation cultures
- Sterile (autoclaved) 250 mL Erlenmeyer flask
- 5 mL sterile pipette tip
- Incubated shaker
- 12 15 mL sterile centrifuge tubes
- Spectrophotometer

PPE:

- Lab coats
- Gloves
- Safety glasses

Fermentation cultures:

Each group will have three cultures:

1. A TA-prepared culture flask of *Xanthamonas campestris* from two days prior to the lab period.
2. A TA-prepared culture flask of *Xanthamonas campestris* from one day prior to the lab period.
3. Student-inoculated sample of *Xanthamonas campestris*.

Each group is expected to properly take note of the culture start times for any necessary calculations.

Innoculation protocol:

1. Using aseptic technique, aliquot 5 mL of culture #3 into media in a 250 mL flask. The media is composed of: (Shu & Yang, 1990)
   a. 25 g/L Glucose
   b. 3 g/L Yeast extract
   c. 2 g/L K_2HPO_4
   d. 0.1 g/L $MgSO_4 \cdot 7H_2O$
   e. ~0.15 mL of Anitfoam A

   <u>*Technique tip 1:*</u> Be sure to touch the tip of the pipette into the broth as you expel. Do not drop from the air high above the surface of the media. You want to be sure that all of the small volume is dispersed into the medium.

Technique tip 2: Do not touch the sides of the flask with the pipettor in order to minimize spread of contamination by pipetting.

2. Place the flask in the incubated shaker at either 25˚C or 30˚C and agitation speed of ~150 rpm.

Data collection

1. Every 45 minutes, collect 3 mL of sample from each culture flask into a centrifuge tube.
2. Label the cell culture and the time of each sample *clearly* on the sample tube.
3. Obtain a minimum of 4 samples of each cell culture.

Spectrophotometer Use

A spectrophotometer measures the relative light absorbance of a sample; therefore, the device must be "blanked" before measuring a given sample. Typically, the chosen blanking solution would be a buffer of similar pH to that of a sample. However, water is a valid choice of blank solution as well. To "blank" a spectrophotometer, place the "blank" cuvette into the sample holder, close the door, and wait for the reading to stabilize. Record the value shown on the device.

Sample Preparation

1. Remove 1 mL of broth from the fermentation flask and place in a microcentrifuge tube. Do this twice so that you have two tubes containing 1 mL each.
2. Place each tube in the microcentrifuge and spin at 12,000 rpm for 25 minutes.
   BE SURE THAT THE CENTRIFUGE ROTOR IS BALANCED
3. The cells are now concentrated in the bottom of the tube and the supernatant will contain the xanthan and the glucose.
4. Transfer the supernatant from each tube into a clean microcentrifuge tube set aside for measuring Glucose and Xanthan concentrations in Week 2. These tubes will be frozen until use in Week 2. You will need to use a microcentrifuge tube rack for storage.
5. Use the cells for measurement of cell growth in Week 1.

Cell Density

The cell density will be measured to be able to determine some growth kinetics of the bacteria. The relative density will be measured, as opposed to the absolute density, so the results will not be exact but will indicate a trend.

1. Remove 1 mL of broth BEFORE inoculation, this will act as your reference blank.
2. Dilute 1 mL of sample with 2 mL of water in a cuvette.
3. Measure the OD at 600 nm in the spectrophotometer.
4. Each time you measure a sample, measure the OD of the reference blank.
5. Estimate the cell density Equation 1

$$\text{Cell Density} = S\text{ample}\text{OD}_{600} - \text{Blank}\text{OD}_{600} \qquad (1)$$

Glucose Concentration

The glucose concentration will be measured using a colorimetric test and quantified using a spectrophotometer. A glucose oxidase/perioxidase (GOPOD) kit will be used for the assay. Glucose oxidase will convert the D-glucose in the broth into D-gluconate and hydrogen peroxide. The resulting hydrogen peroxide is converted into quinoneimine dye via perioxidase. The reagents for the assay will be prepared before the start of class.

1. Pipette 0.1 mL of the stored supernatant into a test tube.
2. Add 0.9 mL of DI water to solution.
3. Pipette 0.1 mL of diluted sample into a second test tube.
4. Add 3.0 mL of GOPOD to the test tube.
5. Incubate in the oven at 40˚C for 20 minutes.
6. Read absorbance in the spectrophotometer at 510 nm.

7. Read reagent blank in spectrophotometer at 510 nm.
8. Use Equation 2 to determine the concentration of D-glucose in your sample.

$$\text{D-Glucose}\left(\frac{\mu g}{0.1mL}\right) = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{D-glucose standard (100 }\mu g)}} \times 100 \qquad (2)$$

Xanthan Concentration

The concentration of xanthan produced during fermentation can be measured using an oven-drying method, with a viscometer, or a spectrophotometer. This lab will explore the use of an acid digestion followed by quantification using a spectrophotometer. A stock solution made up of 0.1 g of xanthan gum in 1 L of water is provided.

Calibration curve

1. Retrieve 6 tubes and create the following solutions. Label each tube *clearly*.
    a. Tube 1: Add 2 mL of the stock solution to the first tube.
    b. Tube 2: Add 0.75 mL of the stock solution and add to the second test tube along with 0.25 mL of water.
    c. Tube 3: Remove 1 mL from tube 1 and add to tube 3. Add 1 mL of water. . Mix well.
    d. Tube 4: Remove 1 mL from tube 3 and add to tube 4. Add 1 mL of water. Mix well.
    e. Tube 5: Remove 1 mL from tube 4 and add to tube 5. Add 1 mL of water. Mix well. Pipette 1 mL from this tube and discard leaving only 1 mL in the tube.
    f. Tube 6: Add 1 mL of water. This will act as your blank or zero concentration tube.
        i. This is known as a serial dilution. Calculate the concentration of the mixture in each tube.
2. IN THE FUME HOOD –add 3 mL of concentrated sulfuric acid to each of the tubes containing the xanthan solution. Mix vigorously for 30 seconds. The reaction will increase the temperature of the tubes within 10-15 seconds (Albalasmeh, Berhe, & Ghezzehei, 2013).
3. Cool each tube in ice for 2 minutes to bring back to room temperature.
4. Carefully pour the contents of each tube into a cuvette and read in the spectrophotometer at 325 nm.
5. Make a plot of xanthan concentration (x-axis) versus absorbance (y-axis). The points should form a line and the equation can be used to determine the unknown concentrations in the fermentation samples.

Fermentation Samples

1. Pipette 0.1 mL of the stored supernatant into a test tube and add 0.9 mL of water. This will be a 10x dilution. You will need to account for this when calculating the actual concentration.
2. IN THE FUME HOOD – wearing a lab coat, gloves, and safety glasses – add 3 mL of concentrated sulfuric acid to the tube. Mix vigorously for 30 seconds.
3. Cool in ice for 2 minutes to bring back to room temperature.
4. Carefully pour the digested contents into a cuvette.
5. Read in the spectrophotometer at 325 nm.
6. Use your calibration curve to determine the concentration of xanthan in each sample. If your absorbance is not within range, dilute your sample with more water and run the procedure again.

DATA ANALYSIS

- Data will be combined from both Monday and Wednesday sections. All students will have access to both incubation temperatures and all concentration samples measured. Data must be entered into the shared spreadsheet by February 23 at 5 PM.
- Make a plot of cell density, glucose concentration, and xanthan concentration as a function of time.

Do this for each temperature. What trends do you see? How does temperature affect cell growth or product formation?

- Use the relative cell density as a function of fermentation time to determine the specific growth rate, μ, of the bacteria. How does μ change with temperature?
- Calculate the xanthan yield both graphically (plot of glucose concentration versus xanthan concentration) and using the final xanthan concentration divided by the initial glucose concentration. How do these values compare?
- Calculate the theoretical and the observed xanthan yield. Comment on why these two may differ at each temperature.

REFERENCES

Albalasmeh, A. A., Berhe, A. A., & Ghezzehei, T. A. (2013). A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. *Carbohydrate Polymers*, *97*(2), 253–261. http://doi.org/10.1016/j.carbpol.2013.04.072

Shu, C. H., & Yang, S. T. (1990). Effects of temperature on cell growth and xanthan production in batch cultures of Xanthomonas campestris. *Biotechnology and Bioengineering*, *35*, 454–468. http://doi.org/10.1002/bit.260350503