

E. coli Strain B Ethanol Production Lab

Apparatus: Sterile 50 mL graduated cylinder, Sterile 250 mL grad cylinder, 2 sterile 125 mL flasks, 1 sterile 500 mL flask, 16 sterile culture tubes (number depends on how long the test will be run; 2 tubes for every hour or test period between taking data points), 8 red caps and 8 blue caps (again number depends on number of culture tubes; red have holes and will be anaerobic), Sterile pipettes, Sterile rubber stoppers, Sterile needles, 1 L flask for Glucose, 500 mL flask for LB (Luria broth), 1 sterile test tube (needs to hold about 5 mLs) (*Is this everything? And should that marked in red be included or tested or stated?*)

This starts with the procedure we used for the first time we ran the experiment (*not sure what this means or if it's important*)

Procedure

In the end we would like our media to be 5% glucose and 1X LB. The number of mLs required depends on how much media you will need in the end. In our first run we made 500 mL (make a little bit extra in case you need more). (*<- Include this?*)

To make 1x LB broth, combine:

1. 10g Bactotryptone
2. 5 g Yeast extract
3. 5 g NaCl

In order for our final concentrations to work out correctly, we will be making 2x LB. Dissolve 20 g bactotryptone, 10 g yeast extract, and 10 g NaCl in house deionized water and bring to 1 L. Autoclave on 20 minute liquid cycle

To make 10% (w/v) Glucose solution:

1. Start with 50 mL Milli-Q deionized water in 100 mL graduated cylinder.
2. Add 10 g Glucose (dextrose) with the sterile stir bar.
3. Once this mixture is dissolved, bring to 100 mL with Milli-Q deionized water
4. Put the final mixture into bottles and autoclave for 20 minutes on liquid cycle.

Making the bacteria culture:

1. Combine 4.75 mL of the 1x LB with .25 mL of the 40% glucose (2% sugar total in final solution).
2. Add one bacteria colony to this solution:
 - a. Heat the scraper with a Bunsen burner
 - b. Let it cool
 - c. Scrape off one colony and place it into the solution
 - d. Heat the top of the solution flask and its cover and then close it
 - e. Heat the scraper again
 - f. Place the tube in a shaking incubator at 37 degrees overnight

Conducting the experiment:

1. Put the 2X LB solution into the 10% glucose solution.
2. Place 5 mL of the bacteria solution into the 5% glucose, 1x LB solution (1/100 ratio)
3. Put specified amounts of this solution into the different flasks and tubes. For example:
 - a. 500 mL flask → 112 mL solution
 - b. 125mL flask → 25 mL solution
 - c. 125mL flask → 112 mL solution
 - d. Blue culture tubes → 5 mL
 - e. Red culture tubes → 20 mL
4. Place all of these tubes and flasks into the shaking incubator at 37 degrees.
5. Take a 1 mL sample out of each of the flasks every hour and one of each of the red and blue tubes as well.
6. Observe absorbance and pH
7. To observe absorbance:
 - a. Cover cuvette with parafilm
 - b. Invert to mix
 - c. Clean with Kimwipe
 - d. Place in holder
 - e. Clean with Kimwipe again
 - f. Place in spectrophotometer
 - g. Observe absorbance
8. To observe pH simply dip a pH indicator stick into the cuvette holding the solution.

Subsequent experiments (all the data and graphs should be on the sustainability server; these should show our findings):

Experiment 2- This was done the exact same as before except only the test tubes were used. Each data point had three aerobic and three anaerobic tubes. The pH was also taken using a pH meter instead of an indicator stick. Seeing as the aerobic samples only had 5 mL we poured this sample into the cap so that the pH probe could get a reading.

Experiment 3- This time E. coli strain B was tested against the strain of E. coli sent to us by the Ingram lab with the plasmid in it. The aerobic and anaerobic tubes were placed in different incubators. The aerobic one was moving at 250 revolutions per minute and the test tubes were stuck in at a slanted angle to facilitate more contact with air. The anaerobic ones were in the water filled incubator and moved at 150 revolutions per minute.

Experiment 4- This experiment was carried out similar to experiment 3 except this time E. coli strain B was tested against E. coli strain B with the plasmid (pLOI 297). The big difference is we also started to collect samples to be tested for ethanol concentration. We filled centrifuge tubes with a sample from each

of the tubes. This was then centrifuged for 2 minutes at a medium speed. This sample was then transferred to another centrifuge tube without the pellet. These were then labeled and set in the fridge.

Further experiments:

In the next experiment I believe most of the method will stay the same except for when you collect samples to test for ethanol. Two and a half milliliters of solution will be placed in a 5 mL GC-MS vial. The only problem I see here is that the solution never gets centrifuged. This may or may not be a big deal. These can then be stored until you run them through the GC-MS (look at GC-MS word document to see how this is done).

The other things to explore would be different ways to maximize ethanol production. You could look at using a buffer to keep the pH at a specific level and hopefully keep up bacteria growth. You could also look at using different sugars in the media to see if they would be better. Different incubator temperatures (all ours were at 37 degrees C) might also have an effect on growth.