Yeast Chemostat
Laboratory 3
BCHE 8210

Introduction

In lecture we discussed continuous growth of microbial cells. This experiment involves conducting steady-state "chemostat" experiments using one strain and at one dilution rate. There is just too much preparation needed to accomplish more!

The volume of the ultimate chemostat will be 1.5 liters. You will not control the pH, and we will not use the pH probe. However, at the end of the experiment we will measure what the pH is. You will not control the DO, but we will measure the DO and therefore will use a DO probe.

The culture will be carbon-limited. The glucose concentration of the feed will nominally be 15 g/L. The medium also contains "Yeast Nitrogen Base without Amino Acids", which is a prepared medium with various macronutrients and vitamins to support yeast growth. From this prepared medium, the feed will also contain 5 g/L ammonium sulfate.

The experiment will have essentially three phases. The first phase will be a shake flask inoculum. The second phase will be a batch phase in a bioreactor. The third phase will be a continuous process using the glucose-limited medium. After steady-state is reached (after at least 24 hours), samples are taken at the end of this third phase.

Preparation of Agar Plates (for 1 liter):
Prepare a 10x stock solution by dissolving 6.7 g yeast nitrogen base without amino acids (YNB-NoAA) and 5 g glucose to a final volume of 100 mL. The solution can be warmed to add dissolution.
Into 900 mL water add 15 g agar, warm with stirring to dissolve the agar. Autoclave.
When the agar is withdrawn from the autoclave (and still warm ~ 75°C), add the 100 mL by sterile filtration under the biological safety cabinet. Pour plates as usual prior to the mixture solidifying.

Preparation of Culture Medium:
Prepare a 10x stock solution by dissolving 50.25 g YNB-NoAA and 112.5 g glucose to a final volume of 750 mL. The solution can be warmed to aid dissolution.

1) Autoclave 90 mL water in 250 mL shake flask
2) Autoclave 1260 mL water in bioreactor with DO probes and calibrated pH meter.
3) Autoclave 5.4 liters water in carboy.

When the vessels are withdrawn from the autoclave (and still warm ~ 75°C), use sterile filtration under the biological safety cabinet to:

1) Add 10 mL of 10x stock to the shake flask.
2) Add 140 mL of 10x stock to the bioreactor.
3) Add 600 mL of 10x stock to the carboy.

Bioreactor set-up:

1) Batch Phase:
   - Volume: 1.5 liters
   - Temperature: 30°C (Yeast will not grow at 37°C)
   - pH: not measured online

- DO: measured but not controlled
- Offgas: measured but not controlled
- Agitation: 200 rpm
- Air flowrate: 0.3 liters/min
- Inoculate and allow cells to grow to saturation.

2) Chemostat:
   - Carboy feed with 6 liters of medium.
   - When the maximum cell density in the batch phase has been achieved, commence feed pump at a dilution rate of 0.10 – 0.15 h^{-1} (range of 2.5 mL/min – 3.75 mL/min)
   - Same conditions as for the batch process
   - Allow the cells to proceed for 24 hours (3.6 residence times).
   - Measurements:
     1) Off gas CO_2 and O_2.
     2) DO
     3) Dry Cell Weight: 3 × 50 mL yeast culture. Centrifuge*; wash; centrifuge; wash; centrifuge. Dry pellet for 24 hours at 60°C in a weighing boat (record mass before and after adding yeast)
     4) Glucose and products: *save supernatant of each of the samples used to measure dry cell weight.
     5) **MAKE SURE you measure the CO_2 and O_2 composition of the influent Air.**
     6) **MAKE SURE you take three samples of the feed to measure for glucose.**

Procedure:

Sunday:
- Evening: Inoculate plate with yeast and incubate at 30°C

Monday:
- Make medium
- Afternoon: Transfer plate culture to shake flask
- Assemble Bioreactor

Tuesday:
- Morning: calibrate DO probe
- Afternoon: Transfer shake flask culture to bioreactor

Wednesday:
- Morning: Initiate feed pump, commencing chemostat

Thursday:
- Morning: 24 hours after initiating feed pump, take samples as described above.