Bioreactor

Introduction

In this protocol the instructions on how to prepare and load a bioreactor are given.

Materials

- 1 L Sartorius Stedim bioreactor
- Control tower
- Off-gas
- YPD media
- Sigma 204 antifoam
- Sterile arginine solution
- Acid (2 M sulfuric acid)
- Base (2 M sodium hydroxide)
- Spore suspensions
- 1 mL syringes
- Lab grease
- White strips

Procedure

Assemble the bioreactor

1. Mount the inoculation, sampling and temperature port from the bottom of the lid and secure them with the bolt from the top of the lid.
2. Screw the impellers at the right heights.
3. Attach the sparger and secure it with a bolt from the top of the reactor.
4. Mount the condenser in one of the available sample ports. Try to put it on the side closest to the control tower.
5. The top of the condenser should be packed with a small amount of glass wool. Do not pack them too hard. Close the condenser top.
6. Mount the temperature probe and the sampling pipe. Attach the sampling tube on the top of the pipe and mount a butterfly clamp and a regular clamp on the tube.
7. Assemble inoculation port. Check that there is a membrane in the thin port. Otherwise, put a membrane in there, and make sure there also is a metal gasket (O-ring).
8. Attach the 4-way connector to one of the empty ports. We need 2 of the connections, so close the third and fourth connection with the piece of knot tubing.
9. Attach a marprene tube to the air inlet in the fermentor, attach the air filter, make sure it is placed in the right direction (writing on the filter should be on the side toward the tower)
10. Assemble the bioreactor. Put a small amount of lab grease on the top rim of the fermentor glass, to help maintain the rubber gasket. Use only your fingers to screw the lid on the fermentor. Finger-tighten the screws just a bit.

11. Connect the empty 250 mL Blue Cap bottles to the 4-way connector with a sufficiently long piece of tubing, so that the tube can pass through the pump. For the part of the tubing that goes into the pump put pump tubing. We do not autoclave the acid and base with the fermentor due to the risk that they will spray out and over the reactors during autoclavation.
12. Clamp the tubes between the fermentor and the bottles. Put the clamps as close as possible to the reactor. Further clamp the tube to the air filter on the glucose bottle.
13. Ensure that all possible tubing is secured with white strips (pay attention to the piece of tubing that hold the air filter).
14. Fill in 1 L of minimal medium with 500 μL Sigma 204 Antifoam, use a funnel and a beaker to measure the right volume.
15. Calibrate the pH-electrode. Connect the electrode to the control tower. Also connect the temperature probe to the tower to have a reference temperature for the pH. On the DCU enter the calibration menu. Press the green Measure box on the right fermentor. In mode, select Calibrate. Select Group Calibrate. In the sub-menu select calibrate zero, select automatic. Make sure the pH is selected at 7.0, and press OK. Rinse electrode of buffer solution with distilled water and place it in buffer with pH=7.00. When the reading is stable press OK. Continue with selecting calibrate slope, follow the method of calibrating zero, but place electrode in buffer pH=4.01. Disconnect the pH-electrode. Put the black cap back on it. Put the pH-electrode into the bioreactor and do not switch off the controller. The pH electrode can easily break if it is not handled with care!
16. Perform the pressure test of the reactor. To do that you should ensure that all clamps and screws are tightly closed so that no air can get in or out of the reactor. Connect the air tubing and turn on the air on the DCU (1.00 vvm). Mount the clamped air tube to the condenser outlet (tubes will be provided by teachers).Wait a few minutes. If the reactor is properly sealed, the amount of air entering the reactor will decrease and get equal to 0, and no bubbles from the sparger, when the system is saturated. If the pressure is not dropping, the leaking spot on the reactor should be found. That can be done by spraying alcohol on different lid elements until bubbles are observed. Be aware that reactor is under pressure so always first release the pressure by removing the clamped air tubing from the condenser!! If the air filter is removed from the tower first, you will lose your media through the filter.
17. Cover all open endings and filters on the outside with tin foil to protect them while autoclaving.
18. Check all clamps: all clamps are closed on the tubing between the bottles and the fermenter. The two clamps on the air inlet is closed as well. Unscrew the big screw in the fermenter lid and place it loosely in the port, put a beaker above it. This is to prevent overpressure in the fermenter during autoclavation.
19. Autoclave the bioreactor.
20. After autoclavation close the fitting that has been kept opened. Mount the tube from the air supply, set flow rate on the DCU to 0.75 vvm. Thereafter remove the clamp from the tubing for the inlet air supply.
21. Connect the condenser tubing, and the temperature jacket tubing (connect out, then in).
22. Connect the pH-electrode and DO electrode.
23. Connect the stirrer and set stirrer speed to 800 RPM on the DCU.

24. Once the medium is cooled down determine the pH with an external pH meter and compare it with the number that pH controller shows. If there is any difference, the pH set points should be adjusted accordingly.
25. Mount the tubing for acid and base addition on the respective pumps. Set the pump to manual and let the acid/base fill the tube and set the pump to Auto as soon as it hits the reactor!
26. Set the timer to zero at the DCU and start your batch at the computer to monitor your process values.
27. Calculate the inoculum volume (final concentration of 10^6 spores/mL) and add it to the fermentor.

Process conditions

Load program with the following ramps for the process conditions:

Process conditions	
Airflow inlet	
Time (min)	ValueVVM (L/L/M)
0	0.1
300	0.16
420	0.4
600	1
pH	
Time (min)	ValuepH
0	3
600	5
Stirrer speed	
Time (min)	ValueRPM
0	100
300	200
420	300
600	500
720	800
Temperature	
Time (min)	Value(°C)
Start-end	30

Sampling

1. Insert syringe in the sampling tube
2. Loosen the clamp on the sampling tube and withdraw approx. 5 mL for waste
3. Instert syringe in sampling tube again and withdraw approx. 10 mL for samples.
4. Close clamp on sampling tube.

5. Add 2 x 1.5 mL sample to two Eppendorf tube.
6. Fill HPLC vial with sample filtered through a 0.45 μm glass fiber filter