

Cell-Cultivation

Fermentation

Aims of the experiment

Cultivation in fermenter is used for preparing bacteria in huge bench with control of pH and pO_2 .

Materials

- fermenter
- 2L 2YT+P media
- Rosetta strain
- Chloramphenicol
- Antifoam

Preparation

1. Fill 2YT+P media into a clean fermenter.
2. Empty all the tubes using the pumps and close all tube clips.
3. Remove all filters and insert desired probe (i.e. pO_2 and pH).
4. Cover all in- and outlets in aluminum foil.
5. Autoclave the fermenter.
6. Prepare an Overnight Culture of Rosetta in 2YT+P of 1:100 culture with 1:1000 Chloramphenicol (Cm).

Procedure

1. Prepare the fermenter by connecting to the machine and computer.
2. Cool down the water bath temperature to 16°C.
3. Install all the motors and connect required probes.
4. Open all tube clips for the pumps that will be used.
5. Set the machine with airflow of 2.000 L/min, air mix on at 21% O_2 and offline parameter as OD 600.
6. Open the air pressure before starting the batch.
7. Add 1ml antifoam by syringe into the fermenter.
8. Make sure the water volume reaches 2L and pO_2 is calibrated to 100%.

9. Add 1:1000 Cm through the septum.
10. Add 1:100 Overnight Culture to the reactor using a 20ml Syringe when everything is settled.
11. Measure and record the OD of the culture from time to time.
12. Occasionally measure pH by using pH indicator stripes (pH 7 – 14).
13. Harvest cells at OD 1.8 – 2.0.
14. Continue with the Harvesting and Washing Protocol.