Experiment – 6

OBJECTIVE:

To understand the temperature control system of bioreactor and to carry out blank sterilization of the reactor.

INTRODUCTION

The cultivation of microorganisms, in bioreactors, is done under sterile conditions. As such, medium and the sensors along with other accessories should be properly sterilised. Before proceeding for fermentation work we must ensure that the fermentor (bioreactor) is thoroughly cleaned.

DESCRIPTION

The bioreactor system consists of a vessel with top and bottom plates, agitator shaft with impellers, baffles, sparger tube, sampling/ harvest valve, port adapters & blind plugs etc. The instrumentation and control system includes pH, temperature, dissolved oxygen and antifoam sensors and monitors/ controllers. Some bioreactors may be fitted with additional instruments.

PROCEDURE

1. Prior to starting sterilization of the bioreactor, check all the silicon rubber tubes for leakage and then sterilize the following in an autoclave:

    a) Hypodermic needles with silicon rubber tubes for delivery of acid and base solutions.

    b) Air delivery needle with silicon rubber tube with inlet air filter.

    c) Calculated amount of distilled water in 2X500 ml flasks for preparation of sterile solutions of acid and base. (After autoclaving and cooling the two flasks, required amount of strong solution of acid/base should be added aseptically into the flasks. The strong solutions are considered sterile by their virtue)

    d) Inoculum transfer bottle with silicon rubber tube and delivery needle.

    e) A 250 ml flask containing antifoam agent with silicon rubber tube and delivery needle.

    f) 500 ml distilled water in 1L aspirator bottle with silicon rubber tube and delivery needle.

Ensure the steps (a) to (f) are completed before starting sterilization of the bioreactor.

2. Working volume in a fermentor is about 65–70% of the fermentor's total capacity. For example, the maximum allowable working volume for 3L total capacity fermentor is about 2.L. The minimum allowable limit of the working volume will be determined by the locations of the various sensors. The

working volume includes both the initial medium volume and the inoculum volume. In our example, if the inoculum is 5% (v/v), the inoculum volume will be 100 ml and the medium volume will be 2.9L. The quantities of medium components to be added are calculated based on the working volume. Now pour the 2.9L medium into the fermentor.

3. Calibrate the pH sensor and fit it on a port of top plate of the fermentor. Fit the DO sensor and the temperature sensors. Fit the exit air filter/ condenser assembly. Put silicon rubber septum on ports with adapters and then close all ports with blind plugs.

4. Adjust the impeller agitation at 400-600 rpm. Ensure availability of water and air through their respective supply lines. Connect the inlet air filter (sterilized in step 1-b) to the air delivery line. This will make the filter dry before it is needed.

5. Switch on the temperature controller and adjust the set point lower than the measured value of temperature. This will ensure that the entire tubing loop of temperature control system is filled with water. Now take the set point to 121^0C and open stem valve. Open exhaust air valve slightly so that air in fermentor's headspace escapes out. Fix protection hood.

6. When the temperature rises and comes to 95-100^0C, pressurize the pH probe to 1.5 bar pressure with an air pump to ensure that the electrolyte in the pH sensor does not boil off. Crack-open the exit air valve so that the steam flushes out slowly through it. When the temperature comes to 121^0C, it should be allowed to stay at this value for the duration of the holding time (30 minutes).

7. After holding time is over the set point temperature is adjusted down to working temperature and the fermentor is allowed to cool by the ambient air. When the temperature comes to 104^0C, inlet air filter is inserted through one of the ports of fermentor's top plate over the sparger tube. Now the head space pressure will rise due to incoming air.

8. When the temperature comes to 85-95^0C, enable cooling by tap water (20-30 ^{o}C). De-pressurize the pH probe and open exit air valve slowly. When the temperature comes to 45^0C enable cooling by chilled water (12-16^0C) and switched on the DO and pH monitors. The initial cooling by tap water minimizes stress on heat transfer surface due to temperature shock.

9. When the fermentor cools down to the working temperature, allow the fermentor to stay as such for at-least 2h for the DO reading to stabilize. Add make up water (sterilized in step 1-f) aseptically, if required. Set pH to the desired value and calibrate the DO probe. Now the fermentor is ready for inoculation.