Experiment No. 9

Continuous mode of operation of bioreactor

Objective:

Continuous production of ethanol using *Saccharomyces cerevisiae*

Introduction:

Continuous cultures perform better than the batch cultures economically by reducing or eliminating downtime, which is lost for cleaning, sterilization of the reactor and medium, and restarting the bioreactor. Furthermore, the levels of parameters (concentration of carbon and nitrogen sources, product formation, pH, redox balance etc) will change with time in the batch culture, whereas the continuous culture afford a steady state growth in which input and output are in perfect balance. There are three main types of continuous cultures, viz., chemostat, turbiodostat and auxiostat. Depending on the set-parameter, chemostat and turbiodostat are also known as nutristat or pHstat. In an auxostat, the feeding rate is adjusted to match the rate of cellular metabolism. The primary form of continuous culture is a steady-state CFSTR or chemostat. A chemostat ensures a constant growth in the reactor. The net growth rate is equal to the dilution rate. A constant cell density is maintained in the turbidostat by adjusting the flow rate. A turbidostat operates well at high flow rates (near the washout point) and is useful in selecting cellular subpopulations that have adapted to a particular stress.

Schematic of a continuous reactor operation is shown in Figure 1. Characteristic operating parameter for continuous reactors is the dilution rate, D.

$$D = \frac{F}{V}$$

Where, D is the dilution rate, F is the flow rate and V is the reactor working volume.

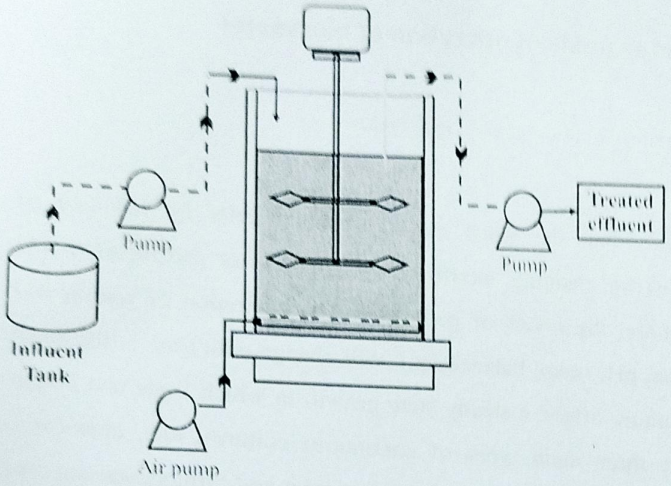

Figure 1: Schematic showing a continuous reactor operation

Methodology:

1. Preparation of medium (YPD):

The YPD medium containing 2% each of Yeast extract, Peptone and Dextrose is used for both seed culture and production medium. The initial pH is adjusted to 5.5. 50 or 100 ml of the medium was taken in a 250 or 500 ml of erlenmeyer flask and sterilized in an autoclave. The medium is inoculated with the slant culture, *Saccharomyces cerevisiae* and incubated in a rotatory shaker at 30^0C and 120 rpm.

2. Preparation of inoculum:

For seed culture preparation, the bacterium is grown in an Erlenmeyer flask (250 ml) containing YPD medium. The flask is then incubated at 30^0C and 120 rpm in an orbital shaking incubator for 48h.

3. Experimental procedure

1. Start/initiate batch fermentation as per earlier method
2. A feed with fresh growth medium or glucose is started, and an equal volume of culture broth is removed from the bioreactor when the culture reaches the exponential growth phase, or when the culture becomes substrate limited

3. Collect the samples from at regular interval of time from the reactor and exit
4. Continuous fermentation is carried out at various dilution rates.

4. Task required:

1. Operate the bioreactor at a particular dilution rate
2. Time profile of biomass growth and glucose concentration in the reactor under batch and continuous modes of operation
3. Determine the growth rate of microorganism and substrate concentration using standard protocols
4. Determine ethanol concentration in the fermentation broth by using ethanol kit or GC

References:

1. Doran, Pauline M. Bioprocess engineering principles. Elsevier, 1995.
2. Shuler M.L., Kargi F., 2002. Bioprocess Engineering, Second ed. Prentice Hall, New Jersey, USA.
3. Paul, Tanushree, et al. "Continuous bioreactor with cell recycle using tubular ceramic membrane for simultaneous wastewater treatment and bio-oil production by oleaginous Rhodococcus opacus." Chemical Engineering Journal 367 (2019): 76-85.