Experiment No. 8

Fed batch bioreactor study

Objective:

Fed batch bioreactor operation for utilization of glucose as carbon source

Introduction:

In a fed-batch operated system, continuous feeding of a substrate is followed for an efficient utilization, in particular for overcoming substrate inhibition in case of toxic substrate or for secondary metabolite production without any undesired product formation. Fed batch operation begins with a relatively low concentration of substrate in a batch operated bioreactor which is highly desired for achieving a maximum biomass growth, followed by continuous substrate loading but without any withdrawal of the contents inside.

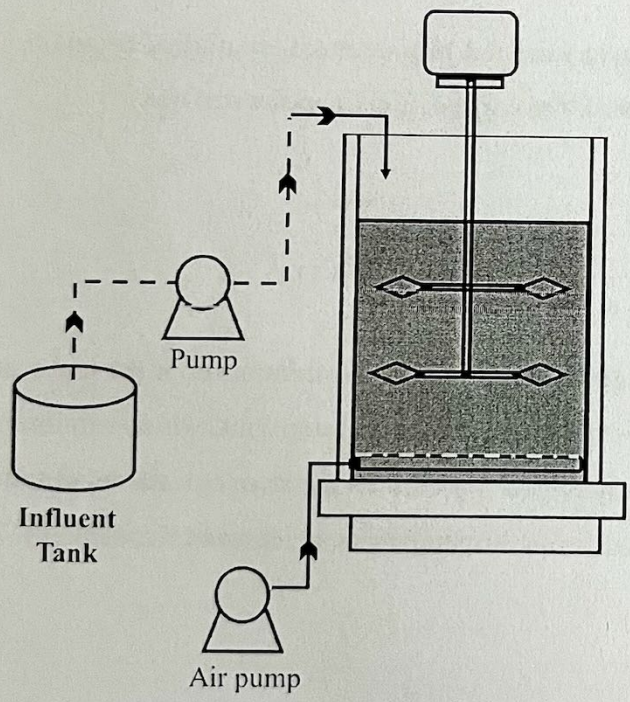

Figure 1: Schematic showing a fed batch reactor operation

This experiment demonstrates a fed-batch operation mode using a CSTR for achieving utilization of glucose without any inhibition.

The bioreactor is operated initially under batch for the growth of *Gordonia sp.*, followed by continuous feeding of glucose in mineral salt media. During the process,substrate is added as required by the growth rate of the organism.

The specific rate at which the substrate is used up, $q_{s/x}$, can be determined as the amount of substrate per cell and unit of time from the growth kinetics:

$$q_{S/X} = \frac{1}{Y_{X/S}}\mu \qquad (1)$$

Here in fed batch operation:

$$XV = X_0V_0e^{\mu t} \qquad (2)$$

Where X and V are the biomass concentration and volume of culture at time t, and X_0 and V_0 are the biomass concentration and volume of growth medium in the reactor at time $t = 0$. μ is the specific growth rate of the organism.

The specific rate of substrate uptake $q_{s/x}$, (g/l·h) in a fed-batch culture is satisfied by addition on demand. The required volumetric feeding rate, Q_s, consist of $q_{s/x}$ and the cell density X.

$$Q_S = q_{S/X}X \qquad (3)$$

This must be identical to the feeding rate:

$$Q_s = \frac{FS_0}{V} \qquad (4)$$

Where F is the rate of feeding (l/h) at the given time, S_0 is the concentration of the input and V is the volume of the reaction. The method of feeding can be either constant, which results in linear growth, or adjusted to increase exponentially so that S is maintained at an optimal level and results in exponential growth. The balance in a fed-batch fermenter may be described as follows.

Biomass:

$$\frac{d(VX)}{dt} = \mu XV \qquad (5)$$

From which

$$\frac{dX}{dt} = (\mu - D)X \qquad (6)$$

$$\frac{dVS}{dt} = 0$$

Since the volume increase as a result of the input is: $\frac{dV}{dt} = F$ (7)

D is the dilution rate as a result of input: $D = \frac{F}{V}$ (8)

For limiting substrate the following is valid: $\frac{d(VS)}{dt} = S_0 F - (\frac{\mu XV}{Y_{X/S}}) = 0$ (9)

Hence:

$$\text{Feed rate (F)} = \frac{\mu XV}{Y_{X/S}S_0} = \frac{\mu X_0 V_0 e^{\mu t}}{Y_{X/S}S_0} \quad (10)$$

Methodology:

1. **Preparation of mineral salt media (MSM):**

Medium with the following composition along with glucose (20 g/L) is prepared. Initial pH of the medium is adjusted to 7 using NaOH/HCL.

Reagent	**Composition (g/L)**
$MgSO_4.7H_2O$	0.409
$CaCl_2.2H_2O$	0.0265
KH_2PO_4	1
NH_4NO_3	1
$Na_2HPO_4.12H_2O$	6
$FeCl_3.6\ H_2O$	0.0833
1 ml trace element solution (g/L) $FeCl_3$ (17), $CaCl_2$ (0.6), $ZnSO_4$ (0.2), $CuSO_4 \cdot 7H_2O$ (0.2), $MnSO_4$ (0.2), $CoCl_2$ (0.8), H_3BO_3 (0.1) and $Na_2MoO_4 \cdot 2H_2O$ (0.3).	

2. **Preparation of inoculum:**

Gordonia sp. used in this experiment is initially grown using 50 ml Luria broth (L.B.) medium at

28 °C and 150 rpm orbital shaking.

For seed culture preparation, the bacterium is grown in an Erlenmeyer flask (250 ml) containing 100 ml MSM and 20 g/L glucose as the sole carbon source.

The flask is then incubated at 28 °C and 150 rpm in an orbital shaking incubator for 48h.

The 84 h grown culture is centrifuged at 5000 × g and the pellet obtained is washed twice with 25 ml distilled water. The washed pellet is then suspended in 100 ml MSM and subsequently used as the inoculum in the fed-batch bioreactor experiment.

3. Experimental procedure

For fed-batch operation mode to achieve steady state utilization of glucose by the bacterium, the reactor is first filled with 1 L MSM containing glucose as the culture media.

The prepared seed culture of the bacterium is then added as the inoculum to the media at the beginning of the experiment.

The reactor is run initially for 96 h under batch mode for achieving maximum biomass growth. The agitation and temperature in the reactor are maintained at 400 rpm and 28°C, respectively.

After the initial batch run, MSM containing glucose is continuously fed to the reactor so as to maintain simultaneous biomass growth and glucose utilization without any inhibition. Feeding starts at the end of exponential growth phase of the bacterium in the reactor. For the feeding, a feeding rate F according to equation 10 is obtained by substituting values of X_0, V_0, S_0, $Y_{S/X}$, and μ as determined previously from the batch operated reactor.

Samples are taken from the bioreactor at 6 h time interval for the analysis of biomass and glucose concentration.

For biomass analysis, OD of the samples at 660 nm are measured by using UV-Visible spectrophotometer.

For determination of glucose concentration in the sample a high performance liquid chromatograph (HPLC)/GOD-POD is used.

4. Task Required

1) Calculation of feed flow rate F

2) Time profile of biomass growth and glucose concentration in the reactor under batch and fed batch mode.

3) Comment on glucose utilization from the media

Table 1: Data for standard curve plotting (biomass and phthalate concentrations)

Values for standard graph between biomass concentration and OD		**Values for standard graph between glucose concentration and area underthe curve**	
Biomass concentration (mg/L)	**OD**	**Glucose concentration (g/L)**	**Area**

Table 2: Values of biomass and glucose concentrations in the samples taken during thebioreactor operation

Time	**OD of the culture**	**Biomass (mg/L)**	**glucose concentrations (g/L)**

References:

1. Doran, Pauline M. Bioprocess engineering principles. Elsevier, 1995.

2. Shuler M.L., Kargi F., 2002. Bioprocess Engineering, Second ed. Prentice Hall, Ne Jersey, USA.

3. Paul, Tanushree, et al. "Continuous bioreactor with cell recycle using tubular cerami membrane for simultaneous wastewater treatment and bio-oil production by oleaginou Rhodococcus opacus." Chemical Engineering Journal 367 (2019): 76-85.