DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY
Indian Institute of Technology, New Delhi-110016
Advanced Biochemical Engineering BEL 850

Experiment No.7

Objective:

To estimate the following kinetic parameters of growth of *E. coli*.

- Maximum specific growth rate (μ_m in h^{-1})
- Biomass yield coefficient ($Y_{x/s}$ in gdw g^{-1})

Materials and Methods:

- 250 ml and 500 ml flasks
- 1ml, 2ml, 5ml and 10 ml pipettes
- A freshly prepared slant of *E. coli*
- DNS reagent for reducing sugar (glucose) estimation
- A bioreactor with control system for pH, temperature, aeration, agitation and monitoring of dissolved oxygen, along with standard accessories.
- A multichannel recorder
- A spectrophotometer

Procedure:

1. Prepare 400 ml seed culture medium (Medium M9) of composition shown in Table 1 in two 500 ml flasks containing 200 ml medium each. Adjust pH to 7.0 with NaOH/ HCL as required. Glucose, $MgSO_4$ and $CaCl_2$ are sterilized separately and added to the remaining components only after cooling.
2. Prepare 200 ml each of 2N HCl and 2N NaOH solutions for pH control. To this end, autoclave required quantity of distilled water in 5000 ml flasks. Aseptically add calculated amount of concentrated HCl or sodium hydroxide which are considered sterile. Do not autoaclave acid or base solutions.

Table 1: Composition of the medium M9 (A standard minimal medium)

S.No.	Component	Concentration (g l^{-1})
1.	Glucose	3.0
2.	Ammonium chloride	2.5
3.	Na_2HPO_4 (anhydrous)	6.0
4.	KH_2PO_4(anhydrous)	3.0
5.	$MgSO_4.7H_2O$	0.246
6.	NaCl	0.5
7.	$CaCl_2.2H_2O$	0.019

3. Usually, the fermenter is inoculated with seed culture @ 10% v/v. Hence, for a fermenter with 4.0 L working volume, the total liquid volume prior to inoculation in the fermenter should be 90% of 4 L (which is 3.6 L). Since glucose, magnesium sulfate and calcium chloride solutions must be autoclaved separately, make their solutions in separate flasks in, say, 100 ml distilled water. With 300 ml volume assigned to these solutions, the liquid volume prior to sterilization in the fermenter becomes 3.6 – 0.3 = 3.3 L.

   Compute the quantities of various constituents of the production medium for 4 L, dissolve them in distilled water in the fermenter, and make the total volume 3.3 L by adding distilled water. Add 10% more water to account for water lost during sterilization. Add a drop of antifoam agent prior to sterilization.

4. After sterilization set the temperature to 30^{o}C and pH to 7.0

5. When temperature reaches 30^{o}C, add 100 ml glucose, 100 ml $MgSO_4$ and 100 ml calcium chloride solutions.

6. Set the aeration rate to 0.5 vvm. Calibrate dissolved oxygen probe.

7. Check if the pH has reached the set value. If yes, the fermenter is ready for inoculation.

8. Inoculate the fermenter with flasks containing 100 ml each of *E. coli* culture.

9. Put the recorder switch on. The chart will move at 20 mm/h. Put a marker line on the chart paper and write down date and time of inoculation.

10. Take samples every hourly. Analyze them for cell mass by taking optical density at 620 nm on a spectrophotometer. For glucose take cell free sample and estimate reducing sugar by DNS method.

Observations:

S.No.	Fermentation Time (h)	Glucose Concentration $s(\frac{g}{l})$	Cell Mass $x,(\frac{g}{l})$

1.
2.
3.
4.
5.
6.
7.
8.
9.
10.

Computations:

- **Maximum specific growth rate**

Plot $\ln x$ vs. t curve, and determine the slope of the linear portion of the curve, which equals to specific growth rate.

- **Biomass yield coefficient**

Average biomass yield coefficient can obtained from the slope of the x vs. s curve.

- **Productivity of cell mass**

Average productivity:

Let x_0 and x_f denote the cell densities at $t = 0$ and $t = t_f$, respectively. Then the average productivity of cell mass is given by $(x_f - x_0)/t_f$ gdw L^{-1} h^{-1}

Point productivity:

The point productivity at any t is given by the slope of the x vs. t curve at that point.

References:

1. Obom, K. M., Magno, A., Cummings, P. J. Operation of a Benchtop Bioreactor. *J. Vis. Exp.* (79), e50582, doi:10.3791/50582 (2013). A video of benchtop video operation.

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