Experiment-2

OBJECTIVE

Estimation of specific growth rate and growth yield in an aerobic fermentation process

INTRODUCTION:

When a sterile liquid nutrient medium is inoculated with a seed culture, the microorganisms grow by selectively take up the dissolved nutrients. A typical batch growth curve exhibits the following phases in order

a) Lag phase
b) Logarithmic or Exponential phase
c) Deceleration phase
d) Stationary phase
e) Death phase

The lag phase appears immediately after the inoculation and is a period of adaptation of cells to a new environment. After this adaptation period, cells have adjusted to the new environment. They can multiply rapidly and the cell number or cell mass increases exponentially with time. This is the phase of exponential growth. The deceleration phase follows the exponential phase, wherein; growth slows down due to depletion of some of the essential nutrients or accumulation of toxic by-products. The duration of this phase is very small. Following this phase, the culture enters the stationary phase. In this phase, the net growth rate is zero or the growth rate is equal to the death rate. After the stationary phase, the death phase begins. In this phase, the toxic products accumulate to such an extent that cells begin to die.

The specific growth rate (μ) given by $\mu=(1/OD)[d(OD)/dt]$, is constant in exponential phase period. It can be obtained from the slope of straight line obtained from a plot of ln(OD) vs time data.

The overall growth yield coefficient ($Y_{X/S}$) is obtained by dividing gm-cell formed by gm substrate consumed upto the end of fermentation.

MATERIALS & METODS:

Microorganism: ***Escherchia*** *coli* (E.coli)

Shaker: at 37^0 C and 220 rpm for the growth of inoculum in LB-medium. Inoculum Culture age 14-16h

Medium for 3L-Fermentor (Working Volume=2.2L)

a) Yeast Extract 1.0 g/l

b)	Citric Acid	1.7 g/l
c)	KH_2PO_4	6.8 g/l
d)	Na_2HPO_4	8.9 g/l
e)	Glucose	3.0 g/l
f)	$MgSO_4$	0.2 g/l

Medium Preparation:

1. Weigh (a), (b), (c), (d) for 2.2L medium volume and dissolve them one after other in a 3L fermentor. Make-up volume to 2.0L. Set pH to 6.8 by 12% NH_4OH.

2. Dissolve (f) in 40 ml distilled water in a 100ml flask.
   Dissolve (e) in 50 ml distilled water in a 100ml flask.

3. Sterilize the fermentor containing contents specified at point-1 above. Also autoclave the items mentioned at point-2.

4. Cool items at point-1&2 to working temperature and aseptically pour contents of items at point-2 into the 3L fermentor. (Now the medium volume in the fermentor will be 2.19L)

Inoculation:

1. Inoculate the 3L fermentor (@5% of 2.2 L) with 110 ml culture from 14 -16h old PC-I (given). (Total medium volume in the 3L fermentor=2.2L)

2. Incubation temp. = 37^oC
   Incubation time = 12h
   Expected max $OD_{600,\ 1\ cm}$ = 6.0
   Shaker rpm = 220

Measurement of Cell OD

The optical density measurement on spectrophotometer is based on light scattering. In accordance with Beer Lambert's Law, the broth sample should be suitably diluted so that its OD_{600nm} is between 0.1-0.4. The OD of undiluted sample can be obtained by multiplying diluted sample OD with the dilution factor.

Measurement of Cell concentration

Weigh 1.5 ml Appendroph tube empty. Transfer 1ml broth sample to it and centrifuge at 10,000 rpm for 5 minutes. Decant the supernant and dry the residue in tube at 40^0C under vacuum for 24h. Now place the tube in a desiccator for cooling. After 30 minutes, weigh the tube again. Subtract the weight of empty tube. Express cell dry weight in (mg dry cell/ml of the sample broth.

Measurement of glucose concentration

1. Preparation of DNS reagent

Solution (i) To 300ml of 4.5% NaOH, 800ml of 10% di-nitrosalicylic acid (DNS) and 225g Rochelle salt (Sodium-potassium tartrate) are added.

Solution (ii) To 10g of crystalline phenol, 22ml of 10% NaOH is added and the volume is made up to 100 by distilled water.

Solution (iii) To 69ml of solution (ii), 6.9g sodiumbisulphite is added.

For making the DNS reagent, solution-(iii) is added to solution-(i) and mixed thoroughly till all the salts dissolve. The DNS reagent so obtained is filtered through glass wool and stored in dark brown bottle to avoid light, which may degrade the reagent.

2. Preparation of standard curve (Glucose/ Sucrose)

The range of sugar estimation by the DNS method is 0.1-4.0 mg/ml sugar in sample. Therefore the standard curves should be made only within this range.

(i) To make a sugar standard solution (SSS) containing sugar concentration of 4 mg/ml, dissolve 400mg sugar in distilled water (DW) and make up the volume to 100ml in a measuring flask.

(ii) In 5X30ml capacity test tubes, pour suitable volumes of SSS and add required volume of distilled water so as to obtain 1ml of diluted SSS in the range .1mg to 4.0 mg in the test tubes. In the sixth test tube (blank), take only 1 ml distilled water (follow tables given in observations)

(iii) In case of sucrose standard, add 0.5 ml 2N HCl to each tube and boil for 10 minutes (this will ensure hydrolysis of sucrose to glucose & fructose). After cooling the tubes, add 0.5 ml NaOH to neutralize the reaction mixture. In case of glucose standard, instead of adding HCl or NaOH, it will be sufficient to add 1ml water to each tube in step-2(iii).

(iv) Add 3ml DNS reagent to all the tubes, and keep the tubes in boiling water bath for exactly 5 minutes. Thereafter, cool all the tubes under running tap. Add 20 ml distilled water to all the tubes and mix them thoroughly.

(vi) Read OD at 540nm wavelength on a spectrophotometer using sixth tube as a blank. Make a plot of OD vs Sugar concentration (mg/ml)

Standard Curve for Glucose

TEST TUBE	SSS Added	DW Added	Sugar in test tube	DW added (ml)	DNS reagent	DW added	OD 540nm

#	(ml)	(ml)	(mg)		added (ml)	(ml)	
1	0.1	0.9	0.4	1	3	20	
2	0.3	0.7	1.2	1	3	20	
3	0.5	0.5	2.0	1	3	20	
4	0.7	0.3	2.8	1	3	20	
5	0.9	0.1	3.6	1	3	20	
6	0	1.0	0	1	3	20	

Make a plot of glucose and standard curve by fitting a regression line.

For measurement of glucose in the sample from broth, centrifuge (10,000 rpm, 10 minutes) the sample and suitably dilute the supernatant. Process as discussed above and read the OD against a blank. Use the standard curve for calculation of glucose concentration in the sample of fermentation broth.

OBSERVATIONS:

Take a sample of 3 ml aseptically from the 3L fermentor at 0h, i.e.; just after the inoculation. Thereafter, take samples at an interval of 1h. Take $OD_{600nm,\ 1cm}$ of the samples. Tabulate the data with time.

Time (h)	**Cell OD**	**Glucose (g/l)**	**Cell dry weight (g/l)**

RESULTS:

Plot ln(OD) vs. time data and fit a linear regression through the data points using Microsoft excel program. Calculate the specific growth rate from the slope of the line
Estimate average growth yield coefficient from the expression:

$Y_{X/S} = (X_F - X_0)/(S_F - S_0)$

Where X_F & S_F represent cell dry weight and substrate concentration at the end of fermentation respectively, and

X_0 and S_0 represent dry cell weight and substrate concentration at the beginning of the fermentation.

DISCUSSION:

Write a discussion on the shape of the OD vs. time graph and your comments on the estimated values of growth parameters.